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NOTE

Atypical lesions and infection sites of larval trematodes in marine gastropods from Argentina

Carmen Gilardoni[1], Gisele Di Giorgio[1], Cristián Ituarte[2], Florencia Cremonte[1]

[1]Laboratorio de Parasitología (LAPA), Instituto de Biología de Organismos Marinos (CCT CONICET-CENPAT), Bv. Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina

[2]Museo Argentino de Ciencias Naturales (CONICET), Av. Ángel Gallardo 470, C1405DJR Ciudad de Buenos Aires, Argentina

ABSTRACT: This study documents the lesions caused by 9 digenean larvae parasitizing 6 marine gastropod species from Patagonia, Argentina. Most digeneans produce a lesion where the parthenitae replace the gonadal tissues and also occupy part of the digestive gland. Three atypical lesions and tropism were observed in (1) *Trophon geversianus* (Muricidae) infected by Renicolidae gen. et sp. 1, where the sporocysts occur inside the gonad acini which retain their general structure due to the persistence of the acinus wall, (2) *Nacella magellanica* (Nacellidae) infected by Renicolidae gen. et sp. 2, where the gonad is not affected and the sporocysts are located only among the digestive gland tubules, and (3) *Siphonaria lessonii* (Siphonariidae) infected by Schistosomatidae gen. et sp., where fully developed cercariae occur inside the digestive tubules. At high infection intensities or in double infections, larvae can invade other organs (mantle, foot or gill) and, on occasion, may elicit hemocytic encapsulation.

KEY WORDS: Digenean trematodes · Lesions · Mollusk

INTRODUCTION

Larval stages of digenean trematodes are the most common parasites of marine gastropods, which act as first intermediate hosts in their life cycles (Lauckner 1980). Generally, young parthenithae (sporocysts or rediae) migrate across the body of the host through large blood spaces or are passively carried by hemolymph currents until they reach the digestive gland and gonad, where they find optimal feeding conditions (Galaktionov et al. 2015).

The phenomenon of parasitic castration in mollusks due to the presence of larval digeneans has been widely reported (e.g. Ginetsinskaya 1988, Galaktionov & Dobrovolskij 2003, Cremonte 2011). It is interpreted as a strategy of parasites to occupy an organ that is not vital for host survival, leading to redirection of energy towards the parasite's growth instead of molluscan reproduction (Galaktionov & Dobrovolskij 2003). In a typical pathology caused by high intensity infections, the characteristic structure of the gonadal acini is destroyed (e.g. Ginetsinskaya 1988, Fried & Graczyk 1997); in some cases, remnants of the gonad may be present although in a reduced condition. According to Sullivan et al. (1985), the parasite may cause regression of fully mature acini, or may simply prevent their maturation depending upon the stage of the host's reproductive cycle.

Numerous studies have also reported complete destruction of the digestive gland along with that of the gonad (e.g. Huffman et al. 2009, Choubisa et al. 2012) and subsequent invasion of gills, mantle and foot in higher intensity infections (Galaktionov & Dobrovolskij 2003, Cremonte et al. 2005). Furthermore, most authors have noted little or no host cellu-

*Corresponding author: gilardoni@cenpat-conicet.gob.ar

lar response to digenean larvae in mollusks, particularly in those used as a first intermediate host, which is interpreted as a evidence of a co-evolutionary process where larval digeneans inhibit the ability of the mollusk to recognize them as foreign (e.g. Cheng & Burton 1965, Cremonte 2011).

The goal of this study was to describe the lesions caused by larval digeneans and infection sites in gastropods from the Patagonian coast, Argentina, with emphasis on those which showed unusual features.

MATERIALS AND METHODS

Gastropods (*Crepipatella dilatata*, *Nacella magellanica*, *Pareuthria fuscata*, *Siphonaria lateralis*, *S. lessonii*, and *Trophon geversianus*) were collected from the rocky littoral near the mouth of the Deseado River estuary (47° 45′ S, 65° 55′ W), Puerto Deseado, Santa Cruz Province, Argentina, along the coast of the southwestern Atlantic Ocean. Information on the number of collected specimens and parasite prevalence was reported in Gilardoni et al. (2018). Parasitized snails (2 *N. magellanica*, 2 *P. fuscata*, 1 *S. lateralis*, 6 *S. lessonii*, and *3 T. geversianus*) and healthy specimens (about 3 of each species) were processed for histology. Shells were carefully removed and soft tissues fixed in Bouin's fluid for 12 h, repeatedly washed in tap water, dehydrated through an ascending ethanol series, and embedded in HistoResin® (Leica™). Sections 3.5 µm thick were stained with hematoxylin and eosin (H&E) and examined under a light microscope. Parasite identifications were based on cercarial morphology as documented by Gilardoni et al. (2018). The infection intensity of each parasitized gastropod was classified as follows: light (few parthenitae containing mainly germinal balls and undeveloped cercariae), moderate (intermediate number of parthenitae containing undeveloped and/or developed cercariae), and high (numerous parthenitae containing mainly developed cercariae).

RESULTS

Typical lesions

In total, 6 out of 9 digenean species studied caused the typical lesions, i.e. replacement of gonadal tissue by parthenitae and/or partial damage of the digestive gland by mechanical compression and stretching of digestive tubules (Fig. 1A–C). Usually, parthenitae were found invading the connective tissue that develops around the gonad acini and among the digestive gland tubules, which may be compressed or deformed in light or moderate infections. This was observed in the case of *Pareuthria fuscata* with a moderate infection by Lepocreadiidae gen. et sp. 2 (Fig. 1B). In gastropods with high infection intensity, the entire intervisceral connective tissue was invaded by parthenitae and the rupture of digestive tubules was frequently observed, as in the case of *P. fuscata* infected by Lepocreadiidae gen. et sp. 2 (Fig. 1C). In *Trophon geversianus* specimens with moderate infection by *Parorchis* sp., the digestive gland tubules were not deformed. Only in cases of high infection intensity were the tubules deformed by compression and, on occasion, the rediae were also found around sections of the seminal vesicle (Fig. 1D) and in the connective tissue of the kidney tubules. In the case of *Siphonaria lessonii* infected by Hemiuroidea fam. gen. et sp., sporocysts were observed in the loose connective tissue between the epithelium that delimits the pseudobranch lamellae, eliciting hemocytic encapsulation (Fig. 1E).

In one case of double infection (the hemiurid and a light infection by the haplosporidian *Halosporidium patagon*), a massive hemocytic reaction was observed in the intervisceral space, such that hemocytosis led to the encapsulation of some sporocysts (Fig. 1F). In a double infection by Lepocreadiidae gen. et sp. 1 and Microphallidae gen. et sp. 1, the rediae were present in lower proportions than the microphallid sporocysts, and they were confined to the connective tissue in the periphery of the digestive gland. In the case of *Crepipatella dilatata* infected by Lepocreadiidae gen. et sp. 1, debris similar to vitellum from the gonad host was observed within the intestinal caeca of the smaller rediae (those not yet containing cercariae) (Fig. 1G).

In the case of *S. lessonii* infected by the microphallid *Maritrema madrynense*, it was not possible to observe the secretory cells that are normally found in the epithelium of the digestive tubules of non-parasitized specimens, or a high vacuolization of the absorptive cells. Sporocysts invaded the connective tissue that develops among the folds of the pallial glandular complex associated with the female reproductive tract (Fig. 1H). In one specimen of *Siphonaria lateralis* with a high infection intensity by *M. madrynense*, the digestive tubules appeared collapsed by compression, without a visible lumen, but the secretory cells persisted; the connective tissue among folds of the pallial glandular complex exhibited massive invasion by sporocysts, resulting in their extreme distension (Fig. 1I).

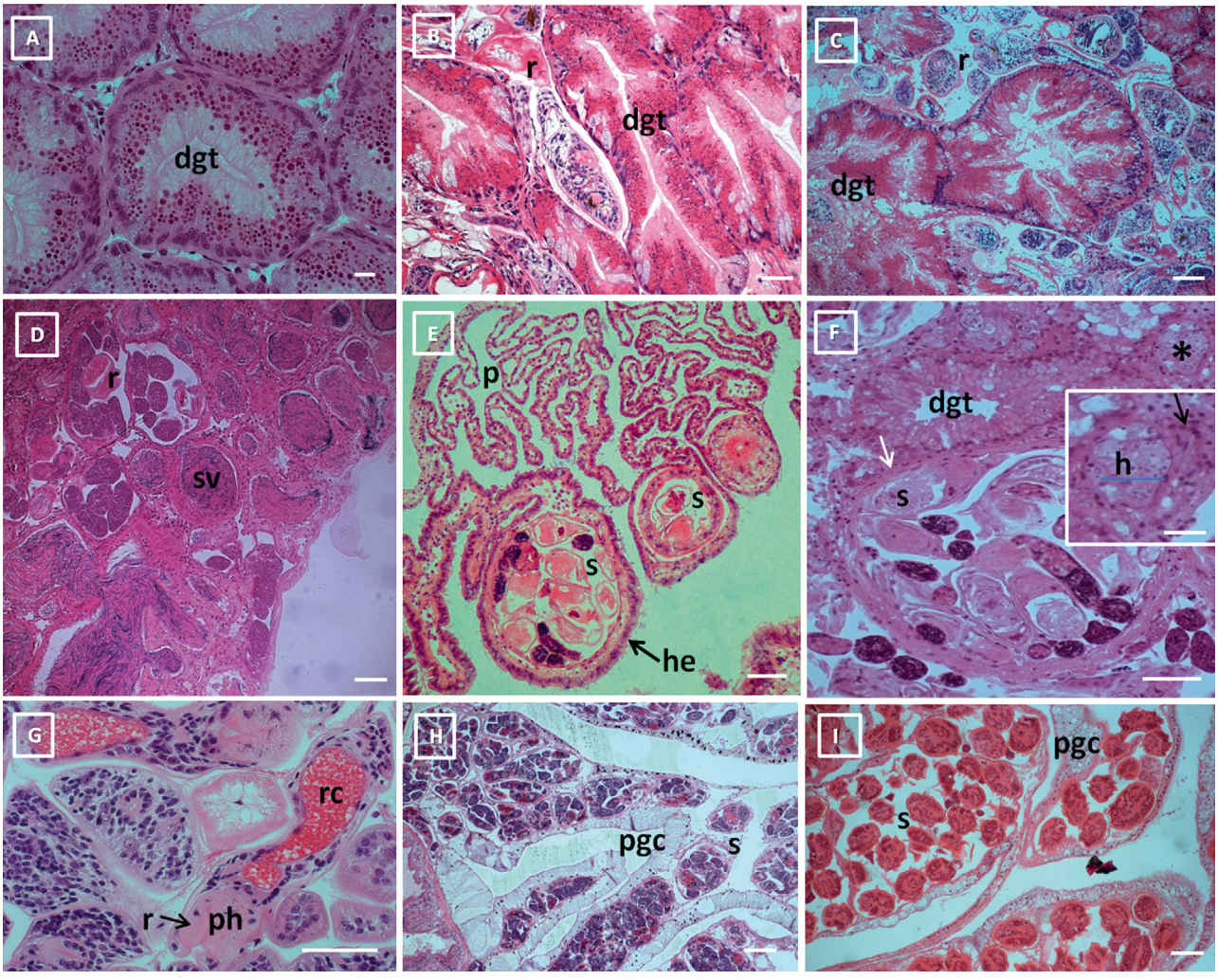

Fig. 1. Histological sections (H&E) of gastropods from Puerto Deseado, Argentina, southwestern Atlantic Ocean. (A) Normal digestive gland tubule of *Pareuthria fuscata*; (B) compressed and distended digestive gland tubule of *P. fuscata* caused by moderate intensity infection by Lepocreadiidae gen. et sp. 2; (C) rupture of digestive gland tubule of *P. fuscata* caused by high infection intensity by Lepocreadiidae gen. et sp. 2; (D) rediae of *Parorchis* sp. surrounding sections of the seminal vesicles of *Trophon geversianus*; (E) hemocytic encapsulation of sporocysts of Hemiuroidea fam. gen. et sp. in the space between the epithelium of the pseudobranch lamellae of *Siphonaria lessonii*; (F) hemocytic encapsulation (white arrow) of sporocysts of Hemiuroidea fam. gen. et sp. and hemocytic infiltration (black arrow) in the digestive gland of *S. lessonii* infected with the haplosporid *Haplosporidium patagon* (indicated on the inset which is an extension of the region indicated with an asterisk); (G) small rediae of Lepocreadiidae gen. et sp. 1 with vitellum granules within the intestinal caeca in *Crepipatella dilatata*; (H) sporocysts (full of cercariae) of *Maritrema madrynense* in the connective tissue among folds of the pallial glandular complex of *S. lessonii*; (I) sporocysts (full of metacercariae) of *M. madrynense* eliciting extreme stretching of the folds of the pallial glandular complex of *S. lessonii*. dgt: digestive gland tubule; h: haplosporid; he: hemocytic encapsulation; ph: pharynx; p: pseudobranch; pgc: pallial glandular complex; r: redia; rc: redia caecum; s: sporocyst; sv: seminal vesicle. Scale bars: 50 µm (A,B,E,F), 100 µm (C,D,G,H,I); 20 µm (inset F)

In the case of *C. dilatata* infected by Microphallidae gen. et sp., the typical lesion was observed, i.e. the gonad tissue was replaced by parthenitae. However, in a case with moderate infection intensity, the sporocysts occupied only the periphery of the digestive gland and therefore the structure of the digestive tubules was not altered. In a case of higher infection intensity, as was observed in a double infection with Lepocreadiidae gen. et sp. 1, the entire digestive gland was invaded by sporocysts. In this case, sporocysts were also observed invading the spaces among the muscle fibers of the foot (Fig. 2A), and occupied the connective tissue lying between the outer and inner mantle epithelium; hemocytic encapsulation was observed.

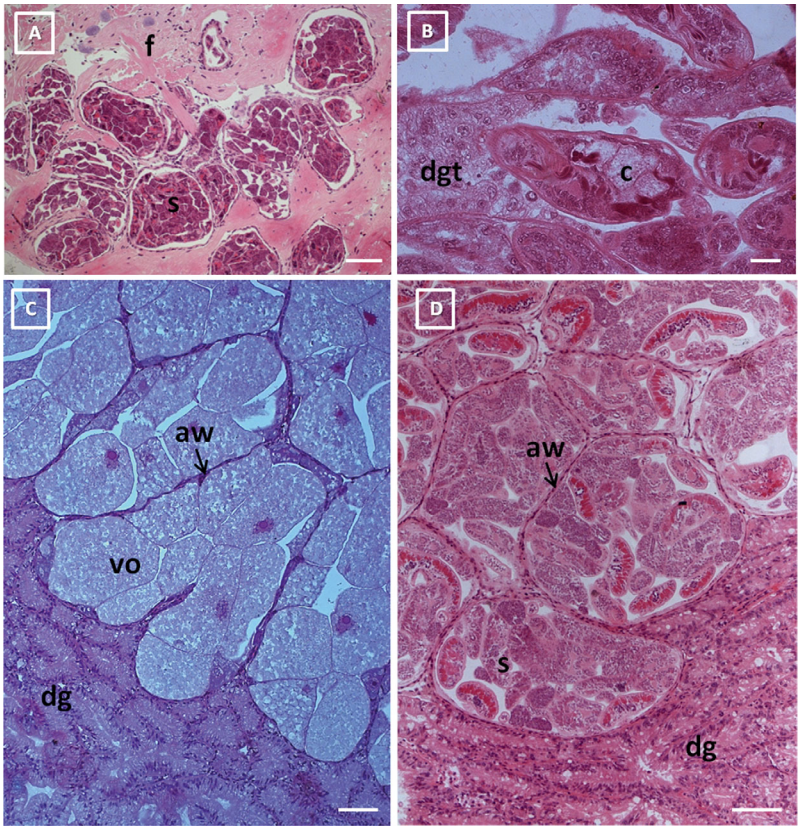

Fig. 2. Histological sections (H&E) of gastropods from Puerto Deseado, Argentina, southwestern Atlantic Ocean. (A) Sporocysts of Microphallidae gen. et sp. invading the spaces between muscle fibers of the foot of *Crepipatella dilatata*; (B) cercaria of Schistosomatidae gen. et sp. within a digestive gland tubule in *Siphonaria lessonii*; (C) normal histology of a female gonad and digestive gland of *Trophon geversianus*; (D) sporocysts of Renicolidae gen. et sp. 1 replacing gonadal germinal cells and surrounded by the acini walls in *T. geversianus*. aw: acinus wall; c: cercaria; dg: digestive gland; dgt: digestive gland tubule; f: foot; s: sporocyst; vo: vitellogenic oocytes. Scale bars: 100 µm (A,B,C), 20 µm (D)

Atypical lesions

Atypical lesions were observed in 3 out of 9 digenean species studied. In the case of *Nacella magellanica* infected by Renicolidae gen. et sp. 2, the gonad was not affected and only the tubules of the digestive gland were deformed, with an enlarged lumen. In the case of *S. lessonii* infected by Schistosomatidae gen. et sp., fully developed cercariae were observed inside the lumen of the digestive gland tubules (Fig. 2B). Furthermore, sporocysts were observed among the lobes of the pallial glandular complex and in the connective tissue of the digestive gland and kidney, whereas the connective tissue surrounding the lobes of the seminal vesicle was not invaded.

In the case of *T. geversianus* infected by Renicolidae gen. et sp. 1, only the gonad was affected and the germinal cells were replaced by parthenitae. Notably, the connective tissue that supports the wall of the acini and the acinar wall itself remained undamaged and surrounded the sporocysts (Fig. 2C,D). The digestive gland was not invaded, and only those digestive tubules found close to the space occupied by the parasites showed a variable degree of compression.

DISCUSSION

In most of the cases described in the present study, the typical lesion was observed in which the gonad was completely replaced by parthenitae. Full or partial castration of the snail host by digenean infection is a well-known phenomenon (e.g. Cheng et al. 1973, Sullivan et al. 1985). Generally, in cases in which some gonadal tissues remain, parthenitae occupy the connective tissue surrounding the acini. Yet parthenitae were also frequently observed invading the connective tissue surrounding the seminal vesicles (cases of *Pareuthria fuscata* and *Trophon geversianus*) or the pallial glandular complex (cases of *Siphonaria lateralis* and *S. lessonii*) without affecting the organs themselves. In the unusual case of *T. geversianus* infected by Renicolidae gen. et sp. 1, the wall of the gonadal acini remained unaffected by parasites, although its lumen was invaded by sporocysts. Thus, the gonad maintained its normal structure even though the acini were full of parthenitae instead of germinal cells. This peculiar reaction is recorded here for the first time in a gastropod and should be supported with more study cases, because only one parasitized snail with the renicolid species was histologically studied. The same retention of gonad structure was recently recorded in the venerid clam *Megapitaria squalida* from the Gulf of Baja California, on the Pacific Ocean, where the sporocysts were observed within gonadal follicles and associated with heavy hemocytic infiltration and the formation of granulocytomas surround the parasite (Yee-Duarte et al. 2017). Another unusual case was that of *Nacella magellanica* infected by Renicolidae gen. et sp. 2, where the gonad was not occupied

by sporocysts. Because the sporocysts mainly contained germinal balls and undeveloped cercariae, it is likely that the infection was recent and the gonad was not yet invaded. According to Rees (1934) and Wright (1966), the first site of infection is the interlobular connective tissue of the digestive gland; the gonad is invaded secondarily by way of the blood sinuses from the digestive gland.

In most of the cases examined in this study, the degree of damage of the digestive gland was mainly correlated with infection intensity. In light infections, the digestive tubules were normal or only slightly deformed by compression; in moderate infections, the tubules showed pronounced mechanical compression or distension; and in higher intensity infections, the normal structure of the tubules was disrupted. Additionally, a correlation between the magnitude of the damage and the size and type of trematode parthenita (redia or sporocyst), and with the presence of a single or a double infection, has been reported in prior studies (e.g. Rees 1936, Ginetsinskaya 1988, Choubisa et al. 2012). In contrast, the severity of reactions observed in this study only appears to be related to parthenitae size and the presence of double infections, but not with the type of parthenite. The hemiuroid and schistosomatid sporocysts from *S. lessonii* were the largest, and they caused the most severe pathogenic effects, namely the rupture of the digestive gland tubules and hemocytic encapsulations. Moreover, the 2 cases of double infections resulted in a higher pathogenicity than single infections. Thus, the host–parasite pair hemiurid plus haplosporidian infecting *S. lessonii* elicited a generalized hemocytic response and the encapsulation of sporocysts, and the pair microphallid plus lepocreadiid infecting *Crepipatella dilatata* caused damage to the mantle and foot, in addition to the typical lesion of a high intensity infection in the gonad and the digestive gland. Although hemocytic reactions appear to be rare in mollusks that act as first intermediate hosts for digenetic trematodes (Cremonte 2011), some hemocytic infiltrations and encapsulations have been recorded (e.g. Sullivan et al. 1985, Ginetsinskaya 1988, da Silva et al. 2002).

A particular case of damage in the digestive gland was observed in *S. lessonii* parasitized by Schistosomatidae gen. et sp., where the cercariae invaded the lumen of the digestive tubules. This unusual site of infection is recorded here for first time and could be explained by disruption of the tissues when the fully developed cercariae are migrating to leave the snail host. During the emergence of schistosomatid cercariae, mechanical damage, such as encapsulation of the host tissues, has been recorded (Soomro et al. 2005). In the present study, sporocysts of Schistosomatidae were observed in the connective tissue of the digestive gland, which is in agreement with several prior studies (e.g. Soomro et al. 2005, Choubisa et al. 2012). However, the finding of digestive tubules disrupted with cercariae within their lumen (outside of the sporocyst) has not been previously recorded.

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