
**PATHOLOGICAL EFFECTS OF *Echinostoma paraensei*
INFECTION ASSOCIATED WITH PYOGENIC
ABSCESSSES CAUSED BY *Pseudomonas aeruginosa*
IN SWISS MICE**

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ABSTRACT

The trematode *Echinostoma paraensei* is an intestinal parasite transmitted by ingestion of the infectious stage of metacercariae. For scientific purposes, its life cycle has been maintained in the laboratory, allowing analysis using various biological approaches. Different parasite isolates have revealed atypical patterns of migration and establishment in ectopic sites in Swiss-Webster mice. During the investigation of the biological life cycle of an *E. paraensei* isolate from the silvatic rodent *Nectomys squamipes* collected in the municipality of Rio Bonito (State of Rio de Janeiro), a bacterial coinfection with *Pseudomonas aeruginosa* was observed, which produced anatomopathological alterations, mainly in the liver, bile ducts, pancreas, and small intestine. The main macroscopic signs were the whitish suppurative pyogenic punctual lesions. The histological sections stained by hematoxylin-eosin showed an intense inflammatory reaction formed by mononuclear cells and macrophages surrounding the bile ducts, although the hepatic parenchyma still presented its normal aspect. Thus, pyogenic abscesses can be associated with *E. paraensei* infection depending on the strain and aggravating pathogenesis in the definitive host.

KEY WORDS: Liver abscess; Swiss mice; trematode infection.

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INTRODUCTION

Echinostomiasis is an intestinal trematodiasis caused by several species of *Echinostoma*, mainly in Asia (Fried & Graczyk, 2000; Sripa et al., 2010; Sah et al., 2018). In Brazil, the life cycle of some species is limited to domestic and wild animals (Maldonado et al., 2003; Maldonado & Lanfredi, 2009), although the presence of *Echinostoma paraensei* eggs in feces has been reported from a mummified human body dated 560 ± 40 years before the present (Leles et al., 2014; Chai, 2019). In addition, *E. paraensei* is one of the most studied species in experimental models (Garcia et al., 2011; Garcia et al., 2012; Tunholi-Alves et al., 2011; Monte et al., 2016; Monte et al., 2019).

The opportunistic pathogen *Pseudomonas aeruginosa* is a bacterium that may be found infecting various taxa of organisms such as plants, nematodes, insects, protozoa and mammals (Morissette et al., 1996; Rehm, 2008). The association between infection by the trematode *Schistosoma mansoni* and bacterial infection by *Staphylococcus aureus* has been reported in human and experimentally acute schistosomiasis, leading to the development of pyogenic liver abscesses (Lambertucci et al., 2001; Teixeira et al., 2001; Lambertucci, 2010). The mechanical interaction established between *S. mansoni* and *Salmonella typhimurium* was previously demonstrated by scanning electron microscopy (Bouillard et al., 1983). *In vitro* analysis has also indicated the possibility of the action of other trematode species, e.g., *Fasciola gigantica*, as carriers of *S. typhimurium* (El Zawawy et al., 2002).

Co-infections with multiple pathogens including bacteria and intestinal helminths occur in different groups of hosts (Chard et al., 2019; Cortés et al., 2020; Su et al., 2014). This interaction can interfere in the dynamics of the disease, in the host's immune response and alter infection virulence (Jenkins et al., 2019; Long et al., 2019; Shen et al., 2019). Jenkins et al. (2019), suggest that the balance between immune-regulatory and pro-inflammatory bacterial taxa during schistosomiasis play a key role in determining the effective establishment of the infection, and/or severity of the disease resulting from the host's immune responses to infection.

Irwin & Fried (1990) first reported the association between bacteria and the surface of encysted metacercariae of *Echinostoma caproni*. Later, Keeler et al., (2007), reported that several species of bacteria were present on the surface of metacercariae of *E. caproni* stored for further experimental use. However, there are no descriptions of the association between the presence of bacteria and the pathogenic effect of *Echinostoma paraensei* parasitism.

Here we describe the histopathological alterations in Swiss Webster mice experimentally infected by *E. paraensei* (Rio Bonito isolate) in association with the bacterium *P. aeruginosa* and the occurrence of pyogenic liver abscesses.

MATERIAL AND METHODS

The isolate of *E. paraensei* utilized was obtained from naturally infected specimens of *Nectomys squamipes* (Cricetidae) captured in the municipality of Rio Bonito, Rio de Janeiro state (22°42'30" S, 42°37'34" W). The life cycle of *E. paraensei* has been maintained in the Laboratory for Biology and Parasitology of Wild Reserve Mammals (LABPMR) at the Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (FIOCRUZ) in Rio de Janeiro, through passages in hamsters (*Mesocricetus auratus*) as definitive hosts, and snails *Biomphalaria glabrata* as intermediate hosts. The experiment was in line with the ethical procedures for animal treatment and the guidelines were approved by the Commission for Ethical Use of Animals at the Fundação Oswaldo Cruz (permit CEUA/L-029/2017).

Twenty adult female Swiss Webster mice were divided into two groups: (i) 15 mice infected with 20 metacercariae per animal by gavage obtained from experimentally infected snails; and (ii) 5 mice forming the control group, free of infection, receiving only dechlorinated water. The animals were euthanized 4 weeks post-infection. For euthanasia, the animals were anesthetized with an intramuscular injection of thiopental sodium, followed by intracardiac administration of potassium chloride, under the supervision of a licensed veterinarian. The intestines were then examined for helminths. The specimens collected from the small intestine were washed in Petri dishes with a physiological solution and stored in 70% alcohol.

Liver, bile duct and pancreas samples from the mice were fixed in Millonig's solution and processed for histopathological examination. Briefly, the samples were embedded in paraffin and sliced into 5 µm sections (Humason, 1979). The material was stained with hematoxylin-eosin (HE) and Gomori's reticulin stain (GR). The slides were studied under a Zeiss Observer Z1 light microscope, and images were obtained using a Nikon Coolpix 4300 digital camera.

For bacterial identification, the purulent material located in the deepest part of the abscess in the liver was collected by aspiration using a syringe and needle. The samples were placed in individual tubes containing brain heart infusion (BHI) nutrient broth and incubated for a period of 24 hours in an aerobic atmosphere at 37°C. After bacterial growth, the samples were spread on solid 5% sheep blood agar medium to obtain bacterial colonies by incubation. The morphology of the specimens was observed, after which these underwent Gram staining. Subsequently, slides were assembled and examined under a light microscope with 100X immersion objective. All selected strains were re-isolated in Hektoen enteric Agar® (Oxoid). Biochemical bench tests were also carried out, followed by identification with the Vitek® automation system.

RESULTS

The anatomopathological aspects were evident in the liver (Figure 1a), bile duct (Figure 1b) and duodenum (Figure 1c). The macroscopic alterations consisted of whitish suppurative pyogenic punctual lesions. These lesions were found in 11 of the 15 livers examined and were less frequent in the small intestine (3/15). In both cases the lesions were associated with the presence of the parasite. There were helminth clusters enlarging the bile ducts and a large quantity of mucus thickening the walls. The bacterial colonies observed in the purulent material were classified as Gram-negative rods, being distinct only in their morphology and arrangement. The colony's morphology and biochemical metabolism identified it as *Pseudomonas aeruginosa*.

The histological sections showed restricted inflammatory reaction around the bile ducts (Figure 2a), with mononuclear cells and low intensity of eosinophils and neutrophils (Figure 2b). Deposition of fibrotic material around the ducts was observed. The basal membrane of the ducts was thickened, with hyperplasia of the cell wall, presenting columnar aspect, together with lymphatic vessel congestion (Figure 2c). The presence of an *E. paraensei* embryonated egg was noted in the intrahepatic duct (Figure 2c). The hepatic parenchyma surrounding the bile ducts was inflamed and contained fibrotic material. The pancreatic ducts showed a similar pattern to that in the hepatic branch, with a slight inflammatory reaction (Figure 2d). No involvement of the Langerhans islands or the endocrine parenchyma was observed.

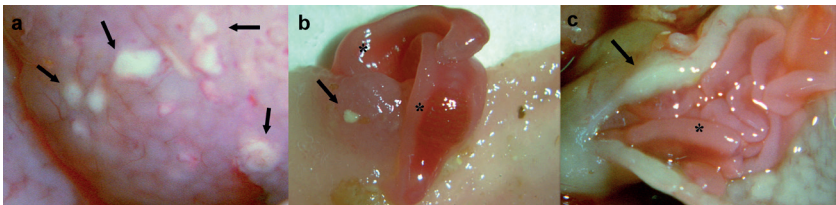


Figure 1. Macroscopic view. (a) Liver showing punctual lesions (pyogenic abscesses) (arrow) under Glisson's capsule. (b) Adult *Echinostoma paraensei* worms (*) attached to the duodenum surface, showing punctual lesions (pyogenic abscesses) (arrow). (c). Macroscopic view of bile duct showing enlargement and thickening of the walls with large quantity of mucus (arrow) and clusters of *Echinostoma paraensei* helminths (*).

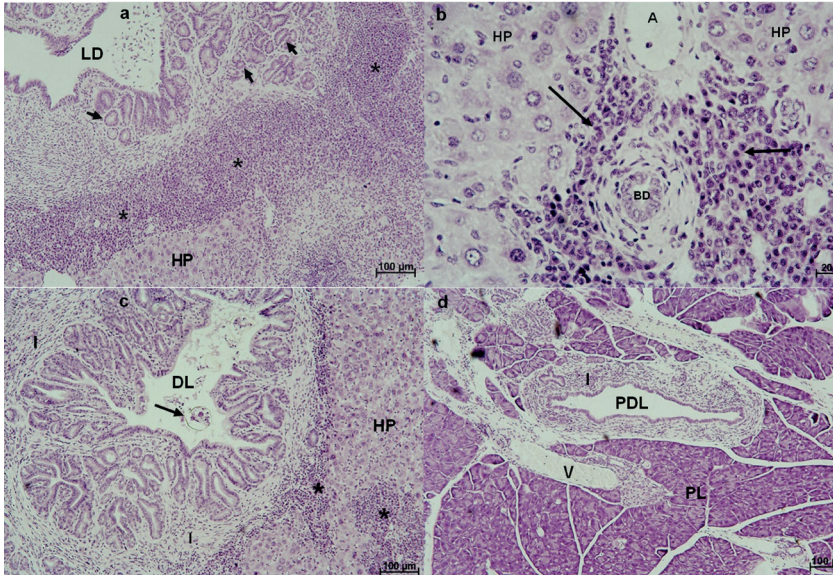


Figure 2. Histological sections. (a) Hepatic duct hyperplasia (arrows) and congestion of lymphatic vessels (*) in the hepatic periportal space. (DL = duct lumen; HP = hepatic parenchyma). (b) Macrophage monocyctic infiltrate involving the duct of the intrahepatic portal space (arrows). (HP = hepatic parenchyma; BD = bile duct; A = artery). (c) Intrahepatic duct hyperplasia with macrophage monocyctic infiltrate and congestion of peripheral lymphatic vessel to the duct (*). Arrow showing *Echinostoma paraensei* egg (I = infiltrate; DL = duct lumen; HP = hepatic parenchyma). (d) Periductal inflammatory infiltrate in the pancreas. (PL = pancreatic lobe; PDL = pancreatic duct lumen; I = cell infiltrate; V = vein).

DISCUSSION

There are relatively few reports of histopathological findings associating bacterial and trematode infections (Abruzzi & Fried, 2011; Lambertucci et al., 2001; Oyarzún-Ruiz et al., 2019).

Most studies report bacteria associated with the surface of encysted metacercariae or the tegument of adult digeneans (Nollen & Nadakavukaren, 1974; Aho et al., 1991; Irwin & Fried, 1990; Keeler et al., 2007). There are no reports on the pathological effects of concomitant infections. Oyarzún-Ruiz et al. (2019) described the presence of gastrointestinal helminths and histopathological lesions in black-necked swans (*Cygnus melancoryphus*). Interestingly, in one swan infected by the nematode *Epomidiostomum vogelsangi* a Splendore-Hoeppli lesion was observed in the muscle wall of the gizzard, containing multiple bacterial colonies surrounded by eosinophils, giant cells and amorphous tissue. Also, in one swan infected by *Echinostoma*

mendax there were Splendore–Hoepli lesions associated with severe fibrosis and an inflammatory response in the serosa of the colon.

In an experimental study with *E. paraensei*, Garcia et al. (2011), determined histological and biochemical parameters in infected *Rattus norvegicus* (Wister). High levels of hepatic enzymes were noted in the first week of infection evidencing the pathogenic effect of the infection. Interestingly, the trematodes were recovered in the first portion of the small intestine (duodenum), differently from the present study in which worms were clustered in the ductal lumen. In addition, helminth migration to ectopic sites may have favored infection by opportunistic bacteria due to alterations in the host's immune system. In fact, helminthic infection can alter immune response to a bacterial pathogen contributing to its dissemination (Louhi et al., 2015; Long et al., 2019; Shen et al., 2019; Su et al., 2014).

Maldonado et al. (2005) reported different biological behaviors in the experimental infection of two isolates of *E. paraensei* collected from naturally infected *Nectomys squamipes* from two municipalities in Rio de Janeiro State, Brazil: Rio Bonito (RB) and Sumidoro (SU). The RB isolate presented a higher worm burden, longer life span and preferential clustering of the trematodes in the duodenum and bile duct, while in the SU isolate they were spread along the small intestine of the infected mice. Indeed, *E. paraensei* infection causes alterations in the hepatic function in experimentally infected rats, possibly due to partial obstruction of the bile duct by helminths (Garcia et al., 2011). These findings demonstrate that parasites express different behaviors, depending on the isolate, which may lead to a worsening of the infection itself or serve as a gateway to concomitant infections. Moreover, Chai et al. (1994), reported a human case of *E. hortense* infection, characterized by ulcerations of the duodenal mucosa. Therefore, this trematode can cause injury at the site of infection, forming a gateway for secondary infections.

This bacterium can exploit breaks in the host's defenses to initiate infection. One of the important entryways leading to septicemia is the intestinal tract. The virulence of the *E. paraensei* RB strain can increase the risk of bacterial infection through the ulcerations produced by the helminth. The opportunistic pathogen *P. aeruginosa* has been found in fecal or cecal samples of laboratory rats (Hayashimoto et al., 2013). On the other hand, the bacterium *P. aeruginosa* has also been isolated from *Biomphalaria glabrata* tissues (Cheng, 1986), which suggests a source of association between bacteria and metacercariae. The *P. aeruginosa* associated with the hepatic and duodenal suppurative lesions observed in the present study probably were transported by metacercariae of *E. paraensei*, since the snail *B. glabrata* is an important intermediate host for this trematode (Lie & Bash, 1967). Thus, pyogenic abscesses can result from *E. paraensei* infection, depending on the trematode isolate.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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