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ABSTRACT: We identified and compared gross and microscopic lesions associated with the cestode, *Parorchites zederi*, in the digestive tracts of three species of penguins (Spheniscidae): the Chinstrap (*Pygoscelis antarctica*), Gentoo (*Pygoscelis papua*), and Adélie penguins (*Pygoscelis adeliae*). The gastrointestinal tracts of 79 recently dead individuals (71 chicks and eight adults) were collected in locations throughout the Antarctic Peninsula during summer field trips in 2006–09. *Parorchites zederi* was found in the small intestine of 37 animals (47%), and 23 (62%) of these had parasite-associated lesions. The cestodes were either free in the intestinal lumen, clustered within mucosal ulcers, or deeply embedded in the intestinal wall. Histopathologic changes were most severe in adult Gentoo Penguins and included transmural fibrogranulomatous enteritis, hemorrhage, and edema. This report of pathology associated with *P. zederi* in the digestive tracts of penguins can serve as reference to monitor health in Antarctic birds associated with environmental changes.

Key words: Cestoda, histopathology, parasite effects, *Parorchites zederi*, *Pygoscelis*.

INTRODUCTION

Parorchites zederi (Cestoda: Dilepididae) is widely distributed among Antarctic penguins (Barbosa and Palacios 2009; Díaz et al. 2013) and is potentially the most pathogenic parasite found in penguins (Hoberg 1984a). It has been isolated in Emperor Penguin, *Aptenodytes forsteri*; Chinstrap Penguin, *Pygoscelis antarctica*; Gentoo Penguin, *Pygoscelis papua*; and Adélie penguin, *Pygoscelis adeliae* (Cielecka et al. 1992; Díaz et al. 2013). The life cycle of *P. zederi* is poorly understood. Because crustaceans are involved in the life cycles of Dilepididae (Hoberg 1994), and krill (*Euphausia superba*) is the main prey of pygoscelid penguins, krill could be the main intermediate host. Fish might also be involved, but more studies are needed.

A literature review of *P. zederi* revealed few descriptions of either their mechanisms for attachment to the intestinal wall or associated pathologic changes in infected hosts. The

attachment modes of different species of cestodes have been described in elasmobranchs and fish (Borucinska and Caira 2006; Williams et al. 2011), in pelagic seabirds (Hoberg 1984a), and White-necked Cormorants (Karstad et al. 1982). The most severe damage from cestodes is usually caused by deeply embedded scolices (Karstad et al. 1982). Several authors have described nodules containing deep diverticula in which the cestode scolex and a characteristic structure, a long neck called the pseudoscolex (Johnston 1937), are located (Harry and Holloway 1989). The number of diverticula associated with the attachment of *P. zederi* is comparatively low (Tzvetkov et al. 1999). We investigated the natural lesions associated with *P. zederi* from three penguin species distributed along the Antarctic Peninsula, the Chinstrap, Adélie, and Gentoo penguins.

MATERIALS AND METHODS

Gastrointestinal tracts from 79 recently dead individuals (within a few hours): 64 Chinstrap Penguins (60 chicks and four adults), 11 Gentoo Penguins (nine chicks and two adults), and four Adélie Penguins (two chicks and two adults), were collected at locations along the Antarctic Peninsula ranging from King George Island (62°15′S, 58°37′W; Gentoo and Adélie), Livingston Island (62°39′S, 60°36′W; Gentoo), Deception Island (63°00′S, 60°40′W; Chinstrap), Ronge Island (64°40′S, 62°40′W; Gentoo), and Avian Island (67°46′S, 68°64′W; Adélie), during the austral summer seasons from December 2006 to February 2009.

All birds had died from causes other than predation but we were unable to establish the specific causes of death (e.g., starvation, diseases). Gastrointestinal tracts were removed and frozen at –20 C until examination. We analyzed sections of the digestive tract separately: esophagus, stomach (glandular proventriculus and muscular gizzard), small intestine, cecum, colon, and cloaca. Intestinal sections were opened longitudinally and examined under a stereoscopic microscope to identify gross lesions and helminths.

Parasites were isolated and stored in 70% ethanol and examined microscopically for identification. Cestodes were stained with Semichon's acetocarmine, mounted in Canada balsam, and identified with the use of dichotomous keys (Yamaguti 1959; Schmidt 1986; Bona 1994; Hoberg 1994) and a specific reference (Cielecka et al. 1992).

We selected 23 infected penguins with lesions. Seven Gentoo Penguins (four chicks and three adults), three Adélie Penguins (one chick and two adults), and 13 Chinstrap Penguins (11 chicks and two adults) were selected for histopathologic study. Infected guts with and without macroscopic lesions were examined.

Small tissue samples were taken and fixed in 10% phosphate-buffered formalin (pH 7.2). The position of helminths found still attached to the gut was recorded before the tissue was processed. Subsequently, a 15×15–mm section of the tissue surrounding the parasite was removed and fixed in 70% ethanol. In accordance with standard paraffin-embedding procedures, 4–5-μ sections were stained with H&E and Masson trichrome, and observed under an Axioskop 40 Zeiss® optical microscope (N.S. Lab, Sevilla, Spain). Microphotographs were taken with the use of the SPOT® program (N.S. Lab).

RESULTS

Distribution of *P. zederi* in Pygoscelid penguins

Thirty-seven (47%) of the 79 penguins examined were parasitized with *P. zederi*, and 23 (62%) of the 37 infected penguins had recognizable lesions associated with the attachment of *P. zederi*. Parasites and lesions were found only in the small intestine. The *P. zederi* observed in the chicks, juveniles, and adults of three species of penguins were more frequent in the duodenum and cranial half of the jejunum. The parasites were free in the intestines, and in occasional small, whitish lesions on the surface of the mucosa or embedded in the intestinal mucosa forming small raised nodules or cysts.

Gross pathology of *P. zederi*

Examination of infected adult penguins showed evidence of gross pathologic changes. In Chinstrap and Adélie penguins, lesions comprising as few as 1–3 tapeworms (Fig. 1a) caused small swellings (Fig. 1b) that were visible from the outside of the intestine prior to dissection. The tapeworm pseudoscolex and the strobilae extended into the lumen of the intestine. We observed raised, rounded nodules surrounding the tapeworms with irregular swelling of the intestine toward the serosa

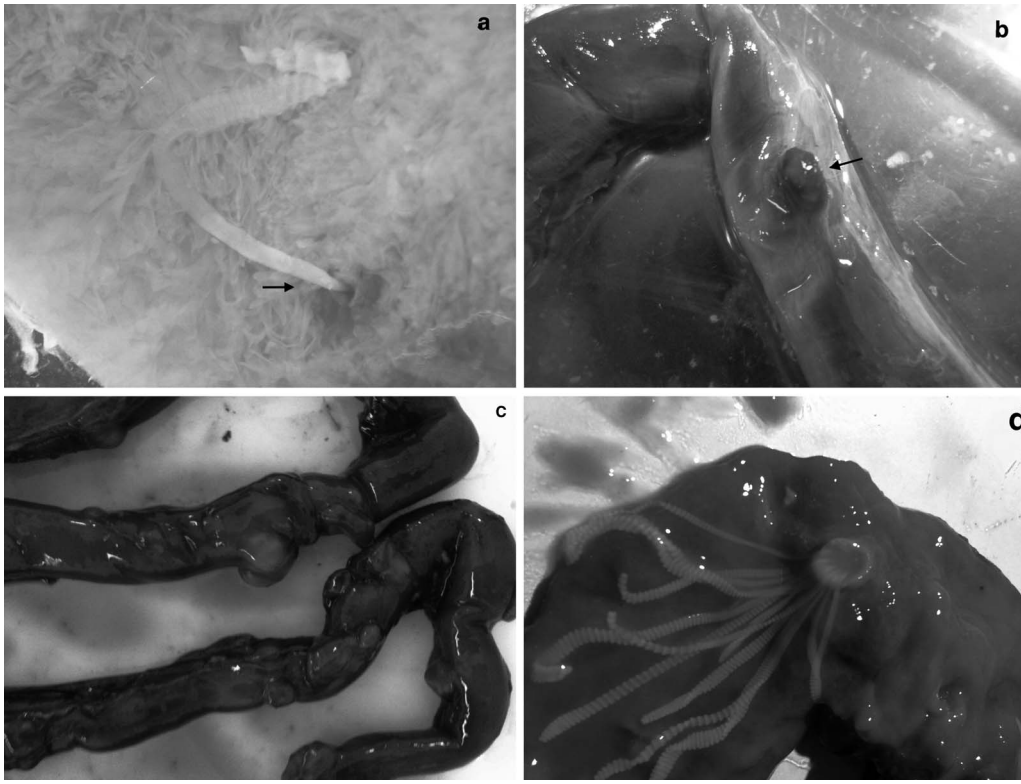

FIGURE 1. Macroscopic lesions in intestinal wall in pygoscelid penguins infected with *Parorchites zederi*, Antarctic Peninsula, 2006–09. (a) One tapeworm (arrow) attached to the intestinal wall resulted in a small, punctuate, whitish lesion on the surface of the mucosa. (b) Pronounced intestinal swelling (arrow) in an adult Chinstrap Penguin (*Pygoscelis antarctica*). (c) Intestine of adult Gentoo Penguin (*Pygoscelis papua*) with irregular raised nodules visible on the serosa. (d) Heavy infection comprising many tapeworms in a single cluster.

(Fig. 1b, c). Nodules had a smooth, well-formed entrance in the mucosa, with a thick white ring-shaped orifice (Fig. 1d).

In adult Gentoo Penguins, parasites usually occurred in characteristic tight clusters of 6–13 tapeworms attached to an ulcer within a nodular swelling of the intestinal wall (Fig. 1d). The heaviest infection showed several yellowish-white, oval-spherical nodules ranging from 1–2 mm to 1.5 cm (Fig. 1c).

Most immature cestodes detected were wholly contained within the cyst. This caused an absence of any noticeable swelling, which, combined with the smaller size and translucency of parasites, impeded detection. Sometimes the locations of attached parasites were indicated by swollen pinpoints in the intestine.

Histopathologic changes

Small cestodes were frequently found inside lesions that had macroscopically been classified as without parasites. The main changes observed were a granulomatous inflammation consisting of lymphocytes and macrophages. In several sections, extensive thinning, disruption, and sometimes total effacement of the muscularis mucosae by granulation tissue and fibrosis were found. Vermiform tracts of necrosis surrounded by granulomatous inflammation extended through the muscularis in some histologic sections. These necrotic tracts showed central hemorrhaging and fibrin mixed with cellular debris. In other sections, the center of the tract showed a perfectly viable cestode (Fig. 2a). The mucosal layer showed signs of

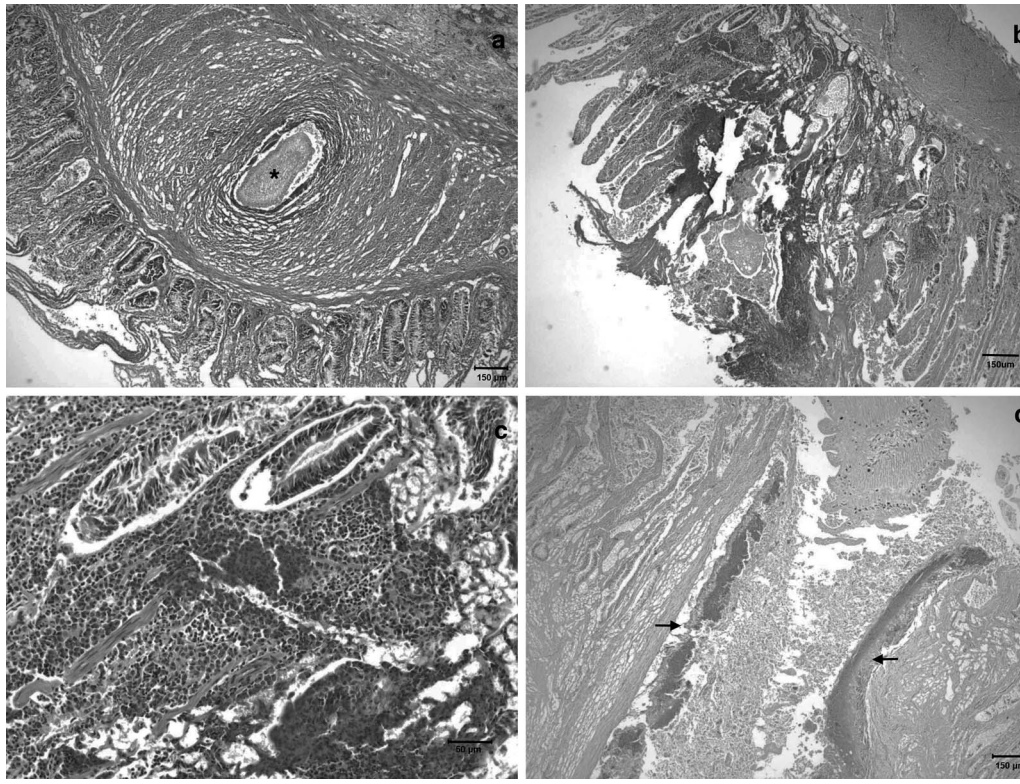

FIGURE 2. Histologic lesions in intestinal wall in pygoscelid penguins infected with *Parorchites zederi*, Antarctic Peninsula, 2006–09. (a) Tract centered on a viable cestode (asterisk). H&E ×5. (b) Intestinal villi with extensive necrosis deep in the lamina propria. H&E ×5. (c) Extensive areas of mucosa totally replaced by infiltrating mononuclear cells. H&E ×20. (d) Focal hemorrhage and necrosis of cells within the lamina propria around the scolex (arrows). H&E ×5.

hyperplasia and hyperemia. We also found hyperplasia and hypertrophy in the crypts. Finally, there was necrosis of the intestinal villi with infiltration by macrophages and lymphocytes (Fig. 2b, c).

Lesions with parasites in situ had scolices attached within deep ulcers over necrotic tracts that extended into the muscular layer. Oblique sectioning revealed a branching of the main necrotic tract into several well-formed irregular compartments/diverticula (Fig. 2d). These compartments were lined with a greyish-white capsule and separated by well-defined septa. Cestodes inside the diverticula appeared in different directions. Lesions associated with immature *P. zederi* differed from those associated with established adult tapeworms. Accordingly, hemorrhage and cell necrosis within the mucosal epithelium and lamina propria were observed around the scolex of immature tapeworms (Fig. 3a). Rod-shaped coccobacilli colonized the surface in some cavities.

The necrotic tracts described earlier were surrounded by concentric layers of multinucleated giant cells and fibroblasts, and sparsely infiltrated with macrophages, eosinophils, and lymphocytes. Although the parasite-induced lesions were transmural and resulted in severe damage to the intestinal wall, no perforation of the gut was observed and the adjacent serosa appeared normal (Fig. 3b). This chronic host reaction was associated with a complete loss of normal gut architecture and replacement of the mucosa, lamina propria, and muscularis with inflammatory tissue.

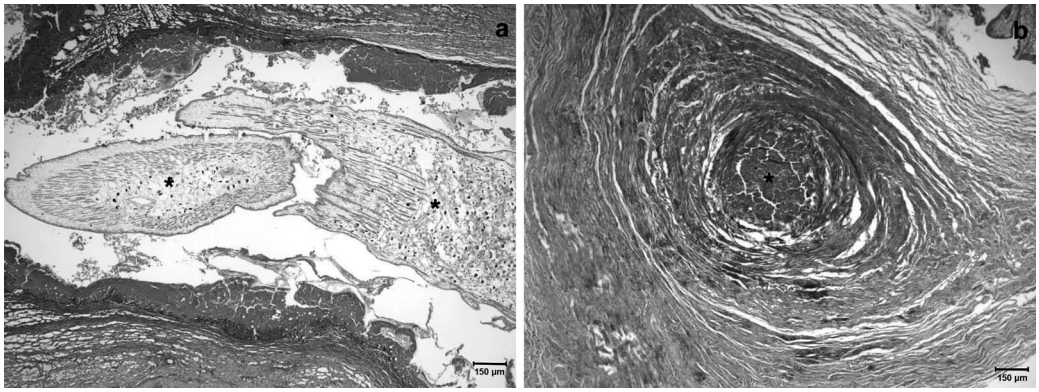

FIGURE 3. Histologic lesions in intestinal wall in pygoscelid penguins infected with *Parorchites zederi*, Antarctic Peninsula, 2006–09. (a) Longitudinal section of one diverticular duct and the inner parts of the parasite nodules covered by hemorrhages, mononuclear and eosinophilic infiltrates. H&E ×5. (b) Chronic lesion with granulomatous inflammation and abundant connective peripheral tissue. Masson's trichrome ×5.

DISCUSSION

Parorchites zederi is a common parasite among Antarctic penguins. Prevalence was 100% in Gentoo Penguin chicks and adults (Díaz et al. 2013), 28% in Chinstrap Penguin chicks, and 75% in adults (Vidal et al. 2012) and 50% in Adélie chicks and 50% in adults (Vidal 2014). *Parorchites zederi,* along with other helminths, is responsible for about a 6% loss of body mass in Antarctic penguins (Palacios et al. 2012). The appearance of macroscopic changes associated with *P. zederi* is similar to those described for several species of cestodes in elasmobranchs and fish (*Monobothrium wageneri*, *Hemigaleus microstoma*, *Heteronybelinia estigmena*; Borucinska and Caira 2006; Williams et al. 2011), pelagic seabirds (*Alcataenia armillaris* and *Alcataenia meinertzhageni*) in murres (*Uria* spp.; Hoberg 1984b), or *Paradilepsis scolecira* in the White-necked Cormorant (Karstad et al. 1982). The attachment of *P. zederi* in the intestinal tract of pygoscelid penguins was associated with severe pathologic changes. We suggest that these changes stem from a combination of factors such as the scolex and pseudoscolex morphology and depth of parasite penetration.

Dissected parasite-induced pseudocysts were composed of up to three compartments. Other authors have observed lesions with only two diverticula (Harry and Holloway 1989; Tzvetkov et al. 1999). We speculate that the number of compartments depends on the age of the host and the number of cestodes in the entrance orifice. The focal attachment of *P. zederi* limits the area of gut damaged, and their tight clustering accentuates the severity of individual lesions. Partial occlusion of the intestinal tract with clusters of 6–13 tapeworms was observed in some lesions. All these significant changes suggest the reduction of normal gut function within the regions affected. Tzvetkov et al. (1999) found clear functional alteration in the glycoprotein profile of the epithelium in the histologic structure where *Parorchites* nodules were located, which could lead to impaired digestive and protective functions.

Railliet and Henry (1912) suggested that cysts are directly involved in cestode reproduction. The presence of several specimens in one cyst has led to the assumption that scolices multiply vegetatively. We found no evidence to support this. Most cysts dissected and analyzed histopathologically contained one cestode. Some histologic sections also contained only one vermiform necrotic tract. These results are confirmed by other studies (Harry and Holloway 1989; Cielecka et al. 1992), where no relationship was found between the cysts and cestode reproduction.

Parorchites zederi exhibits tight clustering, as do a few species of intestinal tapeworms in other hosts such as fish (Mackiewicz et al. 1972). The presence of several cestodes in a cyst could be facilitated by the preference of tapeworms for certain regions of in the intestine. We suggest, as have others (Kennedy 1983), that the mechanisms influencing the clustered attachment of parasites are related to host-derived cues, nutritional gradients within the gut, chemical signaling by conspecific parasites or subsequent gut lesions. In fish, for example, Kennedy (1983) suggested that this behavior may benefit tapeworm reproduction, penetration of the intestine, and nutrition.

Inflammation of the intestinal tract can be provoked by multiple factors, including food, chronic stress, and infectious agents such as parasites (Borucinska 2008). *Parochites zederi* caused obvious inflammatory lesions in the intestinal mucosa, with atrophy, hypertrophy, and compression of the mucosal layer. The layers most severely affected were the submucosa and the muscular layer, which suggest a muscular tropism. There is still no known reason for this, but it might be a conductive microenvironment for the growth of the parasite. In adult Gentoo Penguins, the lesions are often massive and may cause a noticeable swelling on the serosal surface of the intestine.

In Adélie and Chinstrap penguins of all ages, *P. zederi* cestodes were embedded individually rather than in groups. The extensive hemorrhagic and inflammatory areas observed could be due to the parasite moving deeper into the host tissue (Karstad et al. 1982; Hoberg 1984a). The parasitized lesions were found with or without evident proliferation of fibrous connective tissue and without inflammatory infiltrates in the damaged area. Layers of fibrous connective tissue and scarce inflammatory infiltrates surrounding the parasitized sections could be indicative of long-term infections, which normally occur in wildlife (Hoberg 1996). Inflammatory reactions were observed in the damaged mucosa with eventual proliferation of fibrous connective tissue, with or without granulomatous reaction and parasite degradation. No evidence was found to support the assertion that some cysts found in advanced lesions may be mineralized. We did not observe calcium deposits in the advanced lesions of Gentoo Penguin, in agreement with the observations by Tzvetkov et al. (1999).

We observed diarrhea in a small percentage of penguins. This may be related to secondary bacterial infections stemming from parasite immunosuppression (Boots et al. 2009). This assumption is supported by the coccobacilli found in some lesions, although the role of bacteria in the pathogenesis of the parasite-associated enteritis needs to be clarified.

We found differences in *P. zederi* infections among penguin species. In Adelie and Chinstrap penguins, the infection involved a small number of parasites, usually 1–3 tapeworms per cyst and caused fewer cysts infecting the digestive tract. Both species appeared to control the infection by creating a connective tissue capsule to contain the parasites until they are degraded. However, parasites in Gentoo Penguins occurred in tight clusters containing six or more tapeworms, deeply embedded within the mucosa and showing many irregular nodules infecting the digestive tract. This different appearance compared to other pygoscelid hosts was also reported by Fuhrmann (1921) and Tzvetkov et al. (1999), and may be attributed to differences in immunologic reactions. Nevertheless, the probability of infection depends on feeding habits. The diet of the Gentoo Penguin is broader than the Adélie and Chinstrap penguins, including more fish and squid (Williams 1995), which could increase their exposure to parasites (Hoberg 1996). Fish could participate in the life cycle of *P. zederi* if there are two hosts in its life cycle, as postulated by Cielecka et al. (1992). In conclusion, of all the Antarctic parasites detected in penguins, *P. zederi* is potentially the most pathogenic species.

Environmental changes in Antarctica could affect penguins in several ways, including dietary changes and possible exposure to new pathogens (Xavier et al. 2013). Our study provides data on the extent of damage

associated with intestinal cestodiasis in penguins, which could be used as a reference point for future monitoring of the health of these birds and the health to the environment.

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