ABSTRACT

Keywords: liver; cells: stellate, oval, "pit", sinusoidal endothelial, Kupffer; cholangiocytes, Paneth, goblet; bile duct glands; lambs (*Ovis aries*), cows (*Bos taurus*), mice (*Mus musculus*).

Thesis objectives

The liver, among other organs of the animal body, is part of the vital organs without which man or animals can not survive. The importance of the liver in maintaining body homeostasis lies in the multitude of functions it performs.

The functional hepatic complex is the result of collaboration of all constituent cells and in pathological conditions cells interrelate through a cascade of molecular signals with protective (antimicrobial), tissue repair and regeneration purposes of the affected territories.

The domestic animal liver is an important source of protein for humans, confiscating it on the slaughter line leading to significant economic losses.

This research focused on tissue and cell reaction in some liver parasitosis in sheep and cows. A particular interest was paid to special stains in order to identify and confirm the presence of bile duct glands and of oxyphilic granules cytoplasmic cells attached to the bile duct epithelium, which were observed among cholangiocytes in both studied species. Quantifying the area and perimeter of some hepatocytes and oxyphilic granules cytoplasmic cells attached to the bile duct epithelium, as well as statistically interpretating the data represented two of the thesis objectives. Another objective of this research aimed at tracking liver structure cells' reactions in experimental influenza virus infection in mice.

Thesis structure - Doctoral thesis "Studies on the cellular reactivity in hepatopathies of domestic mammals" is structured according to the law in two main parts.

Part I – „Bibliographic study", contains **three chapters** extended to 37 pages, representing 14,86 % of the thesis.

Chapter I - "Morphological, structural and functional aspects of liver parenchymal cells." This chapter mentions literature data related to morphological, structural and functional characteristics of hepatocytes, cholangiocytes and oval cells. It contains a review of identification, through special staining, and immunoblotting methods for specific cell types.

Chapter II - "Morphological, structural and functional aspects of non-parenchymal liver cells"

Chapter II focuses on non-parenchymal liver cells, represented by sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, liver resident NK-cells ("pit") and dendritic cells. Therefore the main morphological, structural, functional and networking features of the cells were synthesized and presented. As in the first chapter, cell specific coloring and/or immunostaining methods were mentioned.

Chapter III - "Bile duct glands". This chapter classifies the bile ducts glands depending on location and morphological aspects, specifies their distribution in relation to the biliary tree. Their role and functions are mentioned, with the review of several pathological conditions that lead to structural and functional alterations. Also, types of monoclonal antibodies used for marking the cells of the bile duct glands are signaled.

Part II - "Personal research" consists of **four chapters** (Chapters IV-VII) having the following structure: introduction and research purposes; material and methods; results, their interpretation and correlations to reported literature data and general conclusions.

The personal research consists of 212 pages, and represents 85,14 % of the thesis.

The thesis contains 263 figures, of which 236 are original pictures and 27 are diagrams or graphics. The total number of tables found in this research is 34.

The purpose of the research had several goals, whose brief presentation will be given in the description of each chapter.

Materials and methods

The study materials of this paper are represented by 108 samples of lamb liver, 66 samples of cow liver and 20 samples of mouse liver (each mouse liver histopathological sample contains 4 to 8 sections, a total of 109 sections being investigated). The histological sample examination was performed using Olympus BX41 optical microscope, equipped with a digital camera (Olympus U-TV1X-2). Capturing and processing of the images was performed using Olympus Cell ^ B software.

The methods used to achieve the objectives and complete the research were:

1. Routine staining methods, used in histopathology: HE and HEA;

2. Special staining methods: PAS, Alcian Blue 2,5 pH, Toluidine Blue, Floxin-Tartrazine (Chapter VI).

3. Immunolabeling with monoclonal antibodies (anti-actin PaB, anti-CD8 and anti-bcl-2) (Chapter IV)

4. Morphometric analysis:

- Sinusoidal capillaries associated cell count (Chapter IV) and porto-biliary areas inflammatory cells count (Chapter VII);

- Determination of area and perimeter of hepatocytes (chapter V) and of cytoplasmic oxyphilic granules cells associated to the bile ducts (Chapter VI).

5. Statistical analysis:

- Tests used: Skewness, Kurtosis, Royston $\chi 2$, Shapiro-Wilk, Shapiro-Francia W' for checking normality and non-parametric Mann-Whitney U test to determine differences between the two species of domestic mammals (Chapters V and VI).

- Parametric tests used: ANOVA and t-Student's test (Chapter VII).

Chapter IV - "The involvement degree of the portal tract and cellular reactions in larval migration hepatitis in lambs (*Ovis Aries*)"

The main objectives of this chapter's study were to determine the involvement of the portal tract within the lesions diagnosed through the liver parenchyma, and also to confirm or infirm the identified lesions by exclusively examining the liver fragment of the gallbladder area. The second objective was to assess the number of capillaries associated sinusoid cells (CASC) and establish a correlation between the number of cells associated to sinusoidal capillaries in the immediate proximity of the lesion and distant from the lesion. Also a possible association between the cell number and the evolutionary stage (acute, subacute and chronic) of the disease was investigated, together with the marking of the cell number variations between batches of paired samples.

The studies in this chapter were materialized by quantifying structural alterations of larval migration hepatitis of lambs with their reporting percentage. Pathological changes were consistent with those reported in the literature.

The study of the involvement of the portal tract in faithfully reflecting the liver pathology showed the accurate mirroring of the parasitary migratory stage (exudative and fibrosis changes) and liver reactivity specific phenomena in case of tissue destructions (bile duct hyperplasia).

The possibility of using the liver fragment attached to the gallbladder (which is confiscated at the time of its detachment on the slaughter line) for monitoring liver injury was confirmed by largely reflecting the histopathological changes that occur in parasitic larval migration hepatitis. The results support the hypothesis of using the liver fragment that is attached to the gall bladder in epidemiologically surveillance of slaughtered animals without causing further economic losses.

The reaction of parenchymal cells and of some non-parenchymal cells (Kupffer, hepatic stellate, "pit" and oval) of liver structure were consistent with the traumatic lesions caused by larval migration through the liver.

By applying some immunohistochemical methods for marking certain cells (stellate, oval and "pit"), only activated hepatic stellate cells were identified. Their location was within the

Disse space and showed quite obvious characteristic cytoplasmic extensions. Their limited number was consistent with the liver lesions in which no pericellular or sinusoidal capillaries fibrosis were observed. Regarding oval and "pit" cells whose presence was sought to be highlighted by immunohistochemistry, there were no positive results in this study.

Quantifying the number of inflammatory cells associated to sinusoidal capillaries did not offer noticeable information due to uniformity of lesion classification, predominantly represented by subacute/chronic evolutionary type.

Chapter V - "Morphological characteristics of cellular reaction in some parasitic hepatopathies in cows (*Bos taurus*)."

Chapter V research focused on lesion diagnosis, evaluation of the major histopathological changes and observing the reaction of different cells in the liver of studied cows. Comparative study of the changes observed in liver lobule with the ones observed within the portal tract was another objective of the research in this chapter. The third objective is the morphometric study (area and perimeter) of hepatocytes and finding a correlation between hepatocyte size, weight status of slaughtered cows and the values of some biochemical parameters.

Histopathological study conducted on cow liver established that the hepatic lesions were caused by trematodes, with bile ducts localization inducing lesions such as chronic cholangiohepatitis, and also by the larval form of *Echinococcus granulosus* which led to outbreaks of multifocal granulomatous hepatitis. Cell reaction, represented by the involvement of parenchymal cells (hepatocytes, cholangiocytes), of some non-parenchymal cells (Kupffer cells, hepatic stellate cells) and inflammatory cells was quantified.

From the results it is clear that hepatocytes were affected (to varying degrees) in all the investigated samples; eosinophilic inflammatory infiltrate was found in higher percentage within the portal tract than intralobulary, and the inflammatory infiltrate without eosinophils was better represented intralobulary than within the portal tract. Mechanical and toxic lesions exercised by the trematodes over the portal tract caused necrosis and cholangiocytes hyperplasia in all cases, in response to changes induced by parasites.

Another parameter that was constantly present in the studied cases was the portal tract fibrosis, induced by the activation of hepatic stellate cells and fibrocytes. Massive accumulation of connective tissue within the portal tract was manifested in different aspects, the most common being the coat-sleeve-like pattern around the bile ducts and in many cases fibrosis expanded, having the tendency to form pseudolobules.

Measuring areas and perimeters of hepatocytes with normal/with morphological changes without being pathological and of dystrophic ones and their comparison between the three groups formed (depending on the health status) along with statistical interpretation allowed the

observation that there are significant differences between the two categories of hepatocytes regardless of the health state. Comparing normal/with morphological changes without pathological implications hepatocytes area of the three groups, it was noticed that in cows with a poor health condition this parameter tends to be higher than in the other two groups. Confronting the average values of the dystrophic hepatocytes areas shows that the differences are reduced and there is no statistically significant difference between groups. No correlations between dosed biochemical parameters and area/perimeter of normal/with morphological changes without pathological implications hepatocytes or the dystrophic ones could be made.

Chapter VI - "Bile duct glands and oxyphilic granule cells associated to the bile ducts epithelium in lambs and cows"

The research of this chapter has focused on the morphology of the bile ducts pursuing two main objectives. First is to reveal and characterize the bile duct glands of sheep and cattle liver, and the second aim was centered on investigating and characterizing oxyphilic granules cytoplasmic cells associated to the bile ducts epithelium.

The research results indicated the presence of unicellular intraepithelial glands (goblet cells) in the various sizes bile ducts from the livers of both studied species. Confirming the existence of these structures was carried out by special stains. Regarding the morphological appearance, lambs liver goblet cells showed the same characteristics as the goblet cells in the intestine and respiratory mucosa (goblet shaped), unlike those seen in cow livers, which had columnar shape.

Extramural bile duct glands were found in only one case for each studied species.

In the liver samples originating from cows, in which the lesions were chronic, a marked intramural glandular cell hyperplasia, and glandular hyperplasia and metaplasia of the bile ducts were observed.

Morphometric analysis of the area of the oxyphilic granules associated with bile ducts epithelium of lamb and cattle liver, as well as statistical analysis indicate that in cows their dimensions are larger than in lambs.

Special staining and morphological aspects of oxyphilic granules cytoplasmic cells, located mainly among cholangiocytes allowed the hypothesis that there is a cellular heterogeneity and consequently the cells have different origins, belonging to several cell populations. Some are Mott cells and were found in the bile ducts, in portal tract but also within some necrotic foci. The second identified cell type were the mast cells, which also have been seen at the base of the bile ducts epithelium but also within the abundantly fibrotic portal tract, inserted into connective tissue. Based on morphological appearance and tinctorial reaction to PAS, Alcian Blue 2,5 pH and Lendrum's Phloxine-Tartrazine stainings the idea of a third cell

type being present, Paneth cells, located exclusively among cholangiocytes, is launched. In addition, using Lendrum's Phloxine-Tartrazine staining, intra- and extraepithelial mast cells were found.

Chapter VII – "Liver cellular and tissular reactions in experimental influenza virus infection in mice (*Mus musculus*)"

The objectives of this study consisted primarily of identifying and quantifying the histopathological changes that occur in the liver of mice experimentally infected with influenza virus strain H_1N_1 (PR8 10^{-1} and PR8 10^{-3}), then comparing the results with data obtained in the control group. A second important objective was to assess the number of inflammatory cells associated to the portal tract and group comparison of inflammatory reactivity in this territory.

The research results of Chapter VII on cell reactivity to influenza infection in liver structure reveals dystrophic changes in hepatocytes. These dystrophies (hydroprotidic/steatosis) are inconsistently observed within groups in which influenza virus was administered, the number being higher in the lethal infection group. Regarding the time the dystrophic processes occur, the data obtained in our study are consistent with the literature, meaning that the onset and installation occurs very quickly after influenza infection and are visible mainly in the first days post-infection. Another type of hepatocyte reaction is noted by the presence of hepatocyte necrosis within the livers of mice, most foci of necrosis being seen in the group with a mild flu. Since necrosis also occurred in the control group this could be interpreted as being produced by other factors than experimental infection. The cause of the outbreaks of hepatic necrosis in mice with influenza virus (FGG and FUG 10-1 10-3 lots) is uncertain. The presence of these lesions can't be made only on account of direct or indirect harmful action of the strain used in the experiment.

The inflammatory process is poorly expressed in the liver of mice with influenza virus infection (influenza virus A PR8 / H_1N_1). There are numerical and statistical differences of the inflammatory infiltrate of the portal tract between group FGG 10-1 (severe form of influenza) and group FUG 10-3 (mild form of influenza) and between FGG group 10-1 and control group. In the group of mice that were induced lethal infection (FGG 10-1), the average number of inflammatory cells / group is almost double comparing to the control group and quite large compared to the group with mild flu (FUG 10-3). The difference in the average number of inflammatory cells within portal tract is not significant between the control group and the FUG 10-3 group, being slightly higher in the group with mild influenza.

The bibliography contains 305 references from which 11 belong to some Romanian authors and 294 belong to foreign authors. From the total number of sources, 241are published after the year 2000 and from these 135 are newer than 2010 (6 titles were published in 2016).