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Selected pathological, immunohistochemical and ultrastructural changes associated with an infection by *Diphyllobothrium dendriticum* (Nitzsch, 1824) (Cestoda) plerocercoids in *Coregonus lavaretus* (L.) (Coregonidae)

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Abstract

The pathological changes induced by an infection of *Diphyllobothrium dendriticum* (Nitzsch, 1824) plerocercoids in powan, *Coregonus lavaretus* (L.), from Loch Lomond, Scotland, were assessed using immunohistochemical and ultrastructural techniques. In a sample of 26 powan, the occurrence of encysted plerocercoids of *D. dendriticum* on the outer surface of the stomach was 38.5% (n = 10) with the number of cysts ranging from 4 to 15 and measuring 4.2 ± 1.0 mm × 3.4 ± 0.9 mm (mean ± SD). Histological examination of intestinal samples also revealed plerocercoids (2–21) encapsulated within a proliferation of mesenteric fibrous tissues of the gastric wall and, occasionally, by the gut lamina propria-submucosa and lamina muscularis. In section, cysts were tri-layered and were formed from a series of concentric whorls of fibroblast and collagen fibre-based connective elements. The extent of necrosis within each muscle layer and the serosa of the stomach differed, notably within the latter that was marked by a chronic inflammatory reaction and fibrosis. Within the cyst and around it, a large number of degranulating mast cell/eosinophilic granule cells were seen, in addition to melano-macrophage centres. Immunohistochemical staining of sections of infected stomach revealed a high density of elements, in close proximity to plerocercoids, staining positive for serotonin, bombesin, substance P and galanin. Uninfected material did not present the same levels of activity. Sections through both infected and uninfected tissue were also tested for elements containing vasoactive intestinal peptide, met-enkephalin, calcitonin gene-related peptide, neuropeptide Y and nitric oxide synthase, but these were absent.

Keywords: Coregonus lavaretus, *Diphyllobothrium dendriticum*, immunohistochemistry, pathology, plerocercoid, stomach.

Introduction

While *Diphyllobothrium* Cobbold, 1858 (Cestoda: Pseudophyllidea) infections of man have received much attention (Bonsdorff 1977; Vaiani, Terramocci, Crotti, Gustinelli, Invernizzi, Fioravanti & Pampiglione 2006), they also represent important infestations of wild and cultured freshwater fish (Hoffman & Dunbar 1961; Wootten & Smith 1979; Halvorsen & Andersen 1984). *Diphyllobothrium dendriticum* (Nitzsch, 1824) uses a copepod as its first host, a planktivorous fish such as those belonging to the Coregonidae, Salmonidae and Gasterosteidae as its second intermediate host and a larid gull as its definitive host (Wright & Curtis 2000). Generally, the most intense reaction to the parasite occurs when the fish acts as an intermediate stage where the metacestode becomes encapsulated within the body cavity or within various body organs.

Mortalities of fish linked to infections of *Diphyllobothrium* have been recorded from North

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America (Becker & Brunson 1967; Bérubé & Curtis 1986), South America (Torres, Franjola, Figueroa, Schlatter, Gonzalez, Contreras & Martin 1981; Revenga 1993; Torres, Lopez, Cubillos, Lobos & Silva 2002), and from Europe (Fraser 1960a,b; Hoffman & Dunbar 1961; Henricson 1977; Halvorsen & Andersen 1984; Rahkonen, Aalto, Koski, Särkkä & Juntunen 1996), and their impact on wild populations of fish, however, is currently unknown (Rahkonen & Valtonen 1997).

The pathology associated with infections of *Diphyllobothrium* spp. has been detailed by a number of authors (Torres *et al.* 1981, 2002; Rahkonen *et al.* 1996), while the immune response and tissue reaction of the host has been comprehensively investigated in naturally acquired infections of *D. dendriticum* and *Diphyllobothrium ditremum* and following the experimental infection of *D. dendriticum* in *Oncorhynchus mykiss* (Walbaum) by Sharp, Pike & Secombes (1989, 1991, 1992).

Enteric helminth infections commonly cause inflammation of the host alimentary canal leading to alterations in gastrointestinal function such as enhanced secretion and gut propulsive motility (Palmer & Greenwood-Van Meerveld 2001; Dezfuli, Giari, Arrighi, Domeneghini & Bosi 2003). Intestinal worm infections in mammals, for example, have been shown to induce alterations in the concentration of several neuromodulators in host tissues and plasma (McKay, Halton, Johnston, Shaw, Fairweather & Buchanan 1991; Fairweather 1997; Eysker & Ploeger 2000). A recent plethora of studies in fish have shown that helminths can induce a marked change in the concentration of certain neuromodulators (Dezfuli, Arrighi, Domeneghini & Bosi 2000; Dezfuli, Giari, Simoni, Bosi & Manera 2002a; Dezfuli, Pironi, Giari, Domeneghini & Bosi 2002b; Dezfuli *et al.* 2003; Bosi, Shinn, Giari, Simoni, Pironi & Dezfuli 2005a; Dezfuli, Giari, Simoni, Shinn, Manera & Bosi 2005). These studies suggest that the presence of a parasite within a host can induce the formation of a network of nervous fibres at the site of inflammation which is demonstrated by the increase in the number of immunoreactive elements [e.g. bombesin, substance P (SP) and galanin] within the newly formed network. Although a similar response was expected for the powan–cestode system, almost nothing was known regarding the nervous system of infected and uninfected fish stomach tissue. The aim of this study was to assess which neuromodulators were present in normal gut tissue of *Coregonus lavaretus* and whether the presence of *D. dendriticum* plerocercoids influenced their distribution and concentration.

The work of Sharp *et al.* (1992), using experimental infections, provided a clear account of the sequential development of *Diphyllobothrium* and its migration through host tissue. A preliminary study of powan–*Diphyllobothrium* infected material by the current authors demonstrated a high number of mast cells/eosinophilic granule cells (EGCs) suggesting that they were part of the host defence mechanism as has been shown within other teleosts (Reite 1997, 2005; Reite & Evensen 2006). A high number of such cell types, which are usually active in chronic inflammation (Reite & Evensen 2006), suggested that the parasite is causing damage by inducing degranulation of mast cells/EGCs with the release of inflammatory cytokines. In addition to these cells, melano-macrophage centres (MMCs) were also observed within the tissues forming the cyst around the *D. dendriticum* plerocercoid. Proliferation of MMCs has been linked to a number of pathological and physiological changes in the host (Vogelbein, Fournie & Overstreet 1987; Wolke 1992; Couillard, Williams, Courtenay & Rawn 1999; Agius & Roberts 2003). The aim of this project was to conduct a detailed study of the infected tissues to elucidate the role of the key cell types (i.e. EGCs and MMCs) in the defence mechanisms of the host and to assess the distribution and a number of a range of immunoreactive elements in uninfected and infected host stomach tissue.

Materials and methods

The guts of 26 powan, *C. lavaretus*, (32.03 ± 0.63 cm fork length; 355.96 ± 17.39 g total body weight) were analysed from powan collected in two gill net samples (July 2003 and August 2004) from Loch Lomond (53°10′N, 4°39.1′W). The powan were given a lethal dose of MS222 (Sandoz, Basel, Switzerland), weighed and measured before they were dissected ventrally, sexed and the alimentary canals observed *in situ*. In parasitized fish, the number and position of each encysted plerocercoid on the outer surface of the stomach were recorded; the plerocercoids were removed *in situ* and fixed in chilled (4 °C) Bouin's fluid for 7 h. The samples were then processed routinely for paraffin embedding, cut in 5-μm-thick sections and stained either with haematoxylin and

eosin (H&E), Azan-Mallory, periodic acid–Schiff (PAS), alcian blue/PAS, or used for immunohistochemistry. The latter was done according to the peroxidase–antiperoxidase method detailed in Dezfuli *et al.* (2002b, 2003). The anti-sera, the working dilution and the incubation times used for each of the neuromodulators are given in Table 1. The controls for the specificity of the immunohistochemical reactions were performed by the preabsorption of each anti-serum with the corresponding antigen (Table 2). The control for the anti-protein gene-product 9.5 (PGP9.5) serum was performed by incubating the sections with rabbit normal serum using the same conditions for the primary anti-serum. Tissue sections taken from a rat and a pig were used as positive controls. Evaluation of the distribution and frequency of the immunoreactive elements were based on subjective estimates after the examination at 20× of five sections of the stomach of 18 powan (10 parasitized and 8 uninfected). Each section was scored depending on whether there was a low (+), medium (++) or a high (+++) occurrence of immunoreactive elements.

For light and electron microscopy, infected stomach tissues measuring up to 8 × 8 mm in diameter were fixed for 2 h in a chilled (4 °C) 2% glutaraldehyde solution buffered at pH 7.2 with 0.1 M sodium cacodylate. Thereafter, the pieces were rinsed for 12 h with 0.1 M sodium cacodylate buffer containing 6% sucrose. The tissues were then post-fixed in 1% osmium tetroxide in the same buffer for 2 h, dehydrated through a graded ethanol series, transferred to propylene oxide and then embedded in an Epoxy-Araldite® mixture (Fluka, Buchs, Switzerland). Semi-thin sections (5 μm) were cut on a Reichert Om U2 ultramicrotome (Reichert-Jung, Vienna, Austria) and stained with methylene blue. Ultra-thin sections (90 nm) were stained with a solution of 4% uranyl acetate in 50% alcohol and Reynold's lead citrate and examined using a Hitachi H-800 electron microscope (Hitachi, Tokyo, Japan). For comparative purposes, the uninfected stomachs of 8 *C. lavaretus* were also processed. Light photomicrographs were taken using a Nikon microscope ECLIPSE 80i and a Nikon stereomicroscope (Nikon, Tokyo, Japan). Morphometric measurements of key features of the

Table 1 The primary anti-sera used in this study

Anti-sera raised in rabbit	Code	Source	Dilution	Incubation
Bombesin	1400-0004	Biogenesis Ltd., Poole, UK	1:200	Overnight at 4 °C
Bombesin	IHC 7113	Peninsula Lab., Inc., Belmont, CA, USA	1:200	Overnight at 4 °C
CGRP	IHC 7181	Peninsula Lab., Inc., Belmont, CA, USA	1:400	24 h at 4 °C
Galanin	T-4330 (IHC 7153)	Peninsula Lab., Inc., Belmont, CA, USA	1:500	Overnight at 4 °C
Met-enkephalin	IHC 8602	Peninsula Lab., Inc., Belmont, CA, USA	1:500	Overnight at 4 °C
Met-enkephalin	AB 1975	Chemicon Int., Temecula, CA, USA	1:500	Overnight at 4 °C
NOS	sc-648	Santa Cruz Biot., Santa Cruz, CA, USA	1:200	Overnight at 4 °C
NPY	6730-0004	Biogenesis Ltd., Poole, UK	1:50	24 h at 4 °C
NPY	IHC 7180	Peninsula Lab., Inc., Belmont, CA, USA	1:500	24 h at 4 °C
Serotonin	AB 938	Chemicon Int., Temecula, CA, USA	1:1000	Overnight at 4 °C
Substance P	T-4107 (IHC 7451)	Peninsula Lab., Inc., Belmont, CA, USA	1:500	Overnight at 4 °C
VIP	CA-08-340	Genosys Biotechnologies, Cambridge, UK	1:1000	Overnight at 4 °C
VIP	9535-0204	Biogenesis Ltd., Poole, UK	1:50	24 h at RT

CGRP, calcitonin gene-related peptide; NOS, nitric oxide synthase; NPY, neuropeptide tyrosine; VIP, vasoactive intestinal peptide; RT, room temperature.

Table 2 Details of the peptides used for the absorption controls

Peptide	Code	Source
Bombesin	B 4272	Sigma Chemicals, St. Louis, MO, USA
CGRP	H 4924	Bachem AG, Bubendorf, Switzerland
Galanin	H 1365	Bachem AG, Bubendorf, Switzerland
Met-enkephalin	H 2785	Bachem AG, Bubendorf, Switzerland
NOS	sc-648 P	Santa Cruz Biotechnologies, Inc., Santa Cruz, CA, USA
NPY	H 6375	Bachem AG, Bubendorf, Switzerland
Serotonin	H 9523	Sigma Chemicals, St. Louis, MO, USA
Substance P	H 1890	Bachem AG, Bubendorf, Switzerland
VIP	V 3628	Sigma Chemicals, St. Louis, MO, USA

CGRP, calcitonin gene-related peptide; NOS, nitric oxide synthase; NPY, neuropeptide tyrosine; VIP, vasoactive intestinal peptide.

plerocercoid-infected tissue were recorded with the aid of light microscopy, using computerized image analyser software (Lucia G 4.8, Laboratory Imaging, Prague, Czech Republic).

Results

Figure 1a shows a histological section through the stomach of an uninfected *C. lavaretus*, illustrating the respective layers of the gastric wall and their integrity. From the 26 *C. lavaretus* examined, the stomachs of 10 (38.5%) fish were parasitized with plerocercoids of *D. dendriticum*. Each stomach had between 4 and 15 plerocercoids within a cyst measuring $4.2 \pm 1.0 \times 3.4 \pm 0.9$ mm (mean $\pm$ SD) (Fig. 1b,c). The larger cysts on the external surface of the stomach were evident as loosely attached nodules (Fig. 1b,c), while others were intramural. Sections through the cysts revealed a fully developed, tightly packaged plerocercoid within (Fig. 1c), or occasionally, alongside necrotic tissue (Fig. 1c). Sections through the stomach wall of infected powan revealed a number of plerocercoids (range 2–21 larvae per infected host) distributed at various depths throughout the tissue (Fig. 1d). Cysts were observed to consist of three distinct areas, an inner fibrous area where necrotic cells were found in close proximity to the plerocercoid (Fig. 2a,b), a middle region consisting of degenerating epithelioid cells which give way to giant cells towards the outer zone of the cyst (Fig. 2b,c) and, finally, the outer layer made up largely of concentrically oriented fibroblasts and collagen (Fig. 2e). Larvae appear to penetrate the stomach wall and migrate to the serosa (Fig. 1d,e) and as they migrate and grow, they elicit a chronic inflammatory response, which results in fibrosis and larval encapsulation (Figs 1d & 2a). By the time most plerocercoids reach the serosa, they have attained their maximum size and remain attached to the serosal surface as a loose nodule (Fig. 1b,c) or as a protuberance within the gastric wall (Fig. 1b,e). As mentioned above, intramural plerocercoids were also found within the gastric wall.

In addition to encapsulation of the parasite, a range of other host responses were evident including the presence of MMC in close proximity to the plerocercoid (Fig. 1f). Also, within the cyst wall and in the fibrous tissues surrounding the cyst, a large number of EGCs were observed (Fig. 2a,b). In several cases where the encysted plerocercoid was found firmly attached to the outer surface of the stomach, a section through the cyst revealed a large number of EGCs in a configuration suggesting that they were passing through the muscle layer and migrating towards the plerocercoid (Fig. 2a,c). An electron microscopic examination of the EGCs scattered among the collagen fibres and fibroblasts (Fig. 2d,e) revealed that, ultrastructurally, they have a large number of granules which were frequently depleted suggesting degranulation (Fig. 2f). These cells, typically, had an undifferentiated cytoplasm, lacked organelles and possessed nuclei that appeared irregular in shape and were often pyknotic (Fig. 2f).

In the present study, the prevalence of immunoreactive elements responding to 13 different anti-sera were assessed on histological sections cut from both uninfected and *D. dendriticum*-infected stomachs of *C. lavaretus*. Although both the uninfected and infected stomachs were positive for four different neuromodulators, the most marked difference between the two sets of samples was the number of positive cells within the lamina propria-submucosa (Fig. 3a) and the muscle layers of the infected stomach. Indeed, in parasitized stomachs high numbers of cells positive to serotonin were seen around the plerocercoid (Fig. 3b,c; Table 3). The uninfected stomachs had a low number of immunoreactive elements positive for serotonin, bombesin, SP and galanin (Table 3). In infected stomachs, we found a high number of bombesin-positive nerve fibres principally on the surface of the cyst as well as bombesin-positive endocrine epithelial cells within the mucosa (Fig. 3d,e,f; Table 3). Similarly, numerous nerve fibres running throughout the serosa (Fig. 3h) of infected tissue and in the tissues in close proximity to the plerocercoid were positive for galanin (Fig. 3i; Table 3). Finally, the use of the SP anti-sera also indicated a large number of positive elements in the muscle layers of the stomach, within the cyst capsule and in the tissues around it (Fig. 3g, Table 3).

Histochemical staining of stomach sections taken from both infected and uninfected hosts with the remaining anti-sera anti-neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), met-enkephalin, calciotonin gene-related peptide (CGRP) and nitric oxide synthase (NOS), did not reveal any positive immunoreactive structures.

Discussion

While many studies have dealt with tissue responses to infections with plerocercoids of *Diphyllobothrium*

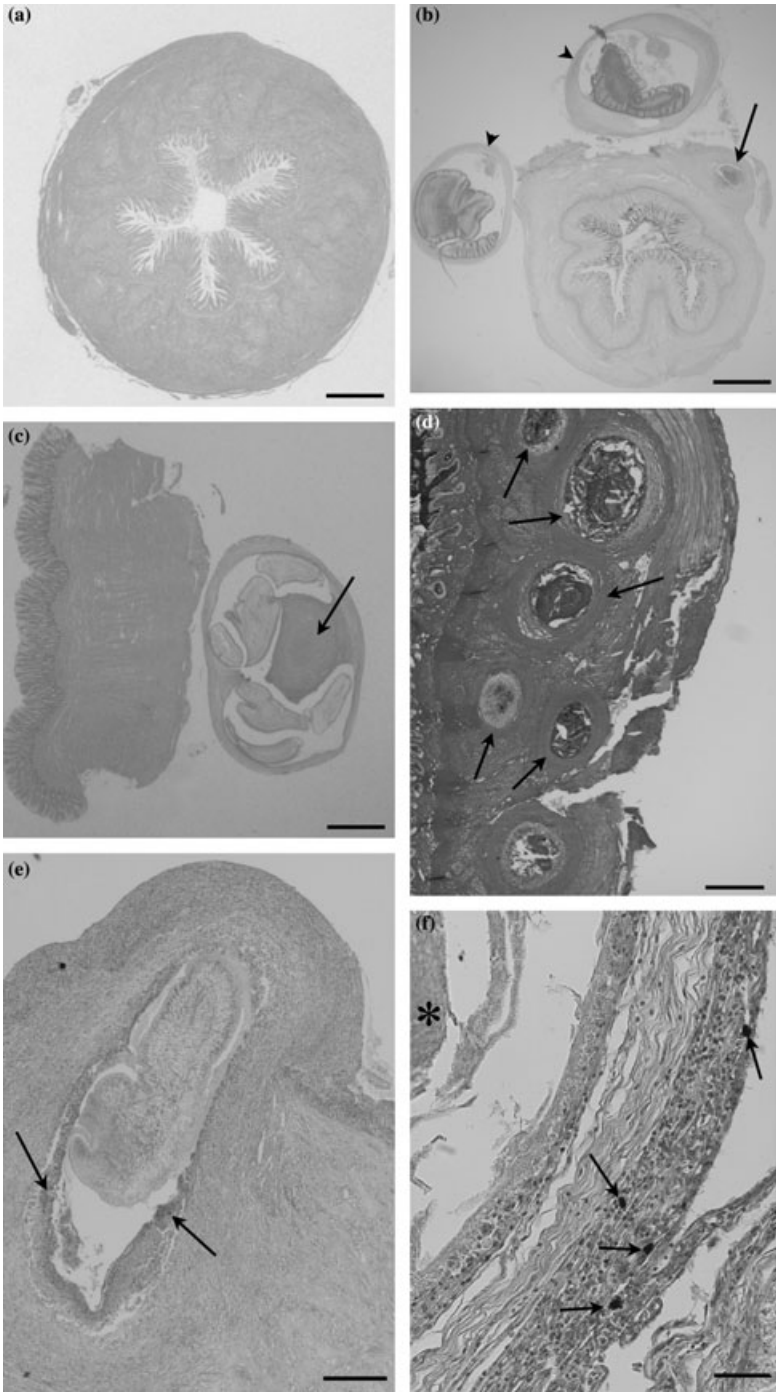

Figure 1 (a) A stereograph image of a section through the stomach of uninfected *Coregonus lavaretus*: note the integrity of the gastric wall (bar = 100 mm). (b) A stereograph image of two large encysted plerocercoids (arrow heads) of *Diphyllobothrium dendriticum* on peritoneal surface of the stomach, the larvae are loosely attached to the organ; one intramural larva (arrow) is visible (bar = 150 mm). (c) Section of infected open stomach, with a cyst apparently free in the peritoneum. Note the size of the cyst and the space occupied by the fully developed plerocercoid: arrow shows necrotized tissue (stereograph image, bar = 120 μm). (d) Plerocercoids (arrows) in different layers of the gastric wall. Host reaction around the cyst is appreciable (bar = 250 μm). (e) A developed plerocercoid within a 'tunnel' migrates toward the peritoneum: near the larval body, presence of necrotic tissue (arrows) is evident (bar = 250 μm). (f) Within the thickness of the cyst and near the parasite body (asterisk) macrophage aggregates (MAs) (arrows) are visible (bar = 50 μm).

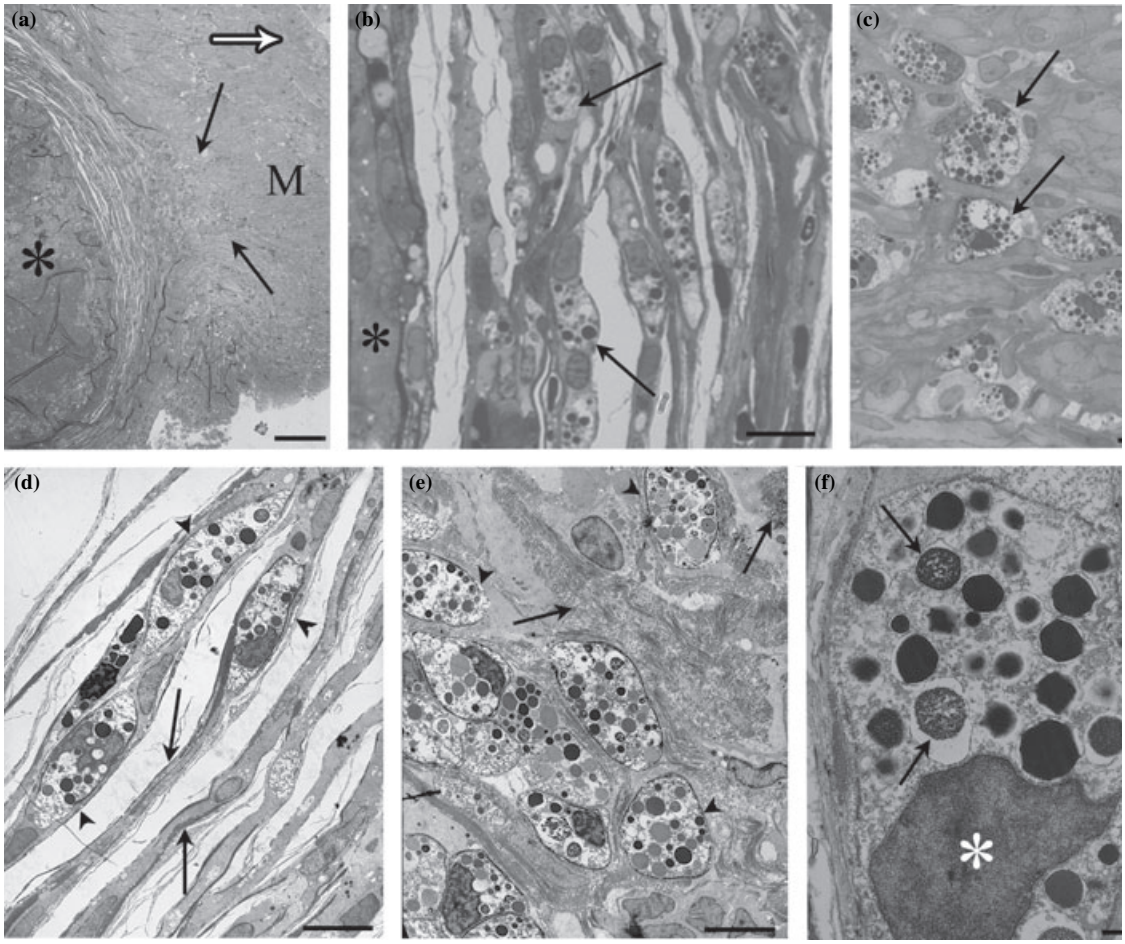

Figure 2 (a) Semithin sections from an infected stomach with a cyst, arrows show the migration of eosinophilic granule cells (EGCs) through the muscle layer (M) toward the plerocercoid (asterisk), white arrow points towards the lumen side of the stomach (bar = 100 μm). (b) Larval body (asterisk) and the cyst wall; arrows indicate two EGCs (bar = 10 μm). (c) EGCs (arrows) within fibrous tissue surrounding the cyst (bar = 10 μm). (d) EM of three EGCs (arrow heads) within connective tissue (arrows) (bar = 4.80 μm). (e) EM of several EGCs (arrow heads) within collagen fibres (arrows) (bar = 4.85 μm). (f) High magnification of a mast cell/EGC: note degranulation (arrows) and pyknotic nucleus (asterisk) (bar = 1.33 μm).

in different species of fish (Halvorsen 1970; Gonzalez, Torres, Figueroa, Contreras & Franjola 1978; Otto & Heckmann 1984; O'Neill, White, Sims & Barber 1988; Weiland & Meyers 1989; Torres *et al.* 2002), the work of Sharp *et al.* (1992) provided clear details of the sequential development of the immune response and cyst development in *O. mykiss* experimentally infected with *D. dendriticum*. From the study of Sharp *et al.* (1992) and the current study, it would appear that, ultrastructurally, the cyst encapsulating the plerocercoids possesses the same features as those produced in response to other endohelminths such as the acanthocolpid digenean *Stephanochasmus baccatus* known to parasitise at least four species of flatfish (Sommerville 1981) and *Rhipidocotyle johnstonei* in *Pleuronectes platessa* (L.) (Pulsford & Matthews 1984).

In the current study, a high number of EGCs were encountered within the walls of the parasite cyst and in the immediate tissues surrounding it. These cells increased in number towards the plerocercoid encysted on the outer surface of the stomach, possibly as a consequence of their migration through the stomach wall towards the site of parasite infection. EGCs are particularly numerous within the gut tissue of salmonids where they form the discrete stratum granulosum (Yasutake & Wales

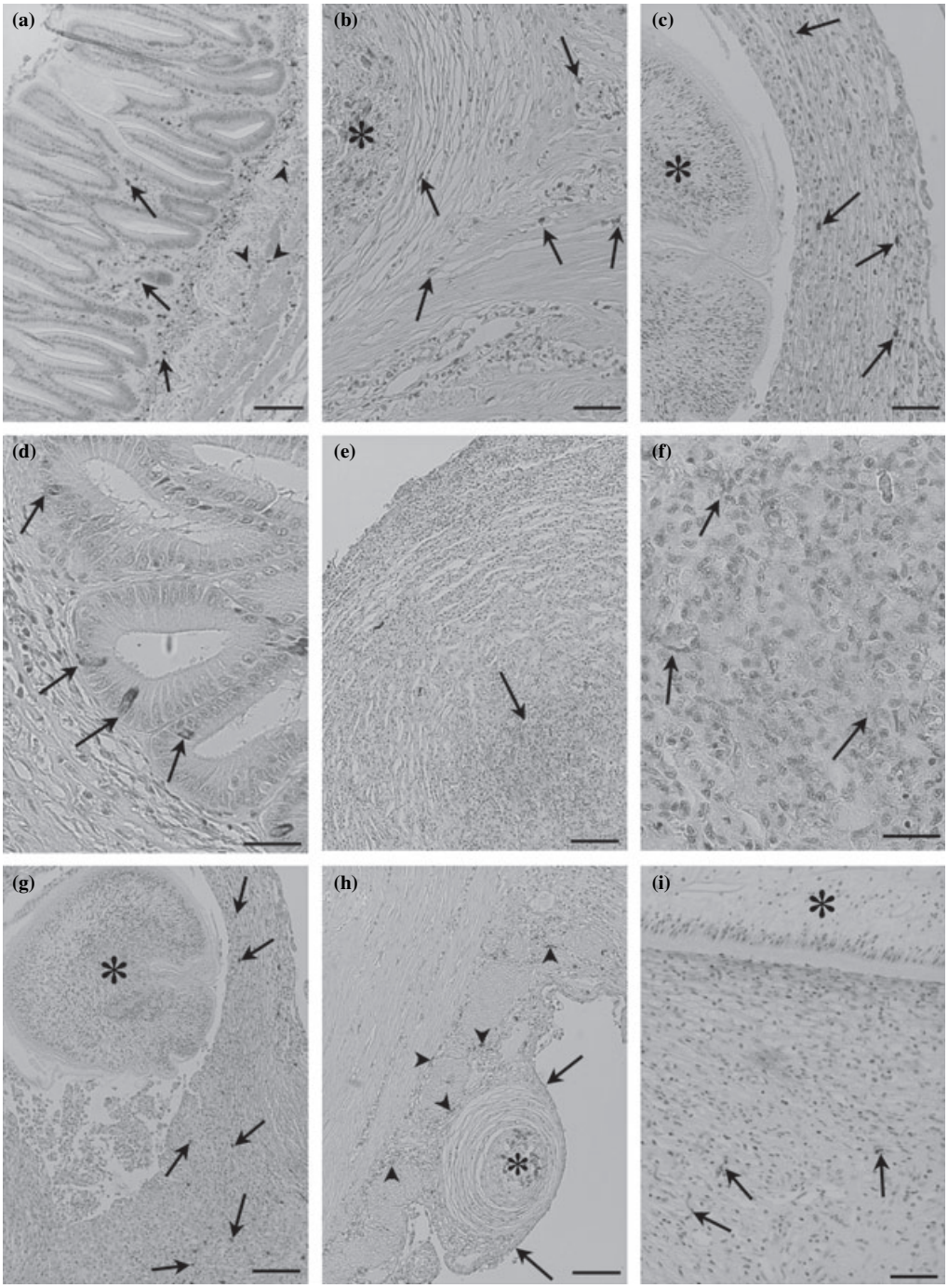

Figure 3 (a) Large number of structures (arrows) immunoreactive to serotonin in the tunica propria-submucosa and positive nerve fibres (arrowheads) within the muscle layer of infected stomach of *Coregonus lavaretus* (bar = 100 μm). (b) Numerous elements (arrows) positive to serotonin around a cyst (asterisk) (bar = 50 μm). (c) Cells (arrows) immunoreactive to serotonin encircle fully developed plerocercoid (asterisk) (bar = 50 μm). (d) Endocrine cells (arrows) containing bombesin in the gastric epithelium of infected powan (bar = 30 μm). (e) Nerve fibres (arrow) on surface of the cyst (bar = 100 μm). (f) Higher magnification of previous micrograph, note numerous bombesin-positive fibres (arrows) (bar = 30 μm). (g) Large number of elements (arrows) reactive to substance P in the muscular layer and in the connective inflammatory tissue around the parasite (asterisk) (bar = 100 μm). (h) Galanin-positive nerve fibres (arrowheads) near the connective tissue surrounding a plerocercoid (asterisk) beneath the serosa (arrows) (bar = 100 μm). (i) Nerve fibres (arrows) immunoreactive to galanin near the parasite body (asterisk) (bar = 50 μm).

Table 3 The response of uninfected and *Diphyllobothrium dendricticum*-infected stomach sections to a range of primary anti-sera

Anti-sera	Uninfected stomach	Infected stomach
Bombesin	++	+++
Galanin	+	+++
Serotonin	+	+++
Substance P	+	+++

The table shows a score of the immunoreactive elements found for each anti-sera in each tissue type.
Key: +: low presence, ++: medium presence, +++: high presence of structures immunoreactive to the specified anti-serum.

1983). They have also been found in high numbers in *Salmo trutta* (L.) infected with the cestode *Cyathocephalus truncatus* (Dezfuli *et al.* 2000) and in 3-spined sticklebacks, *Gasterosteus aculeatus* (L.), parasitized with the microsporean *Glugea anomala* (Dezfuli, Giari, Simoni, Shinn & Bosi 2004). EGCs are known to have an immune function similar to that of mammalian mast cells and their degranulation is in response to acute tissue damage due to pathogens/parasites (Reite 1998, 2005); chronic inflammatory reactions in gills or intestinal tissues induce a local increase in EGC numbers (Reite 1998).

Aggregations of melano-macrophages were also recorded in close proximity to the encysted parasite. Increases in MMCs have been associated with a range of physiological and pathological factors including ageing, starvation, the presence of infectious disease/pathogens and intoxication (Vogelbein *et al.* 1987; Wolke 1992; Couillard & Hodson 1996; Couillard *et al.* 1999; Agius & Roberts 2003). Their response to parasitic infection was comprehensively demonstrated by Vogelbein *et al.* (1987) following the experimental infection of *Rivulus marmoratus* Poey with the protozoan *Calyptospora funduli* (Duszynski, Solangi & Overstreet 1979; Overstreet, Hawkins & Fournie 1984). Thirty days after infection, multifocal granulomatous lesions were noticed within the liver, followed by a progressive increase in melanin and lipofucsin within the resulting MMCs 50–150 days post-infection.

The main detrimental effects of most endoparasitic helminths are localised at the site of infection (Hoste 2001) where, for example, worms induce structural changes to the digestive system, which might include local neuroendocrine structures with resulting alterations in the functions of the gastrointestinal tract (Castro 1992; Fairweather 1997; Fox 1997; Palmer & Greenwood-Van Meerveld 2001). Most of the studies on the above alterations focused on parasitic infections in mammals (Fox 1997; Eysker & Ploeger 2000; Mercer, Mitchell, Moar, Bissett, Geissler, Bruce & Chappell 2000), although there is a growing number of parallel studies in fish (see Dezfuli *et al.* 2000, 2002b, 2003, 2004, 2005; Bosi, Di Giancamillo, Arrighi & Domeneghini 2004a; Bosi *et al.* 2005a; Bosi, Domeneghini, Arrighi, Giari, Simoni & Dezfuli 2005b; Bermúdez, Vigliano, Quiroga, Nieto, Bosi & Domeneghini 2006). Our immunohistochemical analysis showed an increase in the immunoreactive elements responding to the serotonin, bombesin, SP and galanin anti-sera in stomach sections taken from *D. dendriticum*-infected powan. Of these, cells that were immunoreactive to the serotonin (5-HT) anti-serum were found in the lamina propria-submucosa and the muscle layers of the stomach. Information on this neuromodulator and its role in the immune system is scarce (Khan & Deschaux 1997), but its presence has been documented in other fish–parasite systems including *S. trutta* infected with another cestode, *Cyathocephalus truncatus* (Dezfuli *et al.* 2000), an acanthocephalan *Pomphorhynchus laevis* (Dezfuli *et al.* 2003), a microsporean *Glugea anomala* (Dezfuli *et al.* 2004) and also in the hearts of powan infected with the digenean *Ichthyocotylurus erraticus* (Dezfuli *et al.* 2005). A significant increase in serotonin activity in the intestines and muscles of rats infected with *Trichinella spiralis* and *T. pseudospiralis* has also been reported (Terenina, Asatrian & Movsessian 1997). It has been suggested that this biogenic amine affects vascular permeability and lymphocyte function (Lee, Swieter & Befus 1986), exerting a variety of effects that may favourably affect parasite survival (Fairweather 1997). If parallels between these host–parasite systems can be drawn, this suggests that an infection of *D. dendriticum* induces the recruitment of cells to secrete serotonin at the site of infection to ensure their survival.

Elements that were immunoreactive to the bombesin anti-serum were found primarily in the gastric epithelium of infected powan and in the nerve fibres of the tissues forming and surrounding the cyst. Bombesin-positive nerve fibres were also reported by Dezfuli *et al.* (2000, 2003, 2004) within the intestinal folds of *S. trutta* parasitized with *C. truncatus* and *P. laevis* and in *Gasterosteus aculeatus* infected with *Glugea anomala*. These findings suggest that bombesin at the site of tissue

inflammation in fish may act as a putative neurotransmitter in the neo-formed network of nervous fibres. In mammals, bombesin is known to regulate ion transport in the small and large intestine (Kachur, Miller, Field & Rivier 1982; Brown & O'Grady 1997), but more research is needed to determine the precise role of bombesin and its role in uninfected and parasite infected fish.

In mammals, the neuropeptide anti-SP is involved in several neurogenic inflammatory responses such as vasodilatation and plasma extravasation (Abrahamian, Fodor, Gorcs, Galoyan & Palkovits 1991; Onuhoa, Alpar, Chukwulobelu & Nicholls 1999). The work of Sharkey (1992) suggests that the action of SP is not only on the vasculature but also on the mast cells from which histamine and other soluble mediators are released, contributing further to the local inflammatory response. Anti-SP has also been demonstrated in elasmobranchs (Waugh, Wang, Hazon, Balment & Conlon 1993) and teleosts (Davies, Donald & Campbell 1994; Holmgren, Fritsche, Karila, Gibbins, Axelsson, Franklin, Grigg & Nilsson 1994; Waugh, Groff, Platzack, Youson, Olson & Conlon 1995). In the current study, it was observed in the nerve fibres of infected stomach muscle of *C. lavaretus* and in the network of subtle nerve fibres of the fibrous tissues encapsulating the plerocercoids. Although the function of SP within the cyst is currently unknown, the distribution of immunoreactive elements determined in this study suggests that it serves to stimulate blood flow through the vascular system of the developing cyst. Dezfuli *et al.* (2002b) also found this neuropeptide in the connective tissue around the gut of a brown trout, *S. trutta*, infected with *Pomphorhynchus laevis.*

Galanin has been implicated, in fish, to be involved in the olfactory and taste functions, in central visual processing, in somatosensory transmission, in osmoregulation, in sex-specific behaviour and in affecting the cardiovascular system (Cornbrooks & Parsons 1991; Holmqvist & Carlberg 1992; Le Mevel, Mabin, Hanley & Conlon 1998). Although most studies on galanin have focused on its role within the nervous system of vertebrates, it has also recently been reported in the neuroendocrine system of several fish species, both infected and uninfected (see Dezfuli *et al.* 2004; Bosi *et al.* 2004a; Bosi, Shinn, Giari, Arrighi & Domeneghini 2004b; Bosi *et al.* 2005b).

Although the *D. dendriticum*-infected and non-infected stomachs were negative for a number of other anti-sera (VIP, met-enkephalin, CGRP, NPY and NOS), their presence has been shown by Elbal, Lozano & Agulleiro (1988), and Bosi *et al.* (2004a,b) to be species-specific.

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