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Research paper

Oxidative stress associated with pathological changes in the pancreas of cattle naturally infected by *Eurytrema coelomaticum*

Claiton I. Schwertz[a], Mateus E. Gabriel[a], Luan C. Henker[a], Nathieli B. Bottari[b], Guilherme do Carmo[b], Naiara dos S. Guarda[c], Rafael N. Moresco[c], Gustavo Machado[d], Vera M. Morsch[b], Maria Rosa C. Schetinger[b], Fernanda A. Stedille[a], Piotr Baska[e], Vanessa Mattei[f], Aleksandro S. da Silva[b,g], Ricardo E. Mendes[a,*]

[a] *Laboratory of Veterinary Pathology, Instituto Federal Catarinense (IFC), Rod. SC 283, km 08, CP 58, Concórdia, SC 89703-720, Brazil*
[b] *Biochemistry and Molecular Biology Department, Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000, Santa Maria, RS 97105-900, Brazil*
[c] *Clinical and Toxicological Analysis Department, Universidade Federal de Santa Maria (UFSM), Av. Roraima 1000, Santa Maria, RS 97105-900, Brazil*
[d] *College of Veterinary Medicine, Universidade Federal do Rio Grande do Sul (UFGRS), Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil*
[e] *Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), 8 Ciszewkiego, 02-786 Warsaw, Poland*
[f] *College of Veterinary Medicine, Universidade do Estado de Santa Catarina (UDESC), Av. Luís de Camões 2090, Lages, SC 88520-000, Brazil*
[g] *Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Sete de Setembro 77D, Chapecó, SC 89806-152, Brazil*

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ABSTRACT

Although *Eurytrema coelomaticum* is considered a parasite with low pathogenicity, it may be associated with mortality and loss of productive performance in animals due to chronic pancreatitis. The aim of this study was to evaluate the occurrence of oxidative stress caused by *E. coelomaticum* in naturally infected cattle, correlating the biochemical findings with the parasite load and histopathological changes. For this study, blood and pancreas samples from 51 cattle were collected, and levels of the thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and ferric reducing ability of plasma (FRAP) were measured in the serum and pancreas, and superoxide dismutase (SOD) activity was measured in total blood. Parasite burden was determined opening the pancreatic ducts, and then fragments of pancreas were collected and fixed in 10% buffered formalin and routinely processed for histopathology. From the 51 collected pancreas, 33 (63.5%) were parasitized. The average parasite burden per pancreas was 532 (12–2,578). TBARS and FRAP showed higher levels in serum and pancreas of infected animals ($p < 0.05$), with a positive correlation between the histopathological changes and the number of parasites. SOD level in blood was 42% higher in parasitized group compared with control group ($p < 0.05$), as well as AOPP in serum. Based on these results, we concluded that in natural infection by *E. coelomaticum* in cattle, oxidative stress occurs, characterized by the occurrence of protein oxidation, lipid peroxidation and activation of antioxidant system.

1. Introduction

The flukes of *Eurytrema* genus, Looss 1907, belonging to the family Dicrocoeliidae, are parasites of pancreatic ducts of animals and occasionally humans. Among the most important species are *Eurytrema coelomaticum* and *Eurytrema pancreaticum*, the first specie being found in Brazil (Mattos Júnior and Vianna, 1987; Bassani et al., 2007). The parasite has two intermediate hosts, the land snail (*Bradybaena similares*) and arthropods of the genus *Conocephalus*, commonly known in Brazil as "*esperanças*" (Mattos Júnior and Vianna, 1987).

Eurytrema spp. is considered a low pathogenic parasite, but may be associated with mortality and loss of productive performance of animals due to chronic lesions in pancreas. Clinical cases of eurytrematosis in cattle have been reported in Brazil (Ilha et al., 2005; Rachid et al., 2011; Quevedo et al., 2013), and the clinical and pathological pattern observed was chronic and progressive weight loss related to interstitial pancreatitis. The prevalence of parasitism in Brazil varies between regions: Lucca et al. (2015) reported 69% in the west of Santa Catarina State, however, Azevedo et al. (2004)

* Corresponding author.
E-mail addresses: ricardo.mendes@ifc-concordia.edu.br, ricardo.mendes@gmx.us (R.E. Mendes).

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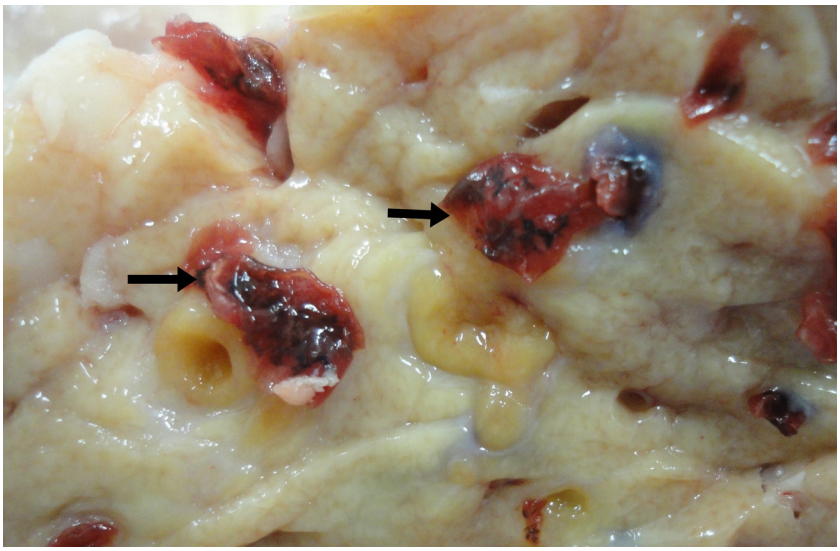

Fig. 1. Cut surface of the pancreas of a bovine naturally infected with *Eurytrema coelomaticum*. There is marked hyperplasia of the pancreatic ducts and various specimens of the parasite (arrow) coming out of sectioned ducts. The parasite has a dark red colour and measures approximately 10–13 mm in length by 6–7 mm wide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

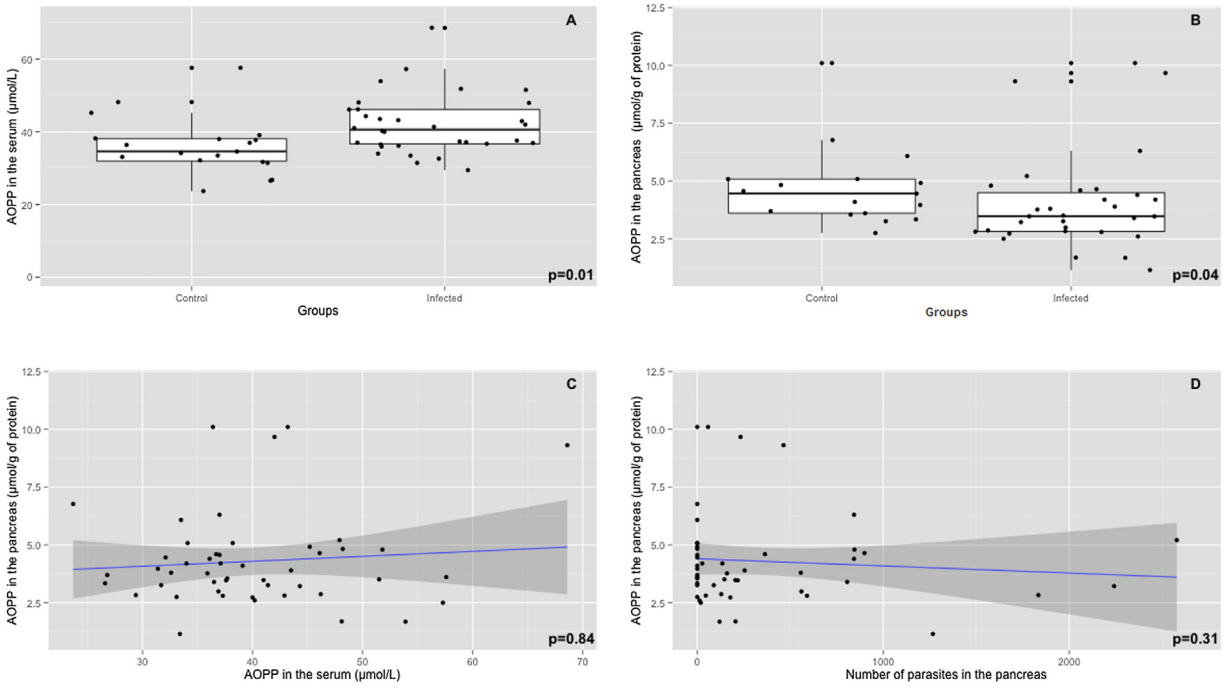

Fig. 2. Advanced oxidation protein products (AOPP) in serum (A) and pancreas (B) of cattle infected or not with *Eurytrema coelomaticum*. Correlation between AOPP in serum and pancreas (C). Correlation between AOPP in pancreas and number of parasites (D).

reported that prevalence varies between 8.3 and 40.5% in different regions in Paraná state.

The main pathological changes described in the pancreas of cattle naturally infected by *Eurytrema* spp. are characterized by thickening of the pancreatic ducts due to hyperplasia and fibrosis, presence of the parasite inside the ducts and inflammatory infiltrate consisting of lymphocytes, macrophages and plasma cells, as well as replacement of pancreatic parenchyma by fibrous connective tissue (Figueiredo, 1928; Belém et al., 1994; Ilha et al., 2005; Schwertz et al., 2015). The islets of Langerhans are generally not affected, but can be replaced by connective tissue in severe cases of disease (Figueiredo, 1928; Belém et al., 1994). In the last decades, numerous studies have been performed to clarify the role of free radicals in inflammatory physiopathological processes (Reuter et al., 2010). Simply, the free radical term refers to an atom or highly reactive molecule that contains an odd number of electrons in its last electronic layer, and the mismatch of electrons in the last layer gives it higher reactivity, which under conditions of an excess of oxidizing agents and/or deficiency of the protective system, leads to an imbalance that characterizes the oxidative stress (Ferreira and Matsubara, 1997). The oxidative stress is evaluated by measuring oxidant and antioxidant markers and among them we can highlight the lipid peroxidation, protein oxidation and antioxidant enzymes (Reuter et al., 2010).

Studies have linked oxidative stress with the clinic-pathological features and pathogenesis of parasitic disease. Among them,