BJVP
Brazilian Journal of Veterinary Pathology

Original full paper

Histopathologic patterns of pancreatic lesions induced by *Eurytrema coelomaticum* in cattle from the central-west region of the State of Paraná, Southern Brazil

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Submitted August 8th 2008, Accepted November 17th 2008

Abstract

Bovine eurytrematosis in Brazil is induced by *Eurytrema coelomaticum* and is endemic in some Brazilian States. The pathology of the disease is not fully understood and there are few descriptions of the histopathological associated lesions. This study analyzed the histopathological patterns of 117 bovine pancreatic samples obtained randomly. Samples were collected during one year from the municipal slaughterhouse of Campo Mourão, Paraná, Brazil, during routine meat inspection; this location is endemic for bovine eurytrematosis. Five predefined histopathological patterns were analyzed. Differences between the types of lesions were analyzed statistically. The pancreas of 11.97% (14/117) animals was normal; 31.62% (37/117) demonstrated initial proliferative periductal lesions; 17% (21/117) revealed severe proliferative periductal lesions; 21.37% (25/117) were characterized as chronic multifocal interstitial pancreatitis; while 17.09% (20/117) demonstrated chronic diffused interstitial pancreatitis. Statistically cases classified as initial proliferative interductal and chronic multifocal interstitial pancreatitis were the most frequently occurring lesions. The pathogenesis of lesions associated with this disease is also discussed.

Key Words: *Eurytrema coelomaticum*, pathology, cattle, pancreas.

Introduction

Bovine eurytrematosis (BE) is induced by trematodes (*Eurytrema coelomaticum* and *E. pancreaticum*) that live principally in the pancreatic ducts and occasionally in the bile ducts of ruminants and other mammals (1, 8, 10, 12). In Brazil, BE has been related to *E. coelomaticum* (9, 12, 13). This parasite requires two intermediate hosts to complete its life cycle; the first is a land snail (*Bradybaena similaris*), and the second are various species of grasshoppers, *Conocephlaus maculatus, C. chimensis, C. melas*, and *C. gladiator* (9, 12).

In cattle, gross pancreatic lesions associated with parasitism induced by *Eurytrema* spp. have been related to pancreatic atrophy (6, 8), while BE clinical signs have been associated with a wasting disease of cattle (7). Histological description includes chronic interstitial fibrosis, periductal parasitic granulomas and fibroplasia,

obstruction of pancreatic ducts, hyperplasia of pancreatic ducts without marked destruction of the Islets of Langerhans (7, 8, 14) and lipomatosis (14).

The pathological alterations induced by *Eurytrema* spp. have been previously classified (3, 4, 14) based on the parasite burden relative to histological pancreatic alterations, with more emphasis on parasitological rather than pathological features and in reduced number of cases. This article describes the histopathological findings of BE and its frequency observed in 117 cattle within an endemic area (2). This paper also discusses possible pathogenesis associated with BE, and complements the results of a previously described epidemiological study (2).

Materials and methods

Sampling

117 randomly obtained samples of pancreas from cattle slaughtered were collected during routine inspection at the city of Campo Mourão, Paraná, Southern Brazil, were used during this study. These samples were retrieved from a previous study realized during June 2003 to May 2004 (2). All samples were collected, labeled, stored individually in 10% formalin solution, and then processed for routine histopathological evaluation.

Histopathological evaluation and identification of trematode

Samples were histologically evaluated and classified in five pre-determined groups based on previous descriptions (3, 14). Group I: normal pancreas. Group II: initial proliferative reactions, characterized by marked proliferation of epithelial cells of pancreatic ducts, discrete inflammation with moderate periductal and interstitial fibrosis. Group III, severe proliferative alterations, represented by moderate obstruction and/or rupture of pancreatic ducts, severe periductal fibrosis and inflammatory response, discrete dystrophic calcification, moderate interstitial pancreatitis, and discrete foci of infiltration of adipose tissue. Group IV, chronic multifocal interstitial pancreatitis, characterized by severe obstruction and/or rupture of pancreatic ducts, severe periductal fibrosis and inflammatory response, moderate dystrophic calcification, severe multifocal interstitial pancreatitis, moderate areas of infiltration of adipose tissue. Group V, chronic diffused interstitial pancreatitis, defined as severe obstruction and or rupture of pancreatic ducts, focally extensive or diffused interstitial pancreatitis, severe dystrophic calcification, and severe infiltration of adipose tissue.

Parasites were identified and classified as *E. coelomaticum* based on typical morphological characteristics obtained from live specimens of trematodes withdrawn from the pancreatic ducts of infected cattle; complete methodology and description can be consulted (13).

Statistical analysis

The number of cases obtained was grouped as previously described and evaluated statistically to determine the possible existence of differences between the frequencies of these lesions. Statistical analysis was based on the Chi-square Test with a significance level of 5% ($p \leq 0.05$).

Results

In cases classified histologically as normal (Group I), there were no alterations to pancreatic parenchyma and/or were there indications of passage of *E. coelomaticum* through pancreatic ducts (Figure 1A). Initial proliferative lesions (Group II) were characterized by extensive proliferation of epithelial cells of pancreatic ducts without, in most cases, corresponding presence of intralesional trematodes (Fig. 1B). Severe lesions (Group III) demonstrated extensive proliferation of epithelial cells of pancreatic ducts, dilation and periductal fibrosis of interlobular pancreatic ducts, mild infiltration of adipose tissue; moderate influx of inflammatory cells (predominantly eosinophils, with macrophages and lymphocytes). There were also intralesional trematodes within pancreatic ducts; in some of these cases there was dystrophic calcification of the wall of some pancreatic ducts (Fig. 1 C-D). Cases of chronic multifocal interstitial pancreatitis (Fig. 1E) revealed severe multifocal interstitial fibrosis, dilation and rupture of pancreatic ducts, periductal fibrosis and moderate dystrophic calcification of interlobular pancreatic ducts, and severe inflammatory infiltrate; in some cases, there were obstruction of pancreatic ducts by intralesional trematodes. Cases of chronic diffused interstitial pancreatitis (Fig. 1 F-H) resulted in severe destruction of pancreatic parenchyma by fibrosis; interstitial presence of eggs of the trematode associated with severe dystrophic calcification; rupture of pancreatic ducts; in some cases, there were classical granulomatous lesions with fagocytosis of eggs by multinucleated giant cells. In all cases observed, irrespective of the extension or severity of pancreatic destruction, there was marked preservation of the Islet of the Langerhans.

Significant differences ($P \leq 0.05$) were observed when the number of cases from Group II (initial proliferative reactions) and Group IV (chronic multifocal interstitial pancreatitis) were compared with the other groups evaluated (Table 1). However, no significant differences ($P \geq 0.05$) were observed when the number of cases of bovine eurytrematosis within Group I (normal tissues), Group III (severe proliferative alterations), and Group V (chronic diffused interstitial pancreatitis) was observed.

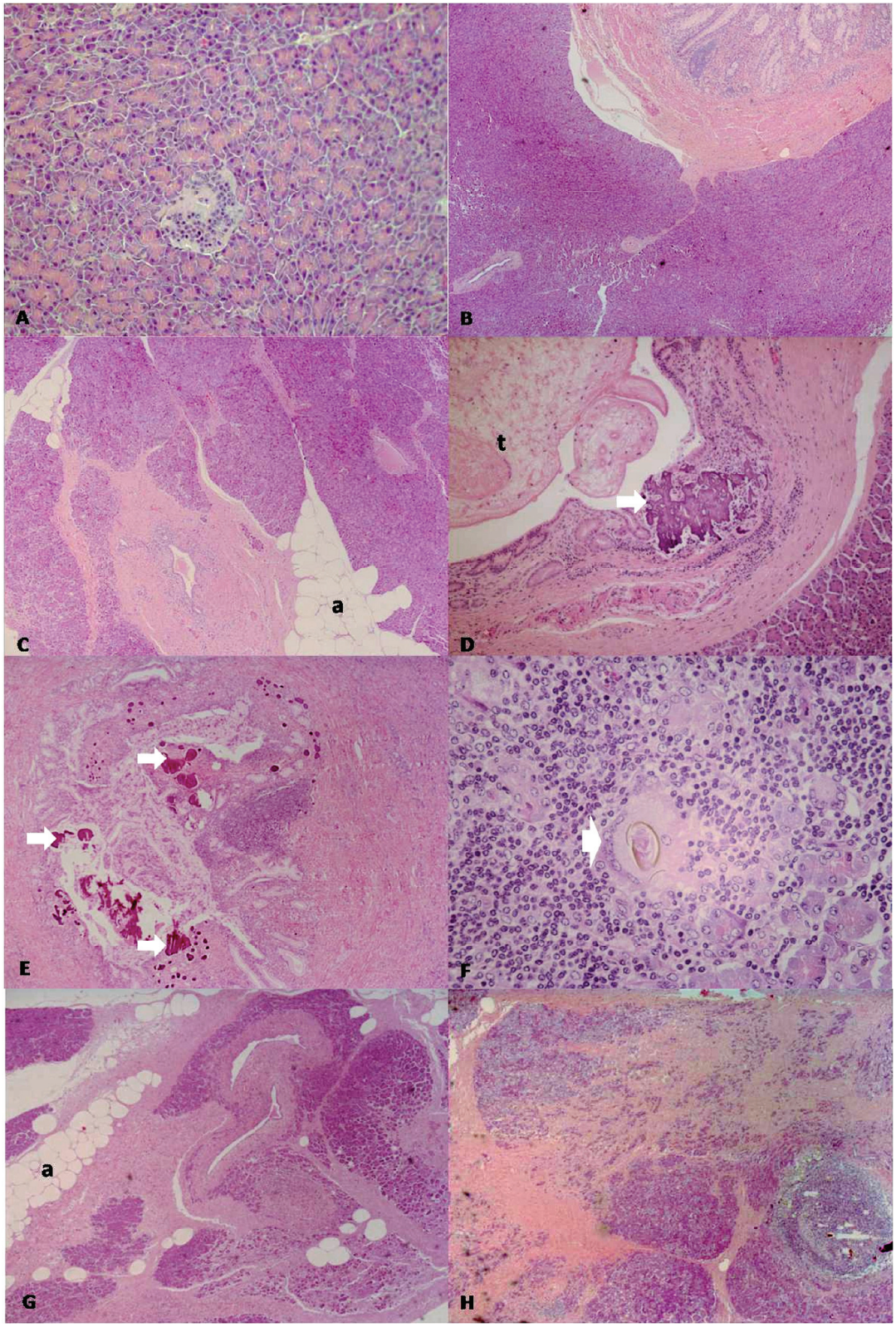

Figure 1 - Photomicrography of principal aspects of histologic lesions associated with bovine eurytrematosis. Normal pancreas, A; initial proliferative intraductal lesions, B; severe proliferative lesions, C and D; chronic multifocal interstitial pancreatitis, E; and chronic diffused interstitial pancreatitis, F-H (HE, Obj. 4x). There are areas of infiltration of adipose (a) tissue within the pancreatic parenchyma (C, G). Observe intraductal trematode (t) with dystrophic calcification (arrow) of pancreatic duct (D). There is rupture and foci of dystrophic calcification of pancreatic duct (E), and classical granulomatous formation around eggs (arrow head) in chronic parasitic pancreatitis (F).

Histopathological patterns	Animals	
	n	%
Group I, normal tissue	14	11.97
Group II, initial proliferative ductal reactions	37	31.62*
Group III, severe proliferative alterations	21	17.95
Group IV, chronic multifocal interstitial pancreatitis	25	21.37*
Group V, chronic diffused interstitial pancreatitis	20	17.09
Total	**117**	**100**

Table 1 - The frequency of histopathological patterns of pancreatic lesions observed in 117 cases of bovine eurytrematosis. *P ≤ 0.05, compared with other groups.

Discussion

This study has demonstrated that initial proliferative ductal lesions (Group II) and chronic multifocal interstitial pancreatitis (Group IV) were the most frequent histological patterns associated with *E. coelomaticum* in cattle within an endemic region of bovine eurytrematosis (BE) in southern Brazil. Although there are previous descriptions (3, 14) of histological patterns of BE, these authors did not evaluate the frequency of the lesions described. Nevertheless, the histological patterns described in this study must not be considered as distinct lesions associated with BE, but as progressive manifestations (Group II to V) of *Eurytrema* spp. infection and corresponding destruction of pancreatic parenchyma. We have recently demonstrated that the number of trematodes within the pancreatic ducts is proportional to pancreatic destruction and not to histological classification (2). In BE, initial lesions represents early infection and as infection persists, or in cases of reinfection, there is corresponding increase in damage to the exocrine pancreas. Further, proliferation of connective tissue induces destruction of the glandular acinus, secondary parenchymal atrophy, obliteration and obstruction of the pancreatic ducts, with accumulation of eggs within these ducts and in cases of ductal rupture, eggs are observed within the exocrine pancreas; in most cases, the Islets of Langerhans are preserved.

The inflammatory infiltrate associated with *E. coelomaticum* induced-pancreatic lesions was predominantly eosinophilic; in some cases, granulomatous reactions within the pancreatic parenchyma were observed when eggs were fagocytised by giant cells; similar results have been previously described (3, 14). However, the degree of associated eosinophilic influx was similar in Group III to V animals; this may indicate that in this disease, eosinophylia is not directly related to the degree of pancreatic destruction induced by the trematode, but represents an immunological antigen-antibody response to the trematode (14).

During this study, animals within Group III to V demonstrated areas of infiltration of fatty tissue within the exocrine pancreas. Fatty lesions have been previously described in BE (14), but these authors have suggested that such alterations might not be induced by the trematode. However, in BE there is marked destruction of pancreatic tissue and obstruction of pancreatic ducts and interstitial fibrosis, which might induce subsequent secondary pancreatic atrophy (8). In cases of secondary pancreatic atrophy due to ductal obstruction, as is very frequent in BE, the pancreas becomes nodular, atrophic, and firm, resulting in areas of fatty infiltration (8); therefore the pathogenesis of fatty infiltration observed progressively in Group III to V animals could be a direct or indirect manifestation of *Eurytrema* spp. infection. Additionally, fatty infiltration in BE might affect the development of interductal trematodes; since the sizes of these parasites were drastically reduced in cases of severe fatty infiltration (14), suggesting that the trematodes feed on substances produced by the exocrine pancreas and in their absence there is growth interference.

The severe rupture of pancreatic ducts, periductal fibrosis, and intralesional presence of eggs within the pancreatic parenchyma observed in Group IV and V animals might be a direct manifestation of irritation induced to one, or a combination, of the following mechanisms. Compressive effect and constant movement of trematodes within affected pancreatic ducts (5, 9), retention of pancreatic juice due to ductal obliteration, and the result of substances liberated by mature parasite or eggs. Additionally, pancreatic fibrosis (interstitial or periductal) is considered as the principal histological manifestation of eurytrematosis in domestic animals (13). Chronic diffused interstitial pancreatic fibrosis as herein described, and referred by some as “pancreatic cirrhosis”, was not observed in a study of BE (3).

In the cases of severe chronic multifocal and chronic diffused interstitial pancreatitis described in this study, the endocrine pancreas, i.e., the Islets of Langerhans were preserved; similar results have been described (3, 14, 15). In another study of this disease, compressive atrophy of the Islet of Langerhans was described, as being proportional to the degree of pancreatic fibrosis (14). However, cats affected with *E. procyonis* in which there is ductal obstruction may develop functional abnormalities (5). The effects of damage to the endocrine pancreas in BE has not been well elucidated. Nevertheless, cattle with chronic pancreatitis have been associated with insulin-dependent diabetes mellitus (11).

Conclusions

In Brazil, initial proliferative interductal pancreatitis and chronic multifocal interstitial pancreatitis are probably the most frequent histopathologic patterns associated with naturally occurring cases of bovine eurytrematosis. These histologic patterns described are not distinct lesions of this disease, but should be considered as

progressive manifestations induced by *Eurytrema coelomaticum* with corresponding destruction of pancreatic parenchyma.

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