Pathomorphological evaluation of hepatobiliary lesions in dogs and cats of Ankara region

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ABSTRACT

The objective of this study was to investigate pathological disorders of the hepatobiliary system in dogs and cats in Ankara using pathomorphological methods, and to determine the types and frequency of the observed lesions. Furthermore, we aimed to evaluate hepatic reparation as a reaction of the liver to injury with different hepatobiliary lesions using immunohistochemical methods. Livers obtained from 56 cats and 74 dogs submitted for post-mortem investigation were examined macroscopically and microscopically. Samples with hepatic fibrosis were stained immunohistochemically with an α-SMA antibody. Lesions were found in 98% of the livers of the examined dogs and cats. The most common histopathological diagnoses were hepatitis (39.28%), hepatocellular lipidosis (16.07%), and cholangitis/cholangiohepatitis (14.28%) in cats. In dogs they were hepatitis (28.38%), passive congestion (25.68%) and proliferative lesions (21.62%). For some hepatobiliary lesions, breed, age and gender predispositions were observed. Immunohistochemically, the α-SMA antibody positively stained parenchymal, portal and septal myofibroblasts. A positive correlation was verified between immunohistochemical α-SMA scores and histochemical fibrosis scores. This is the first study in Turkey documenting both the incidence of hepatobiliary lesions among feline and canine species, and their pathomorphological features. In terms of reparation, the major role of the hepatic myofibroblasts in liver fibrosis was observed. There were variations in the intensity and location of positively stained cells according to the type of lesion. The conclusion of this research indicates the need to pay attention to certain hepatic lesions in dogs and cats, and provides a reference standard for further clinical and histopathological studies.

Key words: cat; dog; hepatobiliary system; immunohistochemistry; pathomorphology

Introduction

Hepatobiliary diseases, commonly reported in dogs and cats, are of great importance due to the dependence of most organs on the metabolic function of the liver (BROWN et al. 2017). Canine and feline liver diseases are frequently underdiagnosed. Although ultrasonographic and biochemical methods are used in the diagnosis, the final diagnostic method remains histopathological (ROTHUIZEN et al. 2006). Despite the retrospective histopathological surveys done in the United Kingdom (BAYTON et al. 2018), United States (WANG et al. 2004) and Japan (HIROSE et al. 2014) on canine and feline hepatobiliary diseases, no similar data reporting the frequency of these diseases have been published in Turkey.

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The World Small Animal Veterinary Association (WSAVA) Liver Standardization Group recommended criteria for histopathological diagnosis of canine and feline hepatobiliary diseases to ensure uniformity of terminology (ROTHUIZEN et al. 2006). Since then, retrospective studies on canine and feline hepatobiliary diseases have been using these criteria as a guide in diagnosis. Liver diseases were classified in general terms into vascular, biliary, parenchymal and neoplastic disorders (ROTHUIZEN et al. 2006).

The liver is an organ with high resistance and regeneration abilities. Following injury, it initiates tissue repair with two balanced reactions, regeneration and reparation, to regain physiological function (BROWN et al. 2017, CULLEN and STALKER 2016). Although the exact mechanism of hepatic fibrosis is not fully known, myofibroblasts are thought to play a major role in its pathogenesis. The best known hepatic myofibroblasts are stellate cells that share the properties of smooth muscle cells. However, there are other important groups of myofibroblasts, such as portal / septal myofibroblasts, bone marrow-derived cells, and hepatocytes, capable of acquiring a mesenchymal phenotype by epithelial-mesenchymal transition (IJZER et al. 2006, SCHOTANUS et al. 2009).

The aims of this study were to: (1) conduct a comparative histopathological evaluation of hepatobiliary lesions in dogs and cats in Turkey (Ankara region); (2) describe the macroscopical and microscopical findings of these lesions; (3) evaluate hepatic fibrosis in different hepatobiliary lesions using histochemical and immunohistochemical methods; (4) investigate the immunohistochemical properties of hepatic myofibroblasts, as well as their role in hepatic fibrosis, if any; and (5) compare the variations of the intensity and localization of these cells according to the type of disease.

Materials and methods

Liver samples were obtained from dogs and cats submitted to the Pathology Department of Ankara University, Faculty of Veterinary Medicine for routine post-mortem examination between April 2017 and September 2018 (ethical approval protocol no-2016-23-197 by Ethical Committee of Ankara University). Fifty-six cats and seventy-four dogs were included. The species, breed, gender and age of the animals were noted. After macroscopical examination of the liver, 2-3 mm thick samples were taken from all lobes. Portal lymph nodes showing lesions were also sampled.

Samples were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin. Sections (4 μm) were routinely stained with Harris's haematoxylin and eosin (H&E). Histopathological evaluation was based on the WSAVA histological criteria. For further characterization of the lesions, special histochemical stains such as Periodic Acid-Schiff (PAS), AFIP method for lipofuscin, Oil red staining, Sudan Black staining and Perl's Prussian Blue staining methods were used. Additionally, liver samples with hepatic fibrosis (18 dogs and 12 cats) were stained with Masson's trichrome stain and scored semi-quantitively using a modified staging system based on the staging systems proposed by GOODMAN (2007), ISHAK et al. (1995) and VINCE et al. (2016) (Table 1). Following this, the same slides were immunohistochemically stained with α-SMA antibody (Thermo Fisher clone 1A4, ready-to-use) using the avidin-biotin complex peroxidase method.

For immunohistochemistry, 4 μm sections were mounted on charged glass slides, dewaxed and rehydrated with xylol and graded alcohols. Heat-induced antigen retrieval was performed: sections were incubated in citrate buffer for 15 minutes in a microwave oven followed by cooling for 15 min at room temperature. Endogenous peroxidase activity was blocked in 3% H_2O_2 (15 min), and unspecific background staining was omitted with blocking serum (35 min). Egg white and milk powder were used to block endogenous biotin (KUTLU and ALCIGIR 2019, MILLER 2001). Sections were incubated with the primary antibody for 1 h at 37 ºC. The biotin-streptavidin based detection system (Ultravision Quanto Detection System HRP, mouse and rabbit IgG, Thermo scientific) was used for secondary labelling (35 min each), and the reaction was visualized with aminoethyl carbazole substrate. Sections were counterstained with Gill's haematoxylin. Positive controls consisted of normal canine and feline liver sections. Negative controls were performed by replacing the antibody with PBS.

In this research, we used the level α = 0.05 for the Pearson Chi-Square statistics to evaluate the independence between fibrosis and α -SMA staining scores when using crosstabulation (also known as a bivariate table). Crosstabulation offers simultaneous distributions of two categorical variables. The Independence Test evaluates whether there is a relationship between the two variables by comparing the observed pattern in the classes with the pattern to be expected if the variables are truly independent from each other.

Table 1. Scoring system for hepatic fibrosis

Stage	Fibrosis
0	No fibrosis
1	Fibrous expansion of most portal tracts, with or without short fibrous septa
2	Fibrous expansion of most portal tracts with bridging (portal portal) septa
3	Marked bridging (portal-portal or portal-central) with architectural distortion
4	Lobular dissection (generalized isolation of hepatocytes into small clusters) or cirrhosis

Results

Of the 74 canine liver samples in this study, various lesions were detected in 73 samples. The three most frequent canine liver histopathological diagnoses were hepatitis (28.38%), passive congestion (27.03%) and proliferative lesions (21.62%). Out of the 56 feline liver samples, lesions were detected in 55 samples. The three feline hepatic histopathological diagnoses encountered most were hepatitis (39.28%), hepatocellular lipidosis (16.07%) and cholangitis/cholangiohepatitis (14.28%). Detailed results of the various encountered hepatic pathological conditions and their frequencies are summarized in Tables 2 and 3.

Non-proliferative liver lesions. Hepatitis, which was the most frequent liver lesion in both dogs and cats, accounted for 28.38% and 39.28% of all canine and feline diagnoses, respectively. The subclassification frequencies of hepatitis cases are detailed in Tables 2 and 3. Histologically, fibrosis was present in all cases of chronic hepatitis. Acute hepatitis cases, with severe necrosis and regeneration, were diagnosed as fulminant hepatitis.

Cirrhosis was detected in four of the 74 dogs. One of the cases that showed excessive shrinking and histologically extensive replacement of the hepatic parenchyma with fibrous and adipose tissue, was diagnosed as atrophic cirrhosis (Fig. 1).

The histological features of non-specific reactive hepatitis were mainly portal inflammatory infiltrations composed of lymphocytes, plasma cells and/or neutrophil leucocytes without hepatic necrosis.

In the examination of all the hepatitis cases, a median age of 10 years old for canine chronic hepatitis and cirrhosis was noticed. Females (7 cases, 63.63%) were more susceptible than males, and the breeds that might be at more risk were German Shepherd (2/11 cases), Golden Retriever (2/11 cases) and crossbreed dogs (2/11 cases). Non-specific reactive hepatitis was found in cats with a median age of 4 months. British Shorthair and Tabby cats were more susceptible (3/6 - 2/6 cases), but no gender predisposition was seen.

Cholangitis/cholangiohepatitis was the third most common diagnosis in cats accounting for 14.28% of all the feline cases. All cholangitis/cholangiohepatitis cases were diagnosed as lymphocytic. Cases confirmed with lymphocytic cholangitis/cholangiohepatitis mostly had a focal lymphocytic infiltration surrounding the bile ducts (Fig. 2A). Two cases, where the lymphocytic infiltration was restricted to the portal tracts, were diagnosed as lymphocytic cholangitis. Six cases, in which the portal lymphocytes infiltrated the adjacent parenchyma as well, were diagnosed as lymphocytic cholangiohepatitis.
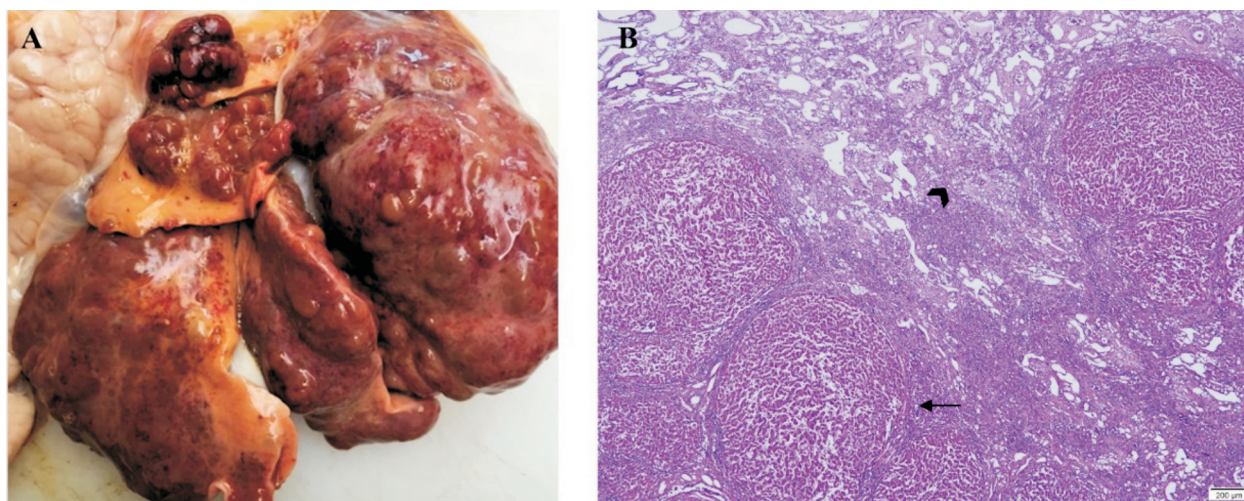
Fig. 1. A - Atrophic cirrhosis, Dog. B - Pseudolobules (arrow) surrounded by fibrous strands and adipose tissue (arrowhead) replacing the normal parenchyma, H&E.

Biliary duct proliferation and peribiliary portal fibrosis were found in most of the cases. In dogs, one case of cholangitis was diagnosed, and it was classified as destructive cholangitis. Histologically, destruction of the bile ducts, peribiliary infiltration of neutrophil leucocytes, lymphocytes and pigment laden macrophages, in addition to cholestasis, were noticed (Fig. 2, B).

Table 2. Distribution of feline hepatobiliary diseases

Histopathological diagnosis	Number of cases	Percentage of total cases
Developmental disorders	**1**	**1.79%**
Congenital polycystic disease	1	1.79%
Hepatic displacements	2	3.57%
Vacuolar hepatopathy	**9**	**16.07%**
Hepatocellular steatosis	9	16.07%
Cholestasis (jaundice)	1	1.79%
Necrosis	1	1.79%
Autolysis	1	1.79%
Circulatory disorders	**6**	**10.71%**
Passive congestion	6	10.71%
Hepatitis	**22**	**39.28%**
Acute hepatitis	5	8.93%
Subacute hepatitis	9	16.07%
Chronic hepatitis	2	3.57%
Non-specific reactive hepatitis	6	10.71%
Cholangitis/Cholangiohepatitis	**8**	**14.28%**
Lymphocytic cholangitis/cholangiohepatitis	8	14.28%
Proliferative lesions	**4**	**7.14%**
Hepatocellular adenoma	1	1.79%
Metastatic lymphoma	3	5.35%
No abnormality	1	1.79%

* percentage of total cases: the number of cases of a diagnosis/the total number of cases ×100

Lymphocytic cholangitis/cholangiohepatitis was diagnosed more frequently in Crossbreed and Tabby cats, with a median age of 2 years 5 months old, and no gender predisposition was observed.

Vacuolar hepatopathy was diagnosed in 7 canine livers (9.46%), and 9 feline cases (16.07%). All feline vacuolar hepatopathy cases were diagnosed as hepatocellular steatosis while those in the canine species were relatively evenly distributed between hepatocellular steatosis, hepatocellular swelling

and hepatic glycogenosis. Grossly, the examined livers with mild hepatocellular steatosis showed an increase in size and a prominent lobular pattern while livers with severe hepatocellular steatosis were yellow and fragile in consistency (Fig. 3, A). On the microscopical level, clear lipid vacuoles were seen within the cytoplasm of the hepatocytes. The remnants of lipid vacuoles were positively stained with special dyes, such as Oil red staining and Sudan Black staining.

Table 3. Distribution of canine hepatobiliary diseases

Histopathological diagnosis	Number of cases	Percentage of total cases
Hepatic displacements	2	2.70%
Vacuolar hepatopathy	**7**	**9.46%**
Hepatocellular steatosis	3	4.06%
Hepatocellular swelling	2	2.70%
Hepatic glycogenosis	2	2.70%
Necrosis	2	2.70%
Autolysis	4	5.41%
Circulatory disorders	**20**	**27.03%**
Passive congestion	19	25.68%
Peliosis hepatis (Telangiectasis)	1	1.35%
Hepatitis	**21**	**28.38%**
Acute hepatitis	1	1.35%
Subacute hepatitis	7	9.46%
Chronic hepatitis	8	10.81%
Cirrhosis	3	4.06%
Non-specific reactive hepatitis	2	2.70%
Cholangitis/Cholangiohepatitis	**1**	**1.35%**
Destructive cholangitis	1	1.35%
Proliferative lesions	**16**	**21.62%**
Hepatocellular adenoma and nodular hyperplasia	1	1.35%
Hepatocellular carcinoma	2	2.70%
Cholangiocellular carcinoma	2	2.70%
Combined hepatocellular and cholangiocellular carcinoma	1	1.35%
Primary hemangiosarcoma	1	1.35%
Metastatic lymphoma	3	4.06%
Metastatic histiocytoma	1	1.35%
Metastatic histiocytic sarcoma	1	1.35%
Metastatic giant cell fibrosarcoma	1	1.35%
Metastatic malignant myeloid tumor	1	1.35%
Metastatic multiple myeloma	1	1.35%
Metastatic malign melanoma	1	1.35%
No abnormality	1	1.35%

* percentage of total cases: the number of cases of a diagnosis/the total number of cases × 100.
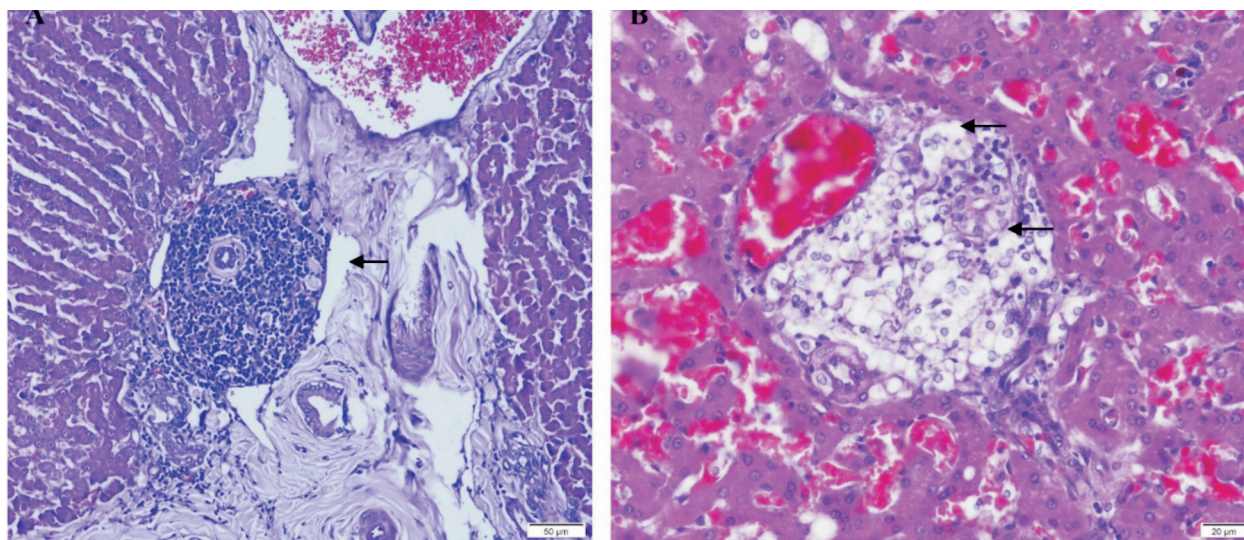
Fig. 2. Cholangitis. A - Lymphocytic cholangitis, Focal accumulation of lymphocytes around the bile duct (arrow), cat, H&E. B - Destructive cholangitis, degenerated bile ducts (arrows), dog, H&E.
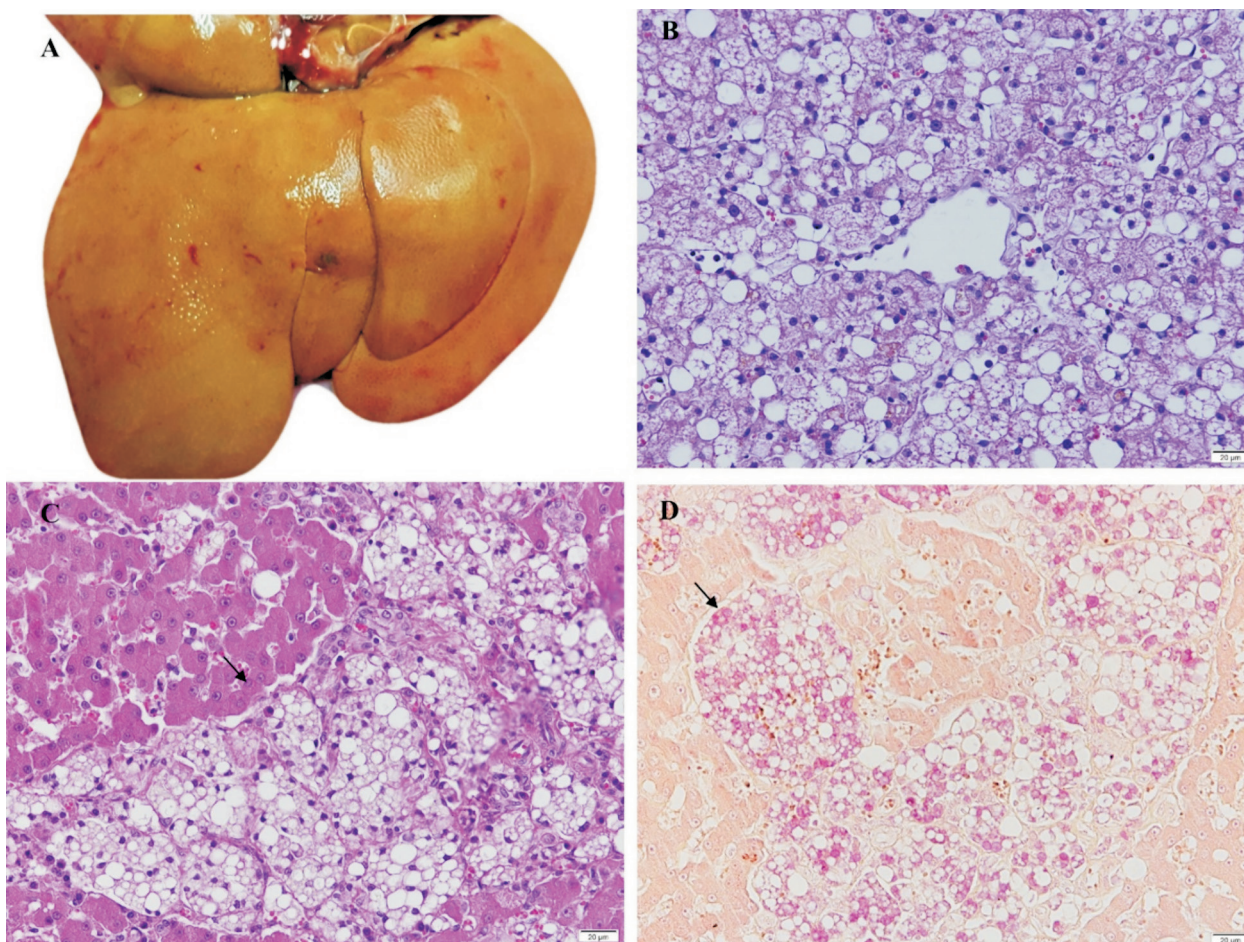

Fig. 3. Severe hepatocellular steatosis, cat. A - Yellow discoloration of the liver. B - Severe microvesicular and macrovesicular lipid vacuoles, H&E. C - Lipogranuloma (arrow), H&E. D - Positive staining of ceroid laden macrophages with AFIP staining method (arrow).
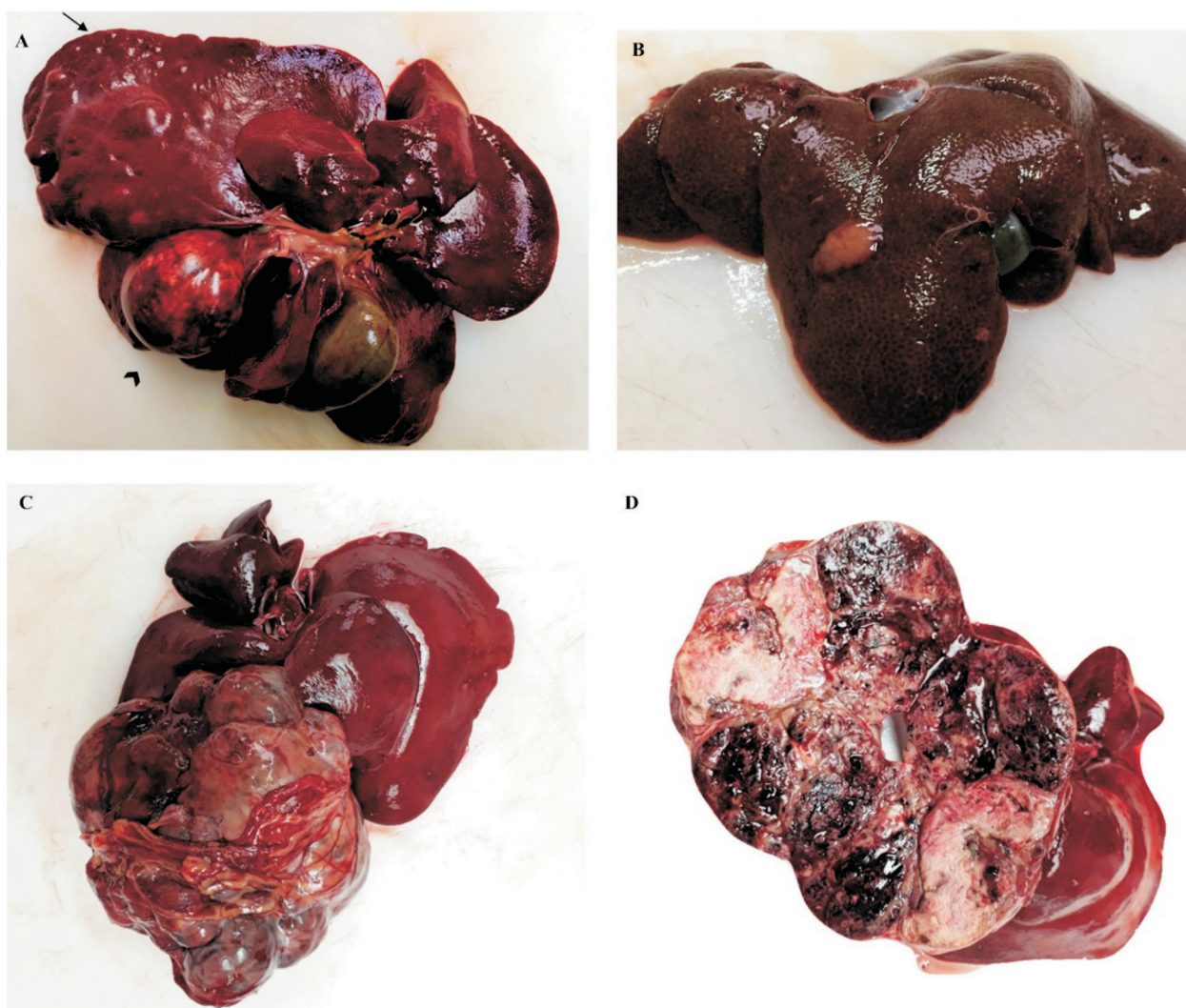
Fig. 4. Gross appearance of hepatocellular tumours. A - Multiple nodules of nodular hyperplasia bulging from the capsular surface of the liver (arrow) and a single white nodule of a hepatocellular adenoma (arrowhead), dog. B - A single white nodule of hepatocellular adenoma raised from the surface. C, D - Hepatocellular carcinoma involving the entire lobe with a multilobulated mottled cut surface, dog.

Cases were classified as either microvesicular or macrovesicular steatosis, according to the size of the cytoplasmic lipid vacuoles. While all canine cases were graded as severe hepatic lipidosis, feline cases were more diverse since there were 2 mild, 3 moderate and 4 severe cases of hepatic lipidosis. In 1 canine and 3 feline cases of severe and diffuse hepatocellular steatosis, lipogranulomas composed of foamy fat aggregations and ceroid laden macrophages were detected. The ceroid pigment was positively stained with PAS and AFIP staining methods (Fig. 3 D). The median age for

feline hepatocellular steatosis was 5 years, and while females were more affected than males (5 cases, 55.56%), there was no significant breed predisposition.

Passive congestion was diagnosed in six cats, which was 10.71% of the feline cases examined. As for canines, it was present in 27.03% of cases (20 of 74) and was the second most frequent liver lesion. Fifty-five percent of passive congestion cases had centrilobular necrosis, periacinar fibrosis, periportal hepatocytic lipidosis and hemosiderosis (hemosiderin was stained with blue pigment using

Perl's Prussian Blue stain). These cases were diagnosed as chronic passive congestion and had a typical macroscopically distinct acinar pattern, known as a nutmeg appearance. Additionally, passive congestion was generally more grave in dogs with 7 severe, 9 moderate and 4 mild cases, compared to cats which showed moderate severity in 4 cases and mild severity in 2 cases. Passive congestion was found in dogs with a mean age of 4 years, and males (12 cases, 63.16%) seemed more predisposed than females. Crossbreed dogs ranked highest (5/19 cases), and German Shepherds (3/19 cases) were second, together with Belgian Malinois (3/19 cases).

An Iranian cat with polycystic kidney disease was diagnosed with congenital polycystic liver disease. Histopathological examination of the case revealed portal-portal bridging fibrosis and prominent proliferation of the bile ducts beside the biliary cysts.

Proliferative liver lesions. Hepatic proliferative lesions were found in 4 cats (7.14 %) and 16 dogs (21.62%). This was the third most frequent category in canine hepatic lesions. The distribution of proliferative lesions in cats and dogs is shown in Tables 2 and 3. Although the difference was not significant, secondary tumours (9 cases/12.16%) were more frequent than primary tumours (7

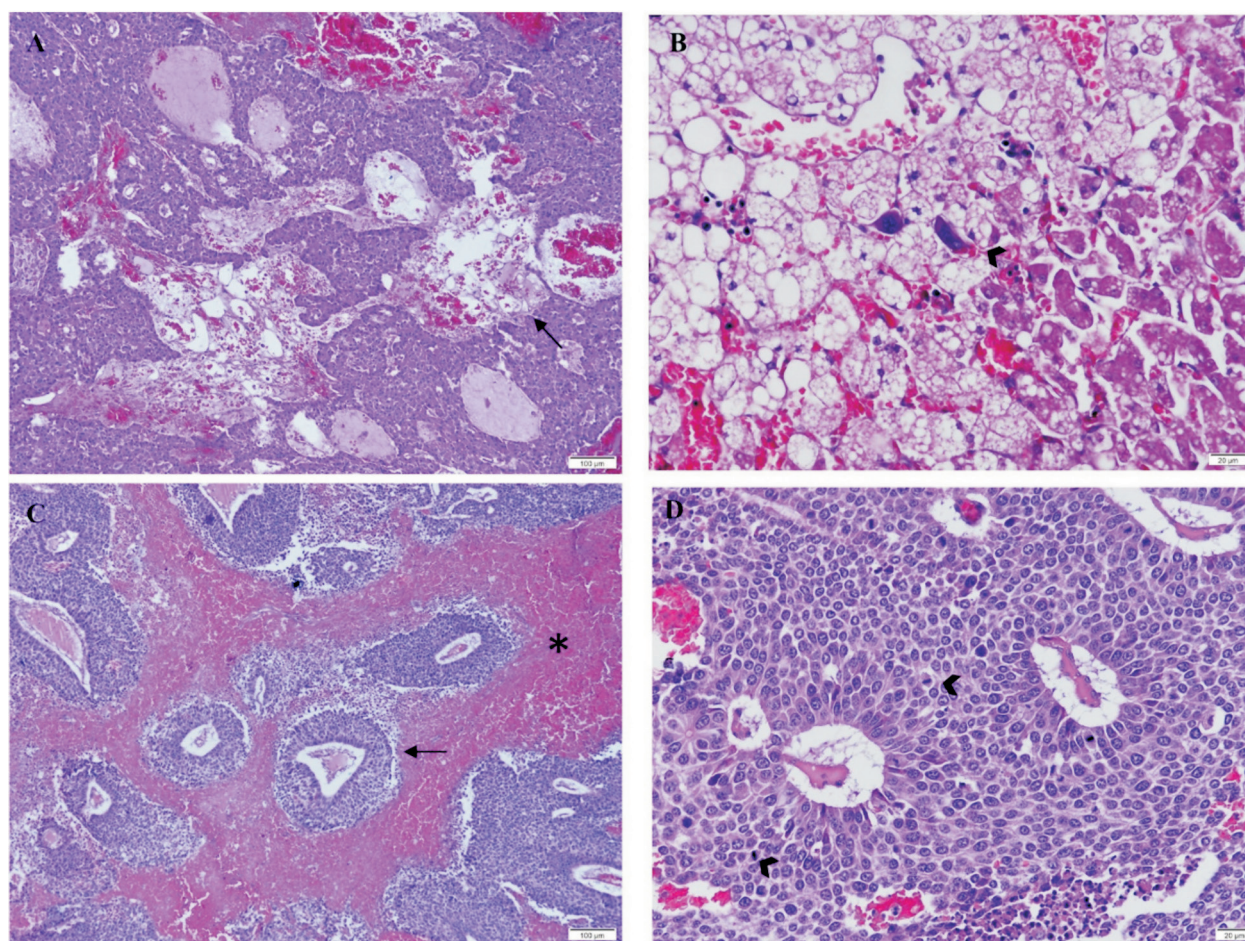Fig. 5. A, B - Hepatocellular carcinoma, dog, H&E. A - Cavern shaped vascular spaces (arrow) separating the hepatocellular neoplastic cells. B - Multinucleated cells most consistent with megakaryocytes (arrowhead). C, D - Cholangiocellular carcinoma, dog, H&E. C - Acini arrangement of biliary neoplastic cells (arrow) and vast necrotic areas (asterisk). D - Frequent mitotic Fig.s (arrowheads), H&E.

cases/9.46%) in dogs. Epithelial tumours accounted for the majority of canine primary hepatic tumours (6 cases), with only one case of primary hemangiosarcoma. Among the secondary tumours, metastatic lymphoma was most prominent for both species.

Primary hepatic tumours exhibited different general and histological appearances (Fig. 4, 5). Combined hepatocellular and cholangiocellular carcinoma was characterized by the presence of hepatocellular and cholangiocellular neoplastic cells. According to the WHO classification, this tumour is considered to be of the classical type, and its metastasis has been reported in the kidneys,

lungs and heart (Bosman et al. 2018). Primary hemangiosarcoma and metastatic tumours of the liver had the same gross and histopathological findings as those arising in other organs. Among the six metastatic lymphoma cases, neoplastic lymphoid cells were localized in five cases in the portal tracts and surrounding the central veins. Only in one case were the neoplastic cells evenly scattered in the sinusoids.

Proliferative lesions appeared common among middle to senior aged dogs and cats, with a mean age of 10 years for both. Golden Retrievers had an increased risk of hepatic primary tumours (3/7 cases).
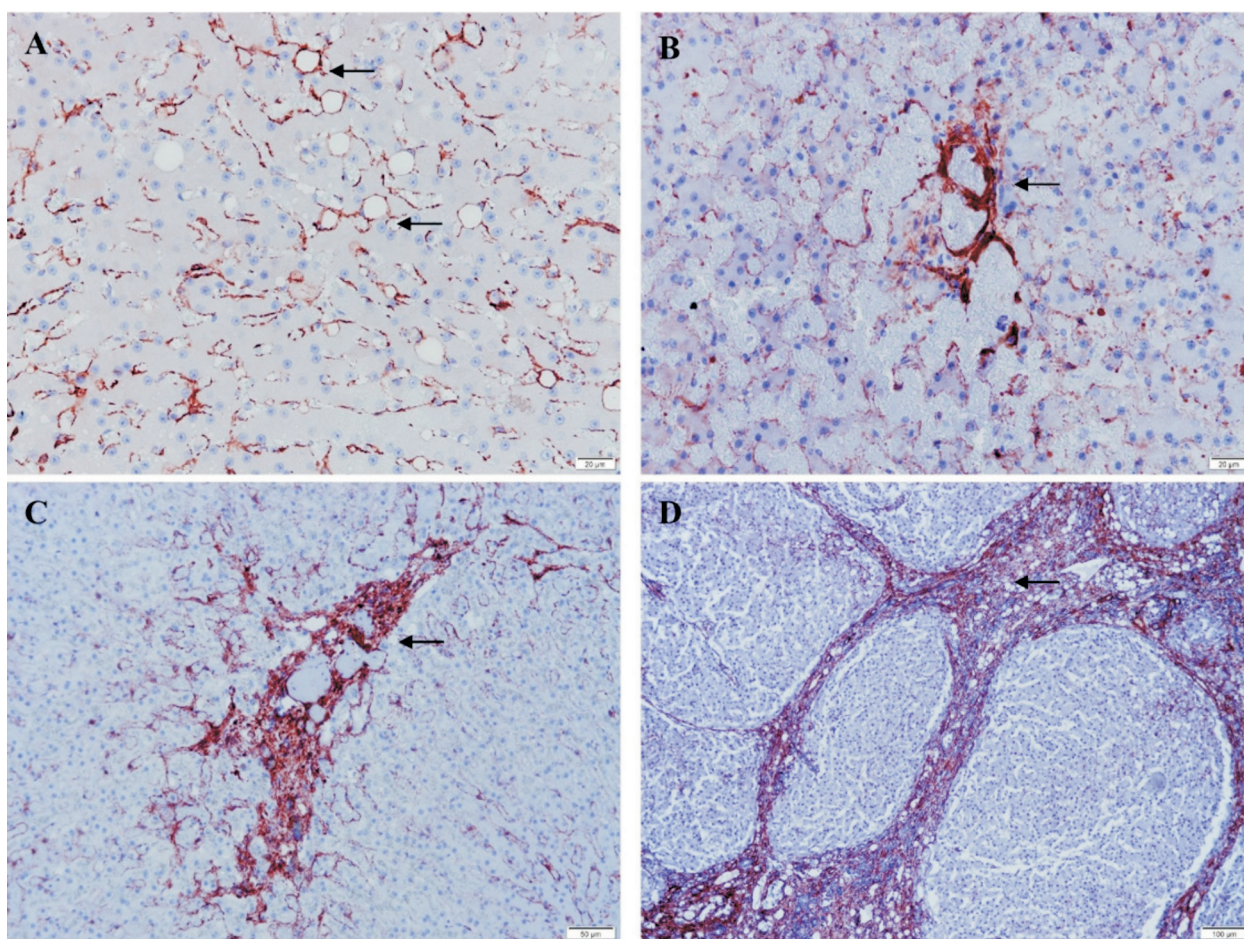
Fig. 6. α-SMA expression in different hepatic diseases. A - Diffuse slight enlargement of the stellate cells (parenchymal myofibroblasts) with cytoplasmic lipid vacuoles in a few of them (arrows), hepatocellular steatosis, cat. B - Perivenular thickening of the stellate cells (arrow), passive congestion, dog. C - Moderate staining of the portal myofibroblasts (arrow), chronic hepatitis, dog. D - Intensive staining of the septal myofibroblasts (arrow) surrounding regenerative nodules, cirrhosis, dog.

Hepatic fibrosis. In normal liver tissue sections, staining with α-SMA antibody revealed mild cytoplasmic positivity throughout the parenchyma, in the form of thin irregular bands along the sinusoids (cytoplasmic extensions of hepatic stellate cells). A closer look revealed that these cells contained cytoplasmic lipid vacuoles. In the portal tracts, α-SMA positive cells were found on the walls of the vena porta, arteria hepatica and around the bile ducts. Moreover, slight positive staining was observed in the borders of the central vein. Some large portal tracts were shown to have slightly positive portal myofibroblasts. Varying degrees of thickening of the parenchymal (stellate cells) and portal myofibroblasts were observed in different pathological conditions. In some cases, α-SMA positive fibrous septa were reported bridging the portal areas and/or the central veins.

In general, cases with the highest α-SMA positivity were canine cirrhosis, and primary and secondary tumours in both cats and dogs. In acute diseases, such as fulminant hepatitis, liquefactive necrosis, hepatocellular steatosis in cats, passive congestion and hepatic displacement in dogs, a slight increase in α-SMA positive cells was observed. These cells were localized in the parenchyma (Fig. 6, A, B), and particularly at the injury sites of each condition. For example, α-SMA positive cells showed perivenular localisation in cases of canine passive congestion (Fig. 6, B).

In feline and canine chronic hepatitis, although the highest number of α-SMA positive cells was situated in the portal tracts (Fig. 6, C), parenchymal cells also showed positivity, but to a lesser extent. Similar to acute diseases, parenchymal positive cells were specifically located at necrotic and inflammatory sites. In some cases, α-SMA positive cells located in the portal tracts, expanded and formed α-SMA positive septa.

In canine cirrhosis, the highest number of α-SMA positive cells was located in the septa surrounding the regenerative nodules, and replacing the damaged parenchyma (Fig. 6, D). Myofibroblasts located in the portal areas, at the interface of portal tracts and septa, were also intensively positive. No α-SMA positive cells were seen in the regenerative nodules (Fig. 6, D). In feline lymphocytic cholangitis/

cholangiohepatitis, α-SMA positive cells were specifically located in the portal areas.

In canine and feline tumour cases, the density and localization of positive myofibroblasts in the tumour sites and the surrounding parenchyma varied depending on the tumour type. The highest positive cell density was located in the portal and septal areas. Whether they were primary or secondary malignant tumours, they showed a higher number of α-SMA positive cells than benign tumours. α-SMA expression was higher in cholangiocellular carcinomas compared to hepatocellular carcinomas. Furthermore, in combined hepatocellular and cholangiocellular carcinomas, while no α-SMA positive cells were encountered at the hepatocellular tumour sites, cholangiocellular tumour sites showed intensive α-SMA expression.

In congenital polycystic liver disease, moderate α-SMA expression was observed. Positive cells were located in the cyst walls, portal tracts, portal-portal bridging septa, and parenchyma.

Statistical analysis. According to Pearson's chi-square test for testing the independence between α-SMA and fibrosis classes, we used 95% significant level and the p-value was 0.02458 for cats and 0.0122 for dogs, which means that α-SMA and fibrosis are dependent on each other for cats and dogs. Thus, there is a significant relationship between these categorical variables. Also, using the same α level for the Pearson correlation test between α-SMA and fibrosis in cats showed that the r value was 0.71, which means that they are 71% linearly related to each other for cats, and the r value for dogs was 0.88 so they are also 88% linearly related to each other for dogs. Thus, α-SMA and fibrosis classes are related to each other.

Discussion and conclusions

Considering the current literature, and on the basis of the WSAVA criteria (ROTHUIZEN et al 2006), this study pathomorphologically evaluated canine and feline hepatobiliary lesions, and simultaneously tracked their incidence in Turkey (Ankara region). Furthermore, hepatic fibrosis was histochemically and immunohistochemically evaluated under these different pathological conditions.

This present study is the first histopathological evaluation of canine and feline hepatobiliary lesions in Turkey. We found a high incidence of hepatobiliary lesions in cats and dogs (98%), proving that the liver is an organ greatly affected by various diseases in the body. The most frequent canine hepatobiliary lesions were hepatitis, passive congestion and vacuolar hepatopathy. In cats, hepatitis, hepatocellular lipidosis and cholangitis/cholangiohepatitis were the most common liver lesions. These results showed similarities with retrospective studies previously performed in different countries (BAYTON et al. 2018, WANG et al. 2004, WARREN-SMITH et al. 2012).

Hepatitis had the highest occurrence in canine and feline hepatobiliary systems, which suggests the major exposure of cats and dogs in Ankara to infectious and toxic agents. In agreement with the reports of HIROSE et al. (2014) and POLDERVAAT et al. (2009), while chronic hepatitis was more common in dogs, acute hepatitis and non-specific reactive hepatitis were more common in cats. Subacute hepatitis had similar prevalence in cats and dogs, while cirrhosis cases were only detected in dogs.

This study, along with previous studies (HIROSE et al. 2014, BEXFIELD et al. 2012), indicates that chronic hepatitis and cirrhosis are diseases that are common among middle-aged and older dogs, with a median age of 10 years. Females outnumbered males, which is consistent with other studies. Breeds with a high incidence of chronic hepatitis and cirrhosis were German Shepherd, Golden Retriever and crossbreed dogs. Breed predispositions, as reported in the literature, include American and English Cocker Spaniels, Labradors, Golden Retrievers, Doberman Pinschers, West Highland White Terriers, English Springer Spaniels, Yorkshire and Jack Russell Terriers (HIROSE et al. 2014, POLDERVAAT et al. 2009, WATSON et al. 2010, KANEMOTO et al. 2013).

Non-specific reactive hepatitis, which was more frequently diagnosed in cats, was histopathologically characterized by the absence of hepatocellular necrosis. Studies investigating non-specific reactive hepatitis reported that it arises in response to diseases affecting the urinary, genital, cardiovascular, respiratory, digestive and endocrine systems (BAYTON et al. 2018, NEUMANN and DANNER 2012). Moreover, the fact that affected cats in the present study had a median age of 4 months can be linked to vulnerability to infections due to an incomplete vaccination program.

Cholangitis/cholangiohepatitis has been shown several times in studies as a disease of feline species (BAYTON et al. 2018, WANG et al. 2004, HIROSE et al. 2014, GAGNE et al. 1996). This was also the case in this study, where cholangitis was the second most frequent lesion in cats, with only one case seen in dogs. All cholangitis/cholangiohepatitis were diagnosed as lymphocytic, which contradicts previous studies that considered neutrophilic cholangitis as the most common form of the disease in cats (BAYTON et al. 2018, HIROSE et al. 2014, CLARK et al. 2011).

The incidence of hepatocellular lipidosis, which has been reported more frequently in cats compared to dogs, was noted to be higher than that reported in Japan and the UK (BAYTON et al. 2018, HIROSE et al. 2014), but lower than in the USA (ARMSTRONG and BLANCHARD 2009). The median age of cats in this study was 5 years old, which is the same as reported by AKOL et al. (1993). KUZI et al. (2017) on the other hand reported a median age of 7 years. An over-representation of females was found by both studies previously mentioned (AKOL et al. 1993, KUZI et al. 2017), which is in alignment with this study, where female cats outnumbered males. No breed predisposition was found, which contradicts a previous association with British Shorthair according to the previously mentioned studies. This may be due to the lower population of this breed in Turkey compared to other countries.

Proliferative lesions of the liver are predominantly reported in dogs in this study. Previous literature on canine hepatic proliferative lesions indicated the predominance of primary tumours versus secondary tumours (CULLEN and STALKER 2016, CULLEN 2017). However, no distinct difference between the ratio of canine primary and secondary hepatic neoplasms was found here. According to HIROSE et al. (2014) and WARREN-SMITH et al. (2012), the most frequent canine primary hepatic neoplasms were hepatocellular adenoma and carcinoma. In the present study, hepatocellular and cholangiocellular carcinomas were the most frequent. Among the metastatic tumours, the most common tumours in both cats and dogs were lymphomas. In accordance with CULLEN (2017),

neoplastic lymphoid cells showed predominant portal and perivenular infiltration of the liver.

Hepatic fibrosis is a common reaction to persistent hepatic injury, and is associated with a wide variety of diseases. Myofibroblasts are cells that play a major role in fibrosis of most organs (CARPINO et al. 2005, VLADIMIR 2014). This study investigated the immunohistochemical properties of hepatic stellate cells and portal/septal myofibroblasts. It also demonstrated, in accordance with previous studies (IJZER et al. 2006), that α-SMA is a suitable antibody to identify hepatic myofibroblasts.

In humans and rats, hepatic stellate cells are only positive when activated (IJZER et al. 2006, VLADIMIR 2014). Unlike in humans and rodents, IJZER et al. (2006) reported in dogs that whether activated or resting, stellate cells and portal myofibroblasts show positive staining with α-SMA antibody. This was explained by the fact that canine myofibroblasts are more involved in the regulation of hepatic sinusoidal flow than in other species. In this study also, resting stellate cells and portal myofibroblasts slightly expressed α-SMA in both feline and canine livers. This finding proved that the distinction between activated and resting hepatic myofibroblasts in dogs and cats is made by examination of their morphological and functional changes.

The severity of fibrosis was staged in canine and feline sections stained with Masson's Trichome stain, on the basis of a modified staging system for fibrosis. The statistical analysis between fibrosis staging and immunohistochemical expression of α-SMA per case showed a definite positive correlation. While MEKONNEN et al. (2007) also found a positive correlation between histochemically staged fibrosis and immunohistochemical myofibroblast expression, IJZER et al. (2006) and BOISCLAIR et al. (2001) reported a negative correlation.

Immunohistochemical investigation of different hepatic lesions showed that stellate cells lost their lipid vacuoles and gained the phenotype of myofibroblasts by transforming into spindle shaped cells. Portal and septal myofibroblasts also showed various degrees of activation. Both the intensity and the localization of positive myofibroblasts changed with lesion type. In acute canine and feline events (fulminant hepatitis, liquefactive necrosis and hepatocellular steatosis), stellate cells (parenchymal myofibroblasts) were greatly activated in the damaged areas. In canine and feline chronic hepatitis, both portal myofibroblasts and parenchymal myofibroblasts located at the damaged areas were activated. As the disease became chronic, portal myofibroblasts became thicker and formed bridging septa. In canine cirrhosis, septal myofibroblasts were predominantly activated, and the absence of myofibroblasts in hyperplastic nodules was observed. These observations are consistent with those reported by SCHOTANUS et al. (2009). In feline lymphocytic cholangitis/cholangiohepatitis cases, portal myofibroblasts were the main activated cells. Activation of myofibroblasts and their densities showed great variation between cases in both feline and canine tumours.

In general, the increase in necrosis and fibrosis (due to the damage to the parenchyma caused by the tumour) was the same for myofibroblasts, especially portal/septal myofibroblasts, which as a result became more active than other myofibroblast groups. In feline congenital polycystic disease, the localization of α-SMA positive cells in the cyst walls, portal tracts, parenchyma and portal-portal bridging septa, suggests that it is due to juvenile or congenital fibrosis. According to the terminology proposed by the WSAVA (ROTHUIZEN et al. 2006), these cases have been defined as juvenile polycystic disease/congenital hepatic fibrosis.

In conclusion, hepatic myofibroblasts proved to play a major cell role in liver fibrosis, changing their type and intensity according to the hepatobiliary lesion. While parenchymal myofibroblasts were more dominant in acute liver diseases, portal and septal myofibroblasts were more prevalent in chronic liver diseases.

This study can be used as a reference point for future pathological surveys since there is a lack of histopathological surveys related to canine and feline hepatobiliary diseases in Turkey. Similar studies are of great importance, not only for diagnosis and prevention but also for an understanding of the incidence and pathological features of hepatobiliary diseases.

Supplementary Table 1. Breed, median age, male-female ratio, gross and histopathological findings and incidence of canine liver lesions.

Diagnosis	Breed	No. of cases	Median age	M : F	Hepatic gross findings	Main hepatic microscopic findings	% of total cases
Hepatic displacement	Irish setter Terrier	1 1	2 y	1:1	Herniated lobes enlarged, indurated and dark red in colour	- Reticular degeneration / Massive necrosis ± Fibrosis	2.70%
Vacuolar hepatopathy							
Hepatocellular steatosis	Bandogge Chow Chow Maltese	1 1 1	3 y	1:2	Hepatomegaly and prominent lobular pattern or yellow and friable liver	- Clear lipid vacuoles within the cytoplasm of the hepatocytes - Lipogranulomas	4.06%
Hepatocellular swelling	Pekingese Crossbreed	1 1	6 m	1:1	Pale liver	- PAS negative cytoplasmic vacuoles in the hepatocytes	2.70%
Hepatic glycogenosis	Pekingese *	1 1	5 y	0:2	Hepatomegaly with wide beige spots	- PAS positive, poorly defined cytoplasmic vacuoles with central nuclei of the hepatocytes	2.70%
Necrosis	Chow chow *	1 1	6 y	1:1	Enlarged liver with white multifocal foci and/ or prominent lobular pattern	- Centrilobular liquefactive/coagulative necrosis - Kupffer cell proliferation	2.70%
Autolysis	Crossbreed Golden Retriever German shepherd	2 1 1	8 y	4:0	Softening of the liver and bile imbibition	- Dissociation of hepatocellular cordons - Autolytic bacilli	5.41%
Circulatory disorders							
Passive congestion	Crossbreed German Shepherd Belgian Malinois Cocker Spaniel Rottweiler English Setter Pointer Kangal Siberian Husky Terrier	5 3 3 2 1 1 1 1 1 1	4 y	12:7	Enlarged liver and dark red in colour with round edges. Nutmeg liver appearance in chronic passive congestion cases	- Portal, central veins and sinusoids filled with erythrocytes ± haemorrhagic foci - Dissociation of hepatic cords and atrophy of hepatocytes - Centrilobular necrosis, periacinar fibrosis, periportal hepatocytic lipidosis and hemosiderosis in chronic passive congestion cases.	25.68%
Peliosis hepatis	Bichon	1	16 y	0:1	Dark-red foci on the capsular surface of the liver	Multiple cavernous enlargement of sinusoids filled with erythrocytes	1.35%

Supplementary Table 1. Breed, median age, male-female ratio, gross and histopathological findings and incidence of canine liver lesions (continued)

Diagnosis	Breed	No. of cases	Median age	M : F	Hepatic gross findings	Main hepatic microscopic findings	% of total cases
Hepatitis							
Acute hepatitis	Doberman	1	2 m	0:1	Hepatomegaly, pale areas on the surface of the liver, prominent lobular pattern (some cases showed no significant gross lesions)	- Neutrophils and/or mononuclear cells (with few neutrophils and predominance of mononuclear cells in subacute and chronic hepatitis) - Hepatocellular degeneration and/or necrosis/apoptosis - Kupffer cell proliferation - Hepatocellular regeneration - Fibrosis in chronic hepatitis cases	1.35%
Subacute hepatitis	Crossbreed / Pomeranian / German Shepherd	5 / 1 / 1	8 m	4:3			9.46%
Chronic hepatitis	German Shepherd / Cocker Spaniel / Golden Retriever / Crossbreed / Rottweiler / Pitbull / Siberian Husky	2 / 1 / 1 / 1 / 1 / 1 / 1	9y 5m	3:5	Previously cited lesions with fibrous grey white depressions on the surface		10.81%
Cirrhosis	Crossbreed / Golden Retriever / English Setter	1 / 1 / 1	10y 5m	1:2	Shrunken liver and hard in consistency with an irregular surface presenting macro or micronodules.	- Pseudolobules separated by thick fibrous strands - Septal mononuclear cell infiltration and regenerative bile ductules	4.06%
Non-specific reactive hepatitis	Bichon / Crossbreed	1 / 1	11 m	1:1	Hepatomegaly, pale areas on the surface of the liver. (some cases showed no significant gross lesions)	Portal inflammatory infiltrations composed of lymphocytes, plasma cells and/or neutrophil leucocytes with absence of hepatic necrosis.	2.70%
Cholangitits/ cholangiohepatitis							
Destructive cholangitis	Bichon	1	9 y	0:1	Hepatomegaly	- Destruction of the bile ducts and peribiliary infiltration of neutrophil leucocytes, lymphocytes and pigment laden macrophages. - Cholestasis	1.35%

Supplementary Table 1. Breed, median age, male-female ratio, gross and histopathological findings and incidence of canine liver lesions (continued)

Diagnosis	Breed	No. of cases	Median age	M : F	Hepatic gross findings	Main hepatic microscopic findings	% of total cases
Proliferative lesions							
Hepatocellular adenoma and nodular hyperplasia	Golden Retriever	1	12 y	0:1	Nodular hyperplasia: Multiple nodules bulging from the capsular surface of the same colour of the liver. Hepatocellular adenoma: single white nodules raised from the surface and soft in consistency.	Nodular hyperplasia: - Uniform hepatocytes organized in trabeculae and forming non-encapsulated but well circumscribed nodules. - Lobular structures and widely separated portal tracts. - Severe lipidosis of the nodules. Hepatocellular adenoma: - Almost similar to nodular hyperplasia with few mitotic Fig.s, but lacking portal tracts and bile ducts. - Severe lipidosis and glycogenosis. - Cystic spaces filled with blood scattered between tumour cells.	1.35%
Hepatocellular carcinoma	Bulldog, Golden Retriever	1, 1	7 y 5 m	1:1	Friable tumour with multilobulated mottled cut section, involving the entire lobe.	- Polygonal anaplastic cells arranged in a solid or trabecular pattern separated by wide cavern shaped vascular spaces. - Few mitotic Fig.s and extramedullary haematopoiesis.	2.70%
Cholangiocellular carcinoma	Crossbreed, Golden Retriever	1, 1	10y	0:2	Multiple firm white nodules diffuse to all the lobes; embedded or bulging from the surface (some were umbilicated).	- Anaplastic cuboidal to columnar cells arranged in acini, tubules or islets and separated by prominent fibrous tissue. - Abundant mitotic Fig.s.	2.70%
Combined hepatocellular and cholangiocellular carcinoma	Siberian Huskey	1	14 y	1:0	Greyish white nodules of different sizes located specially on the margins of the lobes.	Both hepatocellular and cholangiocellular neoplastic cells.	1.35%
Primary hemangiosarcoma	German Shepherd	1	11 y	1:0	Hepatomegaly with dark red coloured foci, bulging from the surface, spread across all the lobes.	Cavern like structures lined with neoplastic endothelial cells.	1.35%
Metastatic lymphoma	Terrier, Bichon, Rottweiler	1, 1, 1	9 y	0:3	Multiple white nodules diffuse to all the lobes; embedded or bulging from the surface.	Neoplastic lymphoid cells localized in the portal tracts and surrounding the central veins.	4.06%

Supplementary Table 1. Breed, median age, male–female ratio, gross and histopathological findings and incidence of canine liver lesions (continued)

Diagnosis	Breed	No. of cases	Median age	M : F	Hepatic gross findings	Main hepatic microscopic findings	% of total cases
Metastatic histiocytoma	Crossbreed	1	10 y	1:0	In general, multifocal white or red nodules of various sizes		1.35%
Metastatic histiocytic sarcoma	*	1	5 y	1:0			1.35%
Metastatic giant cell fibrosarcoma	Rottweiler	1	6 y	1:0		Similar to each primary tumour findings	1.35%
Metastatic malignant myeloid tumor	Golden Retriever	1	6 y	1:0	In general, multifocal white or red nodules of various sizes	Similar to each primary tumour findings	1.35%
Metastatic multiple myeloma	Golden Retriever	1	4 y 5 m	1:0			1.35%
Metastatic malign melanoma	English Cocker Spaniel	1	13 y	0:1			1.35%
No abnormality	Crossbreed	1	*	0:1	A homogeneous red- BROWN organ with smooth capsular surface located at the centre of the cranial abdomen.	- Polygonal shaped hepatocytes arranged in radiating plates. - Absence of hepatocellular necrosis and portal/parenchymal inflammatory cells. - No expansion of portal tracts.	1.35%

*Data absent in the anamnesis

Supplementary Table 2. Breed, median age, male-female ratio, gross and histopathological findings and incidence of feline liver lesions

Diagnosis	Breed	No. of cases	Median age	M : F	Hepatic gross findings	Main hepatic microscopic findings	% of total cases
Developmental disorders							
Congenital polycystic disease	Persian Cat	1	3 y	0:1	Multiple liver cysts filled with clear fluid	- Biliary cysts - Portal-portal bridging fibrosis - Bile duct proliferation	1.79%
Hepatic displacement	Tabby Cat Crossbreed	1 1	4 m	0:2	Pale herniated lobes with large red areas and linear ruptures	- Reticular degeneration - Diffuse lipidosis	3.57%
Vacuolar hepatopathy							
Hepatocellular steatosis	Crossbreed Tabby Cat Himalayan British Shorthair Scottish Fold Van Cat	3 2 1 1 1	5 y	4:5	Hepatomegaly and prominent lobular pattern or yellow and friable liver	- Clear lipid vacuoles within the cytoplasm of the hepatocytes - Lipogranulomas	16.07%
Cholestasis	*	1	4 y	0:1	No significant lesion	- Bile pigment in hepatocytes, bile canaliculi and portal bile ducts - Feathery degeneration of hepatocytes - Portal edema	1.79%
Necrosis	Turkish Angora	1	10 y	1:0	Enlarged liver with white multifocal foci and/ or prominent lobular pattern	- Focal liquefactive necrosis	1.79%
Autolysis	Crossbreed	1	8 m	1:0	Softening of the liver and bile imbibition	- Dissociation of hepatocellular cordons - Autolytic bacilli	1.79%
Circulatory disorders							
Passive congestion	Tabby Cat Crossbreed Siamese Cat British Shorthair Exotic Shorthair	2 1 1 1 1	1 y	4:2	Enlarged liver and dark red in colour with round edges. Nutmeg liver appearance in chronic passive congestion cases	- Portal, central veins and sinusoids filled with erythrocytes ± haemorrhagic foci - Dissociation of hepatic cords and atrophy of hepatocytes - Centrilobular necrosis, periacinar fibrosis, periportal hepatocytic lipidosis and hemosiderosis in chronic passive congestion cases	10.71%

Supplementary Table 2. Breed, median age, male-female ratio, gross and histopathological findings and incidence of feline liver lesions (continued)

Diagnosis	Breed	No. of cases	Median age	M : F	Hepatic gross findings	Main hepatic microscopic findings	% of total cases
Hepatitis							
Acute hepatitis	Tabby Cat Crossbreed Orange Tabby Persian Cat	2 1 1 1	2 y	2:3	Hepatomegaly, pale areas on the surface of the liver, prominent lobular pattern. (some cases showed no significant gross lesions)	- Neutrophils and/or mononuclear cells (with few neutrophils and predominance of mononuclear cells in subacute and chronic hepatitis) - Hepatocellular degeneration and/or necrosis/apoptosis - Kupffer cell proliferation - Hepatocellular regeneration - Fibrosis in chronic hepatitis (two of the acute hepatitis cases with severe necrosis and regeneration were diagnosed as fulminant hepatitis).	8.93%
Subacute hepatitis	Crossbreed Tabby Cat Orange Tabby British Shorthair *	4 2 1 1 1	1 y	3:6	Fibrous grey white depressions on the surface of chronic hepatitis cases		16.07%
Chronic hepatitis	British Shorthair *	1 1	8 y 5 m	2:0			3.57%
Non-specific reactive hepatitis	Tabby Cat British Shorthair Scottish Fold	3 2 1	4m	3:3	Hepatomegaly, pale areas on the surface of the liver (some cases showed no significant gross lesions)	Portal inflammatory infiltrations composed of lymphocytes, plasma cells and/or neutrophil leucocytes with absence of hepatic necrosis. Bacterial clusters in 2 cases.	10.71%
Cholangitits/ cholangiohepatitis							
Lymphocytic cholangitis / cholangiohepatitis	Crossbreed Tabby Cat Scottish Fold *	3 3 1 1	2y 5m	4:4	Pale areas on the surface of the liver (few cases showed no significant gross lesions)	- Focal lymphocytic infiltration surrounding the bile ducts (with infiltration of adjacent parenchyma in cholangiohepatitis cases) - Biliary duct proliferation and peribiliary portal fibrosis ± Destruction of biliary epithelium and cholestasis	14.28%
Proliferative lesions							
Hepatocellular adenoma	Orange Tabby	1	18 y	0:1	Single white nodules raised from the surface and soft in consistency	- Non-encapsulated but well circumscribed nodules of uniform hepatocytes arranged in trabeculae with few mitotic Figs, but lacking portal tracts and bile ducts.	1.79%
Metastatic lymphoma	Bombay Crossbreed Orange Tabby	1 1 1	7 y 6 m	3:0	Multiple white nodules diffuse to all the lobes; embedded or bulging from the surface	- Neoplastic lymphoid cells localized in the portal tracts and surrounding the central veins. - Diffusely scattered neoplastic cells in the sinusoids in one case.	5.35%
No abnormality	Crossbreed	1	3 m	1:0	A homogeneous red- BROWN organ with smooth capsular surface located at the centre of the cranial abdomen.	- Polygonal shaped hepatocytes arranged in radiating plates. - Absence of hepatocellular necrosis and portal/parenchymal inflammatory cells. - No expansion of portal tracts.	1.79%

*Data absent in the anamnesis

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ABOU MONSEF, Y., O. KUTSAL: Patomorfološka procjena hepatobilijarnih lezija u pasa i mačaka uzgojenih na području grada Ankare. Vet. arhiv 91, 359-378, 2021.

Sažetak

Cilj ovog rada bio je istražiti patološke poremećaje hepatobilijarnog sustava u pasa i mačaka uzgajanih na području grada Ankare, te odrediti vrstu i učestalost promatranih lezija. Osim toga cilj je bio utvrditi regeneraciju jetre kao reakciju na ozljedu u različitim hepatobilijarnim lezijama primjenom imunohistokemijskih metoda. Makroskopski i mikroskopski su pretraženo jetre od 56 mačaka i 74 psa nakon postmortalne analize. Uzorci s jetrenom fibrozom imunohistokemijski su obojeni protutijelima α-SMA. Lezije su nađene u 98 % jetara pretraženih pasa i mačaka. U mačaka je najčešća histopatološka dijagnoza bio hepatitis (39,28 %), hepatocelularna lipidoza (16,07 %) i kolangitis/kolangiohepatitis (14,28 %). U pasa je najčešći bio hepatitis (28,38 %), zatim pasivna kongestija (25,68 %) i proliferativne lezije (21,62 %). Za neke su hepatobilijarne lezije promatrani pasminska, dobna i spolna predispozicija. Imunohistokemijski α-SMA protutijela pozitivno su obojila parenhimske, portalne i septalne miofibroblaste. Dokazana je pozitivna korelacija među rezultatima imunohistokemije pomoću α-SMA i histokemijske fibroze. Ovo je prvo istraživanje u Turskoj koje donosi i incidenciju hepatobilijarnih lezija u mačaka i pasa i njihova patomorfološka svojstva. Kad je riječ o regeneraciji, promatrana je važna uloga jetrenih miofibroblasta u jetrenoj fibrozi. Pronađena je varijacija u intenzitetu i lokaciji pozitivno obojenih stanica s obzirom na vrstu lezije. Zaključak ovog istraživanja jest da treba obratiti pozornost na određene jetrene lezije u pasa i mačaka što pruža referentnu normu za daljnja klinička i histopatološka istraživanja.

Ključne riječi: pas; mačka; hepatobilijarni sustav; imunohistokemija; patomorfologija