

Gross and microscopical lesions of the liver in chronic infestation with *Fasciola hepatica* in bovines

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Abstract. Fasciolosis is a hepatic and biliary disease, with seasonal dynamics and with focal character in bovines. The lesions are polymorphic with different aspects, depending on the form of the disease. After macroscopic examination of the liver of 65 cattle with chronic fasciolosis we observed lesions of biliary calculus in 57%, biliary stasis in 18.5%, and calcification associated with fibrosis of the bile ducts in 64.6%. In terms of histopathological lesions, we found the predominant occurrence of fibrous chronic cholangiohepatitis accompanied by compression atrophy of hepatocytes adjacent to the area of fibrosis (70%); we also found dystrophic calcifications, loss of epithelium bile ducts accompanied by infiltration with lymphocytes, plasma cells, eosinophils, macrophages and fibroblast, hepatic fibrosis in portal bridges accompanied by various stage of hepatocytes necrosis and, in rare cases, it was observed hyperplasia and metaplasia of the biliary epithelium, in 20% from examined samples.

Keywords: Chronic fasciolosis; Bovine; Pathology.

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Introduction

Fasciolosis is a hepatobiliary disease, with seasonal dynamics and with focal character affecting herbivores, especially ruminants, being caused by species of the genus *Fasciola*. This parasite is found throughout the world, and appears in animals maintained on the rich pastures in biotopes of snails. Nowadays, fasciolosis is one of the major diseases in many countries (Şuteu and Cozma, 2007).

In bovine fasciolosis, lesions are polymorphic. In cattle the chronic form is the common one; the subacute form is rather rare (Boray, 1969). Characteristic changes occur particularly in bile ducts and in liver parenchyma.

Due to the complex pathogenic action of the parasites, which have together mechanical, irritating, inflammatory, toxic and inoculating effect, the intensity of the lesions is correlated with the number of ingested metacercariae and

with the season, as during the summer, metacercariae are more aggressive than in spring or autumn (Şuteu and Cozma, 2007).

The main aim of this study was to evaluate hepatic pathology in cattle chronically infected with *F. hepatica* in the North-West of Romania.

Materials and methods

The studies have been conducted during October 2007 and June 2009 at the Pathology Department of the Faculty of Veterinary Medicine, Cluj-Napoca, on a number of 67 bovines, slaughtered in the S.C. Agro-Ardeal SRL slaughterhouse, Bistrița-Năsăud County, Romania.

For post-mortem diagnosis of the infestation with *F. hepatica* in cattle, we performed a general inspection of the liver, of the extrahepatic bile ducts and of the gall bladder. The sections of the liver were performed on the visceral side, perpendicularly to the bile ducts in each hepatic lobe. Sections were also made wherever lesions were present. During necropsy, also the gross aspect of the hepatic parenchyma, of the bile ducts and blood vessels was examined. To detect biliary stasis produced by flukes, the gallbladder was opened and we noted the color, the consistency and quantity of the bile, the presence of flukes, the aspect of gallbladder mucosa and the presence of the biliary calculi. We have also recorded the presence of the adults and the immature flukes, as well as the existence of other associated parasitic infections. Histological preparations were also performed in order to evaluate microscopical characteristics of lesions. For histological examination, samples were collected from the livers of 10 cattle (6.5%). The samples were placed in fixing solution (10% formalin, neutral pH) for 24 hours, and subsequently processed by paraffin technique, followed by 5-6 µm sections. The staining methods performed on histological preparations were Trichromic Masson (TM) and Hematoxylin and Eosin (HE). The examination of histology slides was done with an Olympus BX51 microscope. The images were taken with a digital camera Olympus SP 350 and analyzed with an Olympus DP-soft program.

Results and discussion

Necropsy examination revealed in all cases (n=67) liver hypertrophy with thickening of the edges (figure 1), bile ducts with thickened cords aspect, with white-gray color on the surface of the liver parenchyma, as well as thickening of the Glisson's capsule (figure 2). Bile ducts were sclerotic, dilated, rough on palpation, white-gray, with dystrophic calcification that creaked during the cut (n=42, 62.7%). Sections through the biliary ducts revealed the presence of adult flukes (figure 1). Gall bladder volume was increased, with a viscous and blackish content.

Following histopathological examination, the lesions observed were chronic fibrous cholangiohepatitis with hyperplasia and calcifications of the bile ducts (figure 3). Papillary proliferations of bile ducts epithelium and the presence of a chronic inflammatory infiltrate with mononuclear cells were also observed. These lesions were caused by the inflammatory and irritating actions of the adults flukes. In the liver parenchyma we noted the following lesions: periportal fibrosis (figure 4), porto-portal fibrous bridges (figure 5), infiltrate with small number of mononuclear cells dominated by lymphocytes, plasma cells and rare eosinophils. Compression atrophy of hepatocytes from adjacent area of fibrosis was also observed. The hyperplasia of the bile ducts (figure 6) was accompanied in two cases with dilatation of the bile ducts and with epithelial dysplasia (figures 7, 8).

In the chronic evolution of bovine fasciolosis hepatic changes consisting in angiocholitis, with the increase in volume of the liver and with thickening of the bile ducts, periangiocholitis, interstitial hepatitis and atrophic or hypertrophic liver cirrhosis were also reported (Şuteu and Cozma, 2007). The areas with liver cirrhosis are usually disseminated and have a hard texture, and appear as bands or nodules (Baba, 1980). Enlarged bile ducts, with thickened cords aspect, impregnated with calcium salts, reaction due to the chronic irritation caused by adult flukes were found by Ross et al. (1967) and Cătoi (2003).

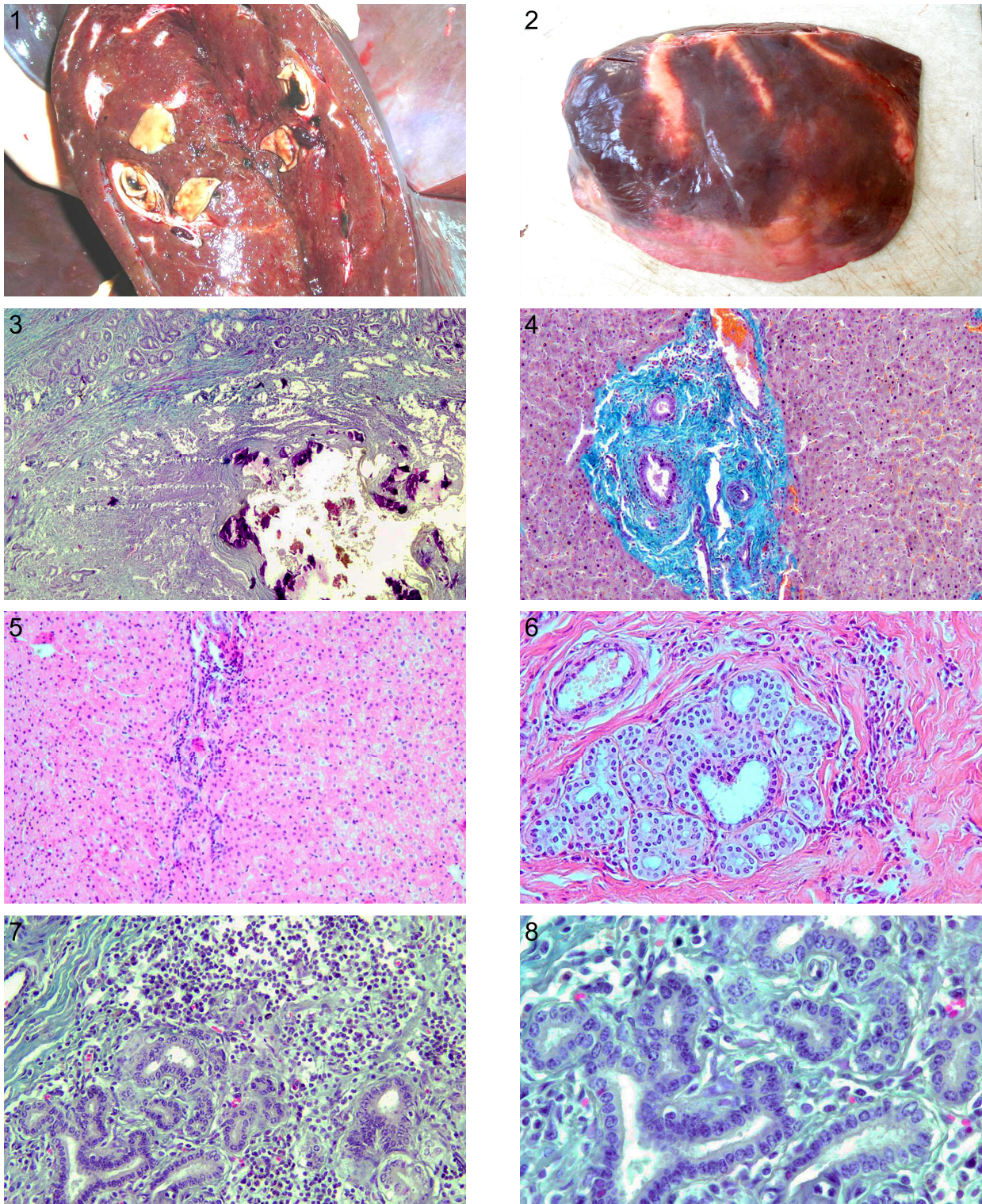


Figure 1. Fibrous cholangiohepatitis; presence of adults parasitic forms of *F. hepatica* in the bile ducts. **Figure 2.** Fibrosis of the bile ducts and atrophy of the liver edge. **Figure 3.** Chronic fibrous cholangiohepatitis, hyperplasia of bile ducts with fibrosis, calcifications, aspects of papillary proliferation of the duct wall (TM, 40x). **Figure 4.** Portal fibrosis, discrete inflammatory infiltrate dominated by mononuclear cells, compression atrophy of hepatocytes adjacent to fibrosis zone (TM, 200x). **Figure 5.** Hepatic fibrosis in porto-portal bridges, hepatocytes in various stages of necrosis (HE, 200x). **Figure 6.** Chronic fibrous cholangitis, bile duct nests- like hyperplasia, discrete inflammatory infiltrate dominated by mononuclear cell and rare eosinophils (HE, 200x). **Figure 7.** Chronic fibrous cholangitis, abundant inflammatory infiltrate dominated by mononuclear and eosinophils, bile duct hyperplasia, (TM, 200x). **Figure 8.** Bile duct hyperplasia, dysplastic cells present in the bile duct epithelium (TM, 400x).

The mechanical actions induced by the parasites are reflected in massive infestations of the bile ducts, by obstructing the biliary drainage with adult flukes, inducing qualitative modification of the bile content (Sinclair, 1967; Paul, 1987). Cholangitis and chronic cholecystitis are often associated with cholelithiasis (gallstone). Biliary stasis may be induced by mechanical obstruction caused by calculi and flukes. The stasis jaundice is secondary to biliary obstruction (Paul, 1987; Şuteu and Cozma, 2007). Sometimes the gallbladder is enlarged, the content is viscous, blackish in color, and in bile ducts adult flukes are present (Paul, 1982; Baba, 1996; Cătoi, 2003).

Following the liver examination of the 67 bovines taken in this study we observed lesions of biliary calculus in 57%, biliary stasis in 18.5%, calcification of the bile in 62.7%. These lesions were accompanied by the liver fibrosis.

The portal stasis or portal hypertension can be appreciated on base of dilatation of portal and mesenteric vein, the ascites and development of systemic shunts. Porto-systemic shunts acquired are associated with chronic liver lesions as atrophy and fibrosis (Şuteu and Cozma, 2007).

When fasciolosis is associated with other hepatic parasites (i.e. echinococcosis, dicrocoeliasis, cysticercosis) the lesions in the liver parenchyma are more polymorphic. In our study 58% from examined animals, had other parasitic diseases associated with fasciolosis as dicrocoeliasis and echinococcosis.

Histologically, compression atrophy of the hepatocytes and of the lobules, with their fibrosis was found in the liver (Şuteu and Cozma, 2007).

Regeneration of necrotic liver parenchyma (the toxic environment is induced by the metabolic

products of flukes) lead to cirrhosis (Dow et al., 1968).

Our study found predominant appearance of chronic lesions to be of fibrous cholangiohepatitis accompanied by atrophy of hepatocytes adjacent the area of fibrosis (70%), dystrophic calcification, loss of bile duct epithelium accompanied by infiltration with lymphocytes and plasma cell with eosinophils, fibroblasts, macrophages, hepatic fibrosis in porto-portal bridges accompanied by hepatocytes in various stages of necrosis, hyperplasia and metaplasia of biliary epithelial cells.

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