

STUDY OF MORPHOLOGICAL CHANGES IN SHEEP FLUKE

Adrian OLARIU-JURCA¹, Valentin CIULAN¹, Atila GÁL¹, Marius Stelian ILIE¹,
Tijana SUICI¹, Liliana Elena TESLICI¹

¹ Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania” from Timisoara, 119 Calea Aradului, 300645
Timișoara, Romania

Corresponding author email: olariujurca_adrian@yahoo.com

Abstract

The aim of this study is to highlight the macroscopic and especially the microscopic tissular and organic lesions of the sheep bodies in order to establish the histopathological diagnosis of fasciolosis. The study was conducted in the period February 2015-April 2017. Nineteen sheep bodies were necropsied. The sheep were aged from 7 months to 3 years and were from the breeds Turcana and Tigaie. Seven of the cases presented hepatic lesions at the necropsy exam expressed through diffuse hepatitis and interstitial fibrous hepatitis (pseudo-cirrhosis). At the necropsy exam, along with the hepatic lesions we highlighted the presence of *Fasciola Hepatica* in a very high number. The hepatic modification caused by *Fasciola hepatica* in the sheep taken into study are complex both in morphopathological shape and in extent being expressed through circulatory modifications, hypertrophy, dystrophy and lymphohistiocytic, eosinophilic and especially fibrous inflammation. The wide range of morphological modifications of the liver parenchyma, which appear in the pathological process end in liver fibrosis and pseudocirrhosis in sheep with repeated infestations. They always end up in death even though we apply repeated treatments with fasciolides. This is the reason why this disease is considered one of the most serious parasitic diseases for this animal species.

Key words: tissular injury, necropsy, *Fasciola hepatica*.

INTRODUCTION

Sheep fasciolosis is one of the most important parasitic diseases signalled in this species. It is a hepatobiliary helminthosis caused by the trematode *Fasciola hepatica*. The economic losses are translated through high morbidity and high mortality, necessity slaughter and disorders of the reproduction function expressed through irregularities of the estrus cycle and abortions. In our country the disease is caused by *Fasciola hepatica*, a foliaceous trematode, 2-3 cm in length and 0.8-1.3 cm wide. Infections with *Fasciola gigantica* and *Fasciola magna* were reported in other countries. *F. hepatica* adults can be seen free or fixed on the walls of the biliary ducts with the help of their oral sucker. The lungs, spleen, subcutaneous conjunctive, serous or intermuscular tissue are considered erratic locations. In sheep, reinfestation or superinfestations are common and this fact leads to modifications in the liver with the aspect of a mosaic. Traumatic-haemorrhagic hepatitis may be overlapped on cirrhosis or angiocholitis. There may be cases when one can see both young parasites and adults in the

same liver (Cosoroabă et al., 1995; Dărăbuș et al., 2006; Paul, 1990).

The liver is the central laboratory of the organism. It is the headquarters of most fundamental pathological processes- almost every type of dystrophy, circulation disorder or inflammation is observed in this organ (Jubb et al., 1993; McGavin et al., 2001; Paul, 1990). Hepatic lesions in sheep fluke have consequences in the entire organism, ending up in lowered milk, meat and wool production but also through mortality.

MATERIALS AND METHODS

The research was based on the necropsy exams of 19 sheep bodies during the period February 2015-April 2017. The animals were aged from 7 months to 3 years and belonged to the Turcana and Tigaie breeds. Seven of the cases presented hepatic lesions expressed through diffuse and interstitial fibrous hepatitis exteriorized through the presence of white ditches on granular, nodular and lobar surface of the hepatic parenchyma. A creak is also present at sectioning. A large number of *Fasciola* parasites can be seen on the

section surface. Samples of 2.5/1.5 cm fragments of lesioned liver were collected from these bodies. The samples were fixed in formaldehyde solution 10% where they were kept for 24 hours. Modelling followed and other formalin baths in the next two days for a definite fixation. The pieces were then prepared for the paraffin method. In order to accomplish the stages of this method we used the following materials and stains: alcohol 50%, 80% and 96%, absolute alcohol, amyl alcohol, paraffin, thermostat, microtome, slides, medicinal alcohol, egg white and gelatine, benzene, dropper, water, absorbent paper, Canada balm, stand. Blocks with samples (fragments) of organs with lesions were obtained through paraffining. Subsequently, the obtained blocks were cut using a microtome at 6 micrometres, after which 2-4 sections from each block were put on slides. The obtained sections were fixed on clean slides with the help of Mayer albumin. The sections were stained using the trichrome Masson method modified by V. Ciurea with methylene blue for general information and with Giemsa stain for cellular details (Olariu-Jurca, et al., 2015).

The histopathological preparations were examined using an Olympus CX41 microscope (acquired through POS CCE, DICES-MVT 2669-145), with increasing objectives. They were then interpreted and microphotographed.

RESULTS AND DISCUSSIONS

From 19 bodies necropsied in the period February 2015-April 2017, seven cases presented hepatic lesions and *Fasciola hepatica* was observed in a high number. This fact lead us to conduct histological tests in order to confirm the morphopathological diagnosis. The simple presence of *F. hepatica* does not confirm the existence of the disease, which has fatal effects on the patient.

According to the exterior exam of the bodies, two of them were in good condition and five of them were in bad condition, cachectic, yellow apparent mucosae, dry skin, dull and friable wool. Pitting edema was identified in the conjunctive tissue from the cervical and lower abdominal region (Figure 1).

After the interior examination of the abdominal cavity, the cases in good condition showed a

considerable quantity (approximately 0.5 litres) of red liquid with blood clots, serohemorrhagic exudate (serohemorrhagic peritonitis). The five cases in bad condition presented 1.2 litres of citrus coloured liquid, transudate (ascites). Lower quantities of serofibrinous exudate were also present in the thoracic cavity and pericardium.

The macroscopic exam revealed physical and structural modifications in the liver (size, colour, aspect, consistency, etc.). The capsule has an irregular surface, cut by ditches, with white stripes and white nodular growths-atrophic cirrhosis (Figure 2). The lesions were also noticed in other parenchymal organs, aspects secondary to the instalment of fasciolosis.

The liver in two of the cases, was macroscopically enlarged, brown-yellowish colour, the Glisson capsule was thickened, uneven because of the ditches and nodular growths. The same colour and a relevant quantity of young fasciola is present on the section surface (Figure 3). The aspect of the lesions leads to the presumptive diagnosis of interstitial fibrous hepatitis, improperly called cirrhosis. Microscopically, we noticed the most characteristic aspects like thickened hepatic capsule, hyperplasia of precollagen and collagen fibres from the hepatic parenchyma, from the capsule and vascular walls, fibrous perihepatitis and perivascularitis (Figure 4). The instalment of this inflammation is the consequence of the liver's response to the action of the young fasciola that perforate or are implanted in the thickness of the hepatic capsule.

In some microscopic fields in the hepatic parenchyma, we found fibrosed biliary tubules and *F. hepatica* in the lumen and haemorrhagic foci - effect of the destruction of hepatic cords and sinus capillaries through the action of the parasites during migration through the hepatic parenchyma (Figure 5). The haemorrhagic and/or haemorrhagic-necrotic foci in some areas of the liver parenchyma alternate with lympho-histioplasmocytic and eosinophilic infiltrations. These are the expression of the host's first reactions to the destructive, irritating and toxic action of the parasites during migration through the liver. The histologic modifications define, in a

morphopathological plan, the superacute and acute phase of the disease expressed through haemorrhagic and/or haemorrhagic-necrotic hepatitis (Figure 6, Figure 7).

In some hepatic lobes, we could see perivascular and peritubular fibrosis and the presence of the parasite, *F. hepatica*. In the lumen of the biliary tubule, there was perivascularitis and fibrous angiocholitis (Figure 8). In most of the microscopic fields, the hepatocytes presented granulations and optically empty vacuoles in the cytoplasm with or without effects in the nucleus. The biliary tubules had hyperplasia, fibrosis and catarrhal exudate in the lumen while in the portal-biliary space there was perivascular fibrosis, granular hepatosis, angiocholitis and perivascular fibrosis.

In three cases, macroscopically, the liver was increased in volume, of yellow-clay colour, high consistency - fact which is perceived as a squeaking sound at sectioning, which is a consequence of fibrosis and of fibrous inflammation. Thickened biliary tubules were also noticed on section, fibrosis and the presence of a very small number of parasites. These structural modifications suggest the instalment of diffuse, fibrous, parenchymal hepatitis, improperly called cirrhosis.

Microscopically, using objective in increasing order - x10, x20, x40 - we noticed hyperplasia of the reticulin and collagen fibres around the hepatocytes throughout the entire hepatic parenchyma. There was an enhanced development of the conjunctive fibres in the Disse gaps, overlapping of hepatocytes, accompanied by atrophy caused by compression, dystrophy, necrobiosis and hepatic necrosis. These histopathological aspects certify the diagnosis of diffuse fibrous hepatitis and hypertrophic cirrhosis (Figure 8, Figure 9).

We could also notice peri hepatocytic fibroconjunctive hyperplasia in the largest part of the parenchyma, steatosis (small optically

empty vacuoles) and hypertrophy of biliary ducts accompanied by pericanalicular fibrosis - hypertrophic cirrhosis (Figure 10, Figure 11, Figure 12).

Some researchers consider that hepatic fibrosis caused by *Fasciola*, as well as hyperplasia of the biliary ducts are the effect of the parasite's proline secretion (Jubb et al., 1993; McGavin, et al., 2001; Paul, 1990). Without denying this possibility, most researchers consider however that the hyperplasia phenomena are the expression of numerous factors induced by parasites including mechanic irritations and their antigens (Cosoroabă et al., 1995; Dărbuş et al., 2006; Dulceanu et al., 1994; Şuteu et al., 2007).

In two cases, the liver was macroscopically decreased in volume, with an irregular surface of yellow-greenish colour of different shades and high consistency. There was a squeaky sound on section-atrophic cirrhosis. Microscopically, we noticed prominent intralobular fibrosis, which divided the lobes into pseudo-lobes, groups of cords without centrilobular veins or with veins in exocentric location (Figure 13, Figure 14). In contact with the hepatic cords, especially around the portal gap we noticed leukocytic infiltrations, predominantly eosinophilic, on a fibrosis background. We also noticed new blood vessels and biliary tubules, aspects, which suggest the instalment of the active phase of the inflammatory process-aggressive cirrhosis (Figure 15).

The microscopic and macroscopic aspects highlighted with the help of colour photographs, which closely show the morphogenesis of the patomorphic aspects in the liver, in different evolution phases in sheep fluke with reflection in the entire organism. The hepatic lesions identified along the research correspond to those described in the specialty literature (Paul, 1990; Cosoroabă et al., 1995; Dărbuş et al., 2006; Dulceanu et al., 1994; Şuteu et al., 2007).



Figure 1. Sheep corpses with fasciolosis.

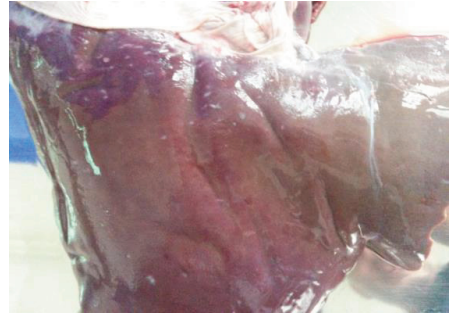


Figure 2. Sheep liver - atrophic, granular and lobar cirrhosis.



Figure 3. Sheep liver - highlighting on the section surface an appreciable amount of young fasciola.

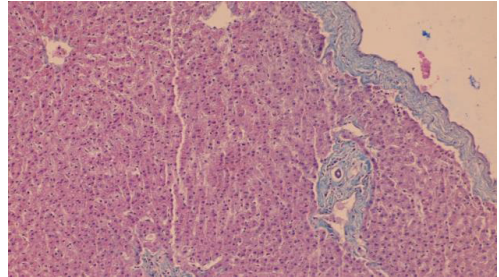


Figure 4. Fibrous perihepatitis and perivasculitis: hyperplasia of precollagen and collagen fibres from the capsule structure and vascular walls. Col. HEA x 40.

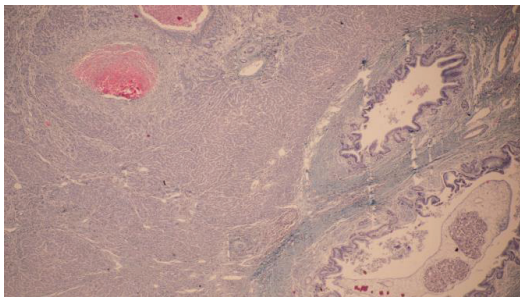


Figure 5. Hemorrhagic hepatitis in outbreaks, hemorrhagic areas, perifocal inflammatory cell infiltration and catarrhal angiocholitis with the presence of *F. hepatica* in the lumen. Col. HEA x20.

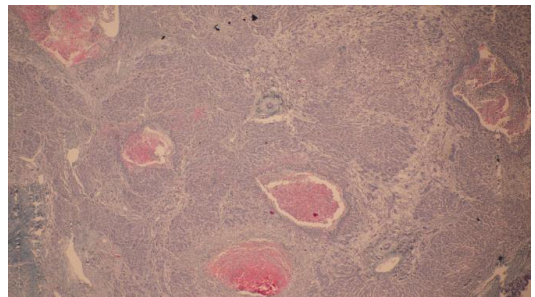


Figure 6. Hemorrhagic hepatitis in outbreaks: hemorrhagic areas and inflammatory cell infiltration. Col. HEA x 10.

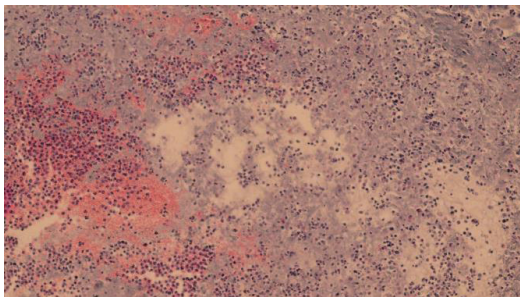


Figure 7. Hemorrhagic hepatitis in outbreaks: highlighting inflammatory cellular infiltrations: lymphocytes, histiocytes and numerous eosinophils. Col. HEA x 40.

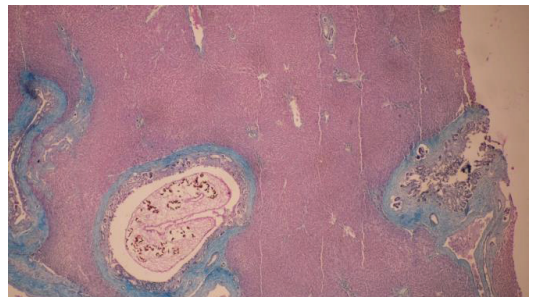


Figure 8. Perivasculitis, fibrous angiocholitis and *F. hepatica* in the lumen of bile canaliculi. Col. HEA x 20.

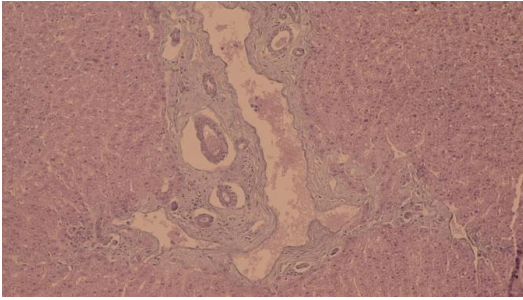


Figure 9. Granular hepatosis, angiocolitis and perivascular fibrosis. Col. HEA x10.

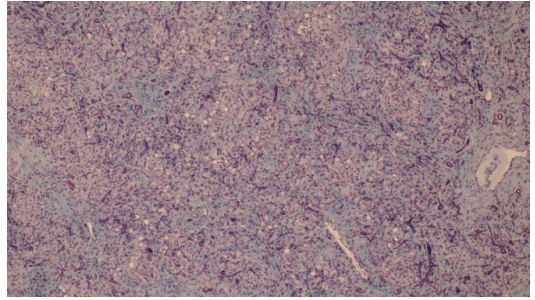


Figure 10. Hypertrophic cirrhosis: intraparenchymal fibrosis (overview). Col. HEA x10.

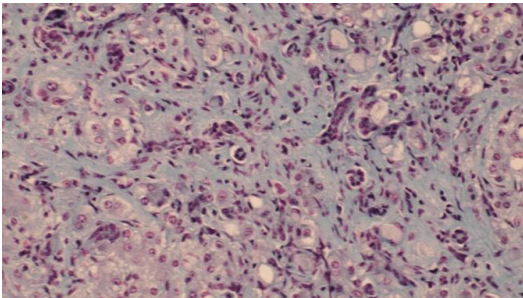


Figure 11. Hypertrophic cirrhosis: diffuse peri hepatocytic fibrosis (detail). Col. HEA x 20.

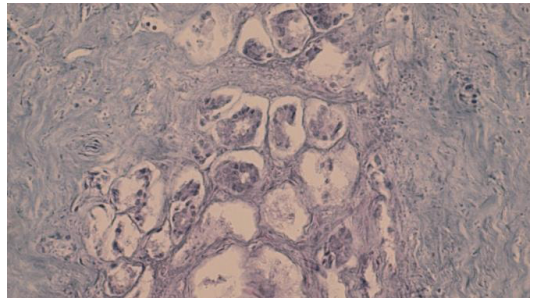


Figure 12. Hypertrophic cirrhosis: hyperplasia of the bile ducts and pericanalicular fibrosis (detail). Col. HEA x 40

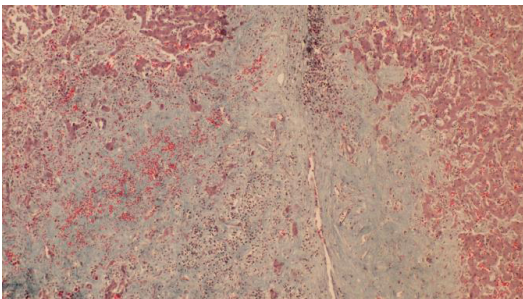


Figure 13. Atrophic cirrhosis: Perilobular massive fibrosis and leukocytic infiltration predominantly eosinophilic. Col. HEA x 20.

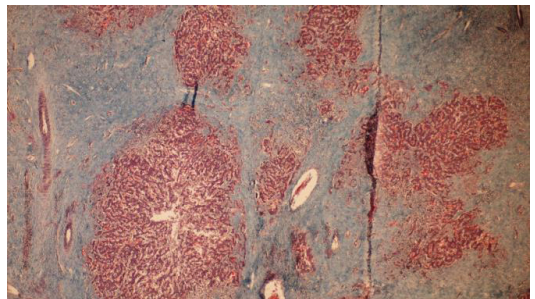


Figure 14. Atrophic cirrhosis: dividing lobules in pseudo-lobes thru fibrosis. Col. HEA x 20.

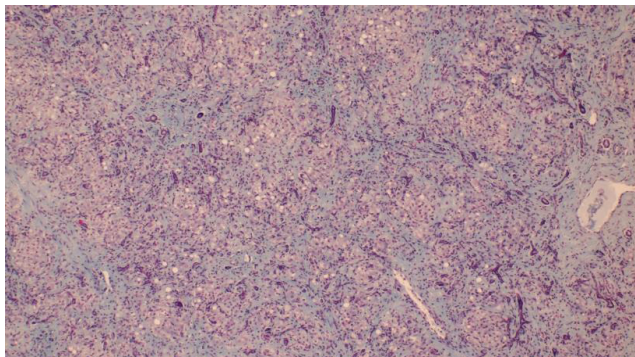


Figure 15. Aggressive cirrhosis: lympho--histo-plasmocytic infiltrations on the background of steatosis and fibrosis. Col. HEA x 10.

CONCLUSIONS

The hepatic modifications produced by *Fasciola hepatica*, in the sheep taken into study are complex both in form and in extent, expressed through circulatory modifications, hypertrophy, dystrophy, lymphohistiocytic inflammation and fibrous inflammation (cirrhosis).

Fibrous hepatitis in foci and diffuse-improperly called cirrhosis is the consequence of the mechanical, irritative and toxic actions of the parasite, continuous and of different intensities, produced by *F. hepatica* in case of periodic reinfestation.

The large range of morphological modifications in the hepatic parenchyma, which appear during the pathological process are finalised through hepatic fibrosis, pseudo-cirrhosis in repeatedly reinfested sheep that always end up with death, in spite of anti-fasciola treatments.

In the same case, in the hepatic parenchyma we noticed circulatory modifications, metabolic modifications-hypertrophy, dystrophy and fibrosis of different intensities due to periodic reinfestations which allow a succession of the evolution forms of the disease (superacute, acute, subacute and chronic) expressed in dynamics through haemorrhagic and/or necrotic-haemorrhagic hepatitis, atrophic cirrhosis and hypertrophic cirrhosis.

The identification in a singular case of a superacute, manifestation form of fasciolosis -

haemorrhagic and/or haemorrhagic-necrotic hepatitis, or of subacute or chronic-fibrous hepatitis or pseudocirrhosis is arbitrary, possible only in experimental fasciolosis where reinfestation and a diverse biocenosis for intermediary hosts are absent.

The diverse patmorphic syndrome installed in sheep fasciolosis ends in most cases with death, fact which makes this disease one of the worst parasitic diseases for this species.

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