Aggregata octopiana (Apicomplexa: Aggregatidae) from *Octopus vulgaris* off NW Spain

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ABSTRACT: Gamogony and sporogony of *Aggregata octopiana* were commonly observed during histological examination of the digestive tract and the connective tissue sheath of wild *Octopus vulgaris* (Cuvier, 1798) from Ria de Vigo (NW Spain). In massive infections, numerous oocysts were found in the muscular tissue.

KEY WORDS: Apicomplexa · *Aggregata octopiana* · *Octopus vulgaris*

INTRODUCTION

Coastal octopus have the longest history of human exploitation of any cephalopod (Boyle 1990). The best known species, *Octopus vulgaris* Cuvier, 1798, is extremely important in fisheries (Roper et al. 1984). It also represents a major protein resource in most Mediterranean countries (Guerra 1992) and is widely used as a biomedical research model (Hanlon & Forsythe 1985).

Despite their importance, little information is available on protozoan-related diseases occurring in wild populations, even in the common *Octopus vulgaris*. A notable exception is the coccidian *Aggregata* Frenzel, 1885 (Apicomplexa: Aggregatidae), which has been reported in the digestive tract of 13 octopus species worldwide (Sardella & Re 1988, Hochberg 1990, Poynton et al. 1992).

This paper reports the first description of an apicomplexan of the genus *Aggregata* in *Octopus vulgaris* from eastern North Atlantic temperate waters.

MATERIALS AND METHODS

Between November 1994 and February 1995, 30 wild octopus were captured in artisanal gear by local fishermen from the Ria of Vigo (NW Spain). Tissues were excised from diverse regions of the digestive tract. Then, fresh material was examined by phase contrast

or fixed in 10% buffered formalin, embedded in paraffin and sectioned using a Microm HM-340 E microtome (Microm, Walldorf, Germany). Sections (4 to 5 µm) were stained with haematoxylin-eosin (Culling et al. 1985). Measurements of parasitic gamogonal and sporogonal stages was made using an ocular micrometer and are expressed in micrometers as ranges followed by the mean and standard deviations in parentheses (n = 30). The number of sporocysts was determined from each individual oocyst by using a Burkner chamber. Terminology employed for parasitic stages follows Hochberg (1983).

RESULTS

Description of life cycle stages of *Aggregata octopiana* in *Octopus vulgaris*

Gamogony. The development of microgamonts was characterized by extensive cytoplasmic membrane folding, cytoplasmic partitioning and an increase in the number of peripheral nuclei. Scores to thousands of flagellated microgametes were produced (Fig. 1).

Macrogamonts became macrogametes after a series of nuclear changes (Poynton et al. 1992). Macrogametes possessed foamy cytoplasm and a nucleus, containing a compact or ring-like nucleolus (Fig. 1D).

Both microgametes and macrogametes were surrounded by a pale homogenous area representing a parasitophorous vacuole.

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Fig. 1. *Aggregata octopiana*. Gamogony in *Octopus vulgaris*. (A to C) Successive stages in the formation of microgametes. (A) Microgamont characterized by extensive folding of the cytoplasmic membrane. Scale bar = 20 μ m. (B) Portion of microgamont (mg) showing the partitioned cytoplasm (Cy) and peripheral nuclei (n). Scale bar = 5 μ m. (C) Microgametes (mt) completely formed. Scale bar = 20 μ m. (D) Macrogamete with eccentric nucleus (n), conspicuous nucleolus and foamy cytoplasm (Cy). Scale bar = 25 μ m. Stained with hematoxylin and eosin

Fig. 2. *Aggregata octopiana*. Sporogony in *Octopus vulgaris*. (A) Sporont showing extensive folding of cytoplasmic membrane, and numerous nuclei at the periphery of the partitions. Scale bar = 25 μ m. (B) Sporont segregating into uninucleate sporoblasts (Sb). Scale bar = 10 μ m. (C) Immature sporocysts (arrows). Scale bar = 10 μ m. (D) Oocyst containing mature sporocysts (Sc) with sporozoites (Sz). Ow: Oocyst wall. Scale bar = 10 μ m. Stained with hematoxylin and eosin

Fig. 3. *Aggregata octopiana* in *Octopus vulgaris*. Fresh microscopic examination of squash preparation, with phase contrast illumination. (A) Isolated sporozoites of *A. octopiana*. Scale bar = 10 µm. (B) Sporocysts of atypical size 'giants' containing from 20 to 26 sporozoites. Scale bar = 10 µm

Sporogony. The union of a microgamete and macrogamete produced a zygote, which then became an early sporont that was characterized by cell membrane folding and alignment of numerous nuclei on the surface of the partitioned cytoplasm (Fig. 2A). Individual nuclei with accompanying cytoplasm later budded off, forming uninucleate spherical sporoblasts (Fig. 2B). The development of sporoblast into sporocyst was characterized by an increase in the number of nuclei, and the further partitioning of nuclei and cytoplasm formed sporozoites (Fig. 2C, D). In fresh preparations, the sporocysts were spherical in shape, 11 to 15 µm (13.34 ± 1.63) in diameter, and possess a smooth surface and a residual body. The sporocysts, which were composed of 2 equal halves joined together by a equatorial suture, opened at the suture, and released the sporozoites. The number of sporozoites for both fresh and sectioned material ranged from 6 to 12 per sporocyst. They were arranged in a spiral fashion inside the sporocyst with all nuclei at one pole. Isolated sporozoites were 16 to 23.5 µm (19.77 ± 2.46) long by 2 to 2.5 µm (2.18 ± 0.25) wide (Fig. 3A).

Oocysts were mainly oval but sometimes almost spherical in morphology and ranged in size from 200 to 1050 µm (612.5 ± 316.75) in length by 150 to 700 µm (402.5 ± 155.99) in width. Multiple sporocysts were found within each oocyst, numbering between <1000

and 60 000. Approximately 10% of the oocysts examined possessed one or more atypically sized sporocysts ('giants') (Fig. 3B) with diameters of 23 to 32 µm (28.83 ± 2.73) containing 20 to 26 sporozoites of sizes 27.5 to 31.5 µm (29.83 ± 1.44) long by 2.75 to 3.5 µm (2.97 ± 0.23) wide.

Prevalence and site of infection

Routine histological examination of the digestive tracts of wild *Octopus vulgaris* from NW Spain demonstrated that gamogony and sporogony were present in all specimens examined (prevalence 100%), most frequently in the digestive tract. The highest percentage of infection was found in spiral caecum, intestine, crop, digestive gland, oesophagus and connective tissue sheath. In one case of massive infection we also found oocysts of *Aggregata octopiana* in the muscle tissue.

DISCUSSION

Several authors (Sprague & Couch 1971, Levine 1985, 1988) have suggested that most species of *Aggregata* in Europe require redescription to determine their validity. Synonymy-related problems have

Table 1. *Aggregata octopiana*. Comparative data on morphology and dimensions of coccidia in *Octopus vulgaris*

Sporocysts (Sc)		Sporozoites		Reference
Surface	Diameter (µm)	Number/Sc	Length (µm)	
Smooth	12–15	9–16	?	Moroff (1908)
	20	16	?	Wurmbach (1935)
	12–15	8–16	25–30	Hochberg (1990)
Smooth	11–15	6–12	16–23.5	Present study

surfaced because *Aggregata* species have usually been differentiated on the basis of host, size and surface appearance of sporocysts, and number and length of sporozoites. With these diagnostic characters, Hochberg (1990) and Poynton et al. (1992) recognize 6 species of the genus *Aggregata* that are associated with cephalopod hosts: *A. eberthi* in *Sepia officinalis* (Dobell 1925), *A. kudo* in *S. elliptica* (Narasimhamurti 1979), *A. dobelli* and *A. millerorum* in *Octopus dofleini* and *O. bimaculoides* respectively (Poynton et al. 1992), and *A. octopiana* and *A. spinosa* in *O. vulgaris* (Moroff 1908).

The characteristic morphological features of our species of *Aggregata* resemble those of the group formed by *A. octopiana* (Moroff 1908, Wurmbach 1935, Hochberg 1990) (Table 1), all having similar gamogonial and sporogonial stages. Both the diameter measurements of the sporocyst and the number of sporozoites were close to those described previously for this species. Sporozoite length, however (16 to 23.5 µm), was less than the length described by Hochberg (1990) (25 to 30 µm), but this may be explained by the presence of abnormally sized 'giant' sporocysts, found in 10% of the oocysts examined, measuring 27.5 to 31.5 µm in diameter. The occasional presence of giant sporocysts had previously been observed by Dobell (1925) in *A. eberthi*, by Narasimhamurti (1979) in *A. kudo* and by Poynton et al. (1992) in *A. dobelli*. Our finding of *A. octopiana* confirms the great extent of its geographical distribution and high specificity for the host.

Previously described *Aggregata octopiana* from octopus produced generalized infections in the digestive tract. The caecum appears to be the preferred site of infection. The unusual presence of the coccidians in the musculature, described here for the first time, is likely a result of the massive infections that occurred in the examined specimens. This unusual phenomenon has been described for other protozoan infections (Estévez et al. 1992).

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LITERATURE CITED

- Boyle PR (1990) Cephalopod biology in the fisheries context. *Fish Res* 8:303-321
- Culling CFA, Allison RT, Barr WT (1985) Cellular pathology technique. Butterworth & Co, London
- Dobell C (1925) The life-history and chromosome cycle of *Aggregata eberthi* (Protozoa: Sporozoa: Coccidia). *Parasitology* 17:1-136
- Estévez J, Iglesias R, Leiro J, Ubeira FM, Sanmartín ML (1992) An unusual site of infection by a microsporean in the turbot *Scophthalmus maximus*. *Dis Aquat Org* 13:139-142
- Guerra A (1992) Mollusca, Cephalopoda. In: Ramos MA (eds) *Fauna Ibérica*, Vol. 1. Museo Nacional de Ciencias Naturales CSIC, Madrid
- Hanlon RJ, Forsythe JW (1985) Advances in the laboratory culture of *Octopus* for biomedical research. *Lab Anim Sci* 35(1):33-40
- Hochberg FG (1983) Parasites of cephalopods: a review. *Mem Natl Mus Victoria* 44:109-145
- Hochberg FG (1990) Diseases of Mollusca: Cephalopoda. Diseases caused by protists and metazoans. In: Kinne O (ed) *Diseases of marine animals*, Vol. III. Biologische Anstalt Helgoland, Hamburg, p 47-202
- Levine ND (1985) Phylum II. Apicomplexa Levine, 1970. In: Lee JJ, Hutner SH, Bovee EC (eds) *An illustrated guide to the Protozoa*. Society of Protozoologists, Lawrence, KS, p 322-374
- Levine ND (1988) *The protozoan Phylum Apicomplexa*, Vol I. CRC Press, Boca Raton, FL
- Moroff T (1908) Die bei den Cephalopoden vorkommenden Aggregatarten als Grundlage einer kritischen Studie über die Physiologie des Zellkernes. *Arch Protistenkd* 11:1-224
- Narasimhamurti CC (1979) The eimeriid *Aggregata kudo* n. sp. from *Sepia elliptica*. *Angew Parasitol* 20:154-158
- Poynton SL, Reimschuessel R, Stoskopf MK (1992) *Aggregata dobelli* n. sp. and *Aggregata millerorum* n. sp. (Apicomplexa: Aggregatidae) from two species of *Octopus* (Mollusca: Octopidae) from the Eastern North Pacific Ocean. *J Protozool* 39(1):248-256
- Roper CFE, Sweeney MJ, Nauen CE (1984) *FAO species catalogue*, Vol. 3. Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. *FAO Fisheries Synopsis* 125
- Sardella NH, Re ME (1988) Coccidian parasitosis by the genus *Aggregata* in Patagonian shallow water octopuses. I. *Aggregata* sp. in *Octopus tehuelchus* d'Orbigny. *Physis (B Aires) Secc A* 46(111):51-60
- Sprague V, Couch J (1971) An annotated list of protozoan parasites, hyperparasites and commensals of decapod crustacea. *J Protozool* 18:526-537
- Wurmbach H (1935) Über die Beeinflussung des Wirtsgewebes durch *Aggregata octopiana* und *Klossia helicina*. *Arch Protistenkd* 84:257-284

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