

## Hepatic Fibrosis in Four German Shepherd Dogs Idiopathic, Genetic Predisposition or a Familial Relationship?

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### ABSTRACT

Idiopathic hepatic fibrosis (İHF) is an inevitable result of chronic liver disease progressing to cirrhosis. Idiopathic Hepatic fibrosis is characterized as congenital or juvenile form in German Shepherd Dogs. However, few data have been published on the incidence and prevalence of İHF in canine species. The case report presented here describes idiopathic hepatic fibrosis in four neutered German Shepherd dogs with a familial relationship. The main clinical complaints were refractory ascites, variable appetite and progressive weight loss. Hepatic fibrosis was confirmed with diagnostic applications and liver biopsy. Nevertheless, all dogs died in spite of supportive and aggressive treatment. Necropsy was performed in the dogs according to written owner consent. In conclusion, the cases presented here reflect the congenital condition of idiopathic hepatic fibrosis in young German Shepherd Dogs with histopathological evidence. However, there was evidence of familial relationship in the cases presented here.

**Keywords:** Dog, fibrosis, genetic, liver, idiopathic

**Dört Alman Çoban Köpeğinde Hepatik Fibroz İdiyopatik, Genetik Yatkınlık ya da Ailesel İlişki mi?**

### ÖZ

İdiyopatik hepatik fibroz (İHF) siroza kadar gidebilen kronik karaciğer hastalığının kaçınılmaz bir sonucudur. İdiyopatik hepatik fibroz, Alman Çoban Köpeklerinde juvenil ya da doğmasal olarak şekillenebilmektedir. Bununla birlikte, köpeklerde İHF'nin prevalansı veya insidansı hakkında az sayıda veri bulunmaktadır. Sunulan olgu serisi, ailesel ilişkisi bulunan dört Alman Çoban Köpeğindeki idiyopatik hepatik fibroz tanımlamaktadır. Olgulardaki esas klinik şikayetler; tekrarlayan asites, değişken iştah durumu ve ilerleyici kilo kaybıydı. Hepatik fibroz tanısı diyagnostik uygulamalar ve karaciğer biyopsisi ile doğrulandı. Destekleyici agresif tedaviye rağmen tüm köpekler kaybedildi. Hasta sahibinin izni ile köpeklerde nekropsi gerçekleştirildi. Sonuç olarak, sunulan olgular Alman Çoban Köpeklerinde histopatolojik kanıtı olan konjenital idiyopatik hepatik fibroz yansıtmaktadır. Bununla birlikte, sunulan olgularda ailesel ilişki kanıtı da söz konusudur.

**Anahtar Kelimeler:** Fibroz, genetic, idiyopatik, karaciğer, köpek

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## INTRODUCTION

Hepatic fibrosis, as a consequence of chronic liver disorders, is a dynamic process including complicated interactions between several hepatic cell types and accumulation of obvious extracellular matrix (ECM) proteins (Brown et al. 2010, Eulenberg and Lidbury 2018). The hepatic remodeling with deterioration of intrahepatic blood flow can lead to development of portal hypertension (PHT). Although increased resistance to portal blood flow can cause the formation of supportive acquired portosystemic shunts (PSS), animals with PHT can also increase abdominal lymphatic fluid production resulting with ascites (Desmet 1998).

Hepatic fibrosis based on the location of fibrosis has been categorized into three forms: central perivenous fibrosis, diffuse pericellular fibrosis and periportal fibrosis (Brown et al. 2010). German Shepherd dogs have appeared to be predisposed to central perivenous and diffuse pericellular fibrosis (Rutgers et al. 1993). It has been reported that idiopathic form of hepatic fibrosis in young German Shepherd dogs has not associated with underlying inflammatory conditions. The cases presented here reflect idiopathic hepatic fibrosis with biliary cirrhosis in four German Shepherd dogs with a familial relationship.

## MATERIAL and METHOD

Four German Shepherd dogs with a history of variable appetite, progressive weight loss and abdominal distention for two weeks referred to Veterinary Teaching Hospital of Ankara University. The dogs had referred to Hospital within three weeks at different times. All dogs were in familial relationship and from the 1<sup>st</sup> and 2<sup>nd</sup> generation of the same parents (Figure 1). All dogs had also essential antiparasitic therapy and routine vaccination against the infections of rabies, distemper, parainfluenza, herpes virus, parvoviral enteritis and leptospirosis. The dogs were feeding with super-premium commercial dry food. In physical examination, all dogs were alert. Signalment and detailed physical examination findings were shown in Table 1. They also had pinky mucosal membranes and moderate dehydration with abdominal distention associated with ascites (Figure 2).

Peripheral blood smears and heartworm antigen test kit (IDEXX® Snap 4DX plus test) revealed any abnormalities for rickettsial infection and dirofilariasis in all dogs. Complete blood count results were presented in Table 2. Elevated levels of total and direct bilirubin, severe increase in ALP,

ALT, AST and GGT activities, mild increase in ammonia concentration, severe increase in pre- and post-prandial bile acids were remarkable. The serum copper and total iron binding capacity (TIBC) were in reference ranges. Analysis of intraabdominal fluid also indicated transudate because of low protein concentration, hypocellularity and lack of blood and bacteria. Microbiological culture of the abdominal fluid revealed no pathogen. The result of serum profiles and intraabdominal fluid analysis were presented in Table 3.

For the possibility of any food poisoning in dogs, dry food of animals were evaluated in the food analysis laboratory. The results of food analysis revealed no microbiological and toxic contents including mycotoxins or others (Table 4).

Radiographic and echocardiographic examinations of the thorax ruled out for congenital and acquired heart disease in all dogs. Abdominal ultrasonography revealed severe free fluid (Figure 3a), microhepatica with diffuse heterogenous liver tissue (Figure 3b), portal hypertension (12 mm Hg, only in case 2) and cholecystitis (Figure 3c).

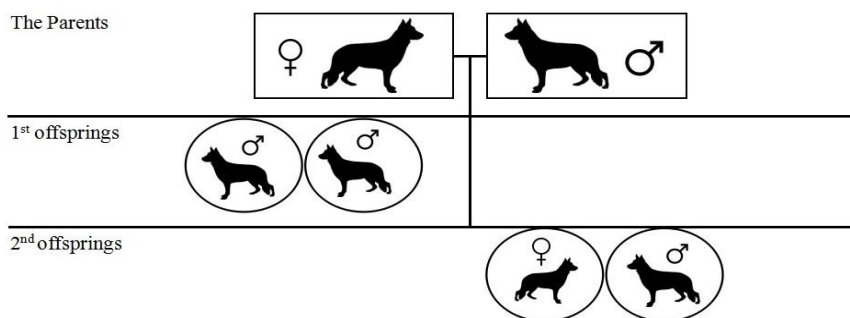
Cholangiohepatitis with portal hypertension was diagnosed considering clinical signs and diagnostic applications. All dogs was initiated the following medications: Ampicillin-sulbactam (25 mg/kg, bid, iv for 14 days, Alfasid, Yavuz İlaç), Enrofloxacin (5mg/kg, bid, sc for 14 days, Baytril K, Bayer), L-carnitine (500 mg, sid, po, Maxi L Carnitine, Solgar), vitamin E (10 IU/kg/day, po, Natural Vitamin E, Solgar), vitamin K (0.5 mg/kg, sc, weekly, Hemadur K, Alke), Silymarin (50 g/kg/day, po, Milk Thistle, Solgar), Ursodeoxycholic acid (7.5 mg/kg, bid, PO) and supportive fluid therapy with electrolyte and aminoacid solutions. Portal hypertension was also controlled by applying the Spironolacton (1mg/kg, bid, PO, Aldacton, Pfizer) and ascites was relieved by Furosemide (2 mg/kg, bid, PO, Lasix, Sanofi aventis). The clinical signs decreased within two weeks following the medication in all dogs and clinical improvement was confirmed by clinical examinations.

Three weeks later the clinical symptoms including anorexia, malaise, dehydration and severe abdominal distension relapsed in all dogs. The same paraclinical results were seen in the dogs. Severe ascites with serious cholecystitis were detected by ultrasonography in cases 2 and 4. Surgical cholecystectomy was employed in case 2. Liver biopsy was also performed during the cholecystectomy operation. The same treatment were repeated in dogs. It has not been observed

any improvement in clinical signs in spite of surgical cholecystectomy and mentioned treatment in following days. Nevertheless, all dogs died and necropsy was performed in case 2 according to written owner consent.

In the necropsy, about 400 cc of free fluid in the abdomen and old petechial hemorrhage in the fundic mucosa of the stomach was remarkable. Marked dilatation of mesenchymal vessels was also observed. Liver tissue samples collected from necropsy fixed in neutral-buffered formalin,

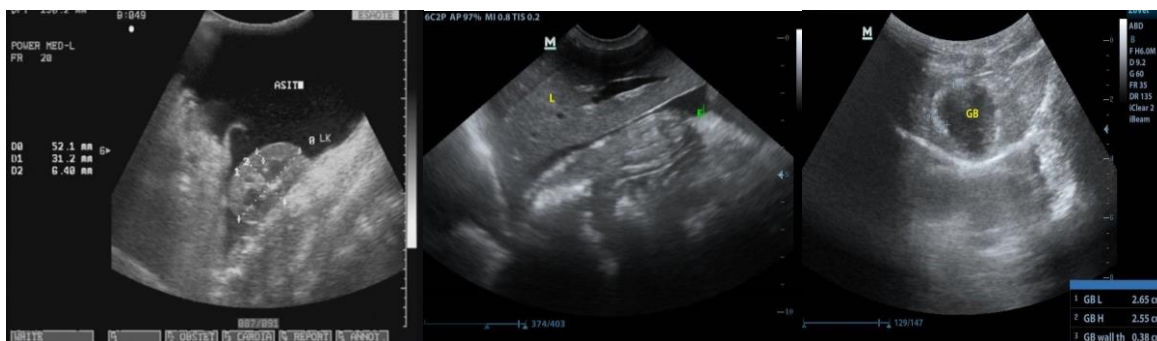
routinely processed and embedded in paraffin. The sections were cut at 5  $\mu$ m and stained with Haematoxylin and Eosin. The histological sections showed typical biliary cirrhosis with replacement of the regular pattern by pseudo-lobules. Internal lober structure of the liver were also abnormal. It was observed that the most of the erythrocyte and bile pigments were within the liver sinusoids. Proliferation of the connective tissue cells were surrounding the portal and interior areas of the lobules. (Figure 4a and 4b).



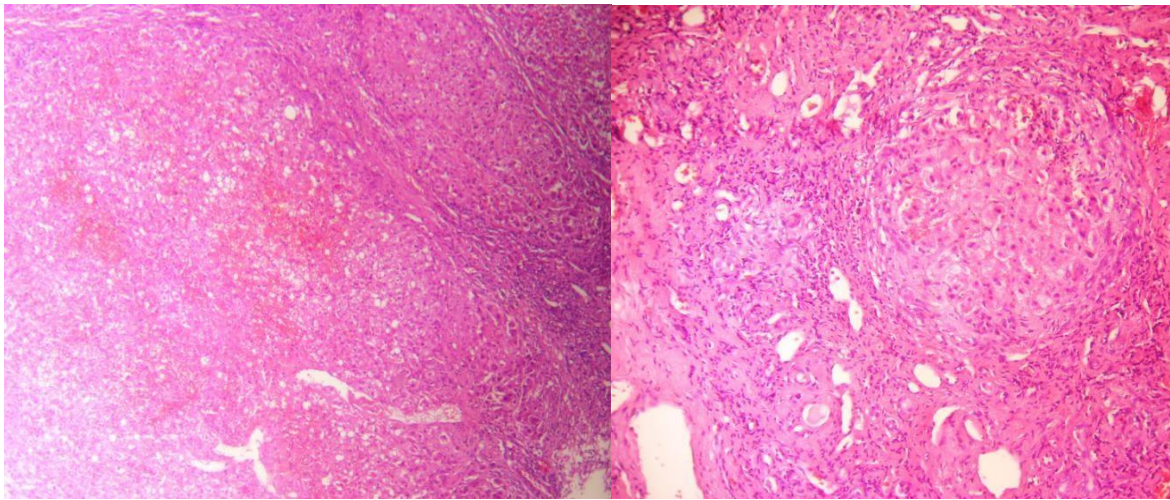
**Figure 1.** Family tree of the dogs: the 1<sup>st</sup> and 2<sup>nd</sup> offsprings from the same parents



**Figure 2.** The German Shepherd Puppy with abdominal distension in sternal (a) and standing position (b).



**Figure 3.** Abdominal ultrasonography in a German Shepherd dog with abdominal distension. a) Severe abdominal free fluid (ascites). b) Free fluid surrounding liver with mild increase in echotexture. c) Severe cholecystitis in 6 month old German Shepherd puppy.



**Figure 4.** Histopathological sections (a,b)

**Table 1.** Signalment and physical examination findings in dogs

<b>Signalment</b>	<b>1st generation puppies</b>		<b>2nd generation puppies</b>	
<b>Case Numbers</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>Sex</b>	Male	Male	Male	Female
<b>Age (month)</b>	21	21	6	6
<b>Weight (kg)</b>	22,3	28	15	19
<b>Posture</b>	Alert and standing position	Alert and standing position	Alert and standing position	Alert and sternal recumbency position
<b>Physical Examination</b>	<b>1st generation puppies</b>		<b>2nd generation puppies</b>	
<b>Case Numbers</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>Body Temperature (°C)</b>	39.0	39.3	38.2	39.2
<b>Heart Rate (bpm)</b>	83	110	88	79
<b>Respiration Rate (rpm)</b>	34	37	34	32
<b>Estimated Dehydration (%)</b>	6 – 8	6 – 8	6 - 8	8 - 10
<b>Mucosal Appearance</b>	Pinky	Pinky-red	Pinky	Pinky-red

bpm: beat per minute; rpm: respiration per minute; s: second.

**Table 2.** Results of complete blood count in dogs

	<b>Case Numbers</b>			
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>WBC (10<sup>9</sup>/l)</b>	28.97	21.10	14.73	15.48
<b>LYM (10<sup>9</sup>/l)</b>	2.34	2.48	2.87	5.66
<b>MON (10<sup>9</sup>/l)</b>	1.28	1.01	0.97	0.1
<b>GRA (10<sup>9</sup>/l)</b>	25.35	19.88	10.89	9.73
<b>LYM(%)</b>	8.1	15.3	19.5	36.5
<b>MON (%)</b>	4.4	3.6	6.6	0.6
<b>GRA(%)</b>	87.5	77.3	73.9	62.8
<b>RBC (10<sup>12</sup>/l)</b>	3.65	4.41	5.75	6.10
<b>HGB(g/dl)</b>	9.0	11.0	13.0	14.0
<b>HCT(%)</b>	26.54	33.31	36.01	39.43
<b>MCV (fl)</b>	57	59	59	61
<b>MCH(pg)</b>	24.6	25.2	22.6	22.9
<b>MCHC(g/dl)</b>	33.8	33.1	36.2	35.5
<b>RDWc(%)</b>	17.5	16.6	16.0	15.4
<b>PLT(10<sup>9</sup>/l)</b>	104	325	277	424

Reference Ranges: WBC: 6-17; LYM: 0.9-5; MON: 0.3-2.5; GRA:3-12; GRA %: 35-70; LYM %: 12-30; MON %: 2-13; RBC: 5.5-8.5; HGB: 12-18; HCT: 37-55; MCV: 60-72; MCH: 19.5-25.5; MCHC: 32-38.5; RDWc: 12-17.5; PLT: 200-500

**Table 3.** Results of serum biochemistry profiles and intra-abdominal fluid analysis in dogs

	Case Numbers			
	1	2	3	4
<b>Glucose (mg/dl)</b>	84.5	101	112	87.4
<b>Urea (mg/dl)</b>	18.0	21.2	8.8	46.0
<b>Creatinine (mg/dl)</b>	1.1	0.6	0.88	1.62
<b>Total Protein (g/dl)</b>	6.7	7.7	7.4	4.5
<b>Albumin (g/dl)</b>	3.2	2.7	2.4	2.5
<b>Total Bilirubin (mg/dl)</b>	0.9	0.7	0.31	0.41
<b>Direct Bilirubin (mg/dl)</b>	0.66	0.5	0.3	0.37
<b>Cholesterol (mg/dl)</b>	331	117	145	223
<b>Triglycerides (mg/dl)</b>	125	98	59	77
<b>ALP (IU/L)</b>	61.5	322.6	347.8	370.0
<b>ALT (IU/L)</b>	65.7	198.9	448.5	292.0
<b>AST (IU/L)</b>	102.6	212.2	196.7	337.8
<b>GGT (IU/L)</b>	5.0	22.3	14.0	8.0
<b>Creatine Kinase (IU/L)</b>	214	188	311.0	197
<b>LDH (IU/L)</b>	55	41	42.0	240.0
<b>TIBC (µg/dl)</b>	317.0	413.9	214.5	246.1
<b>Total Calcium (mg/dl)</b>	11.2	10.1	10.1	7.1
<b>Copper (mg/dl)</b>	Not measured	0.07	0.05	0.04
<b>Ammonia(µmol/l)</b>	0.92	0.81	0.88	0.73
<b>Pre-prandial bile acid concentration(µmol/l)</b>	6.8	5.4	6.6	5.7
<b>Post-prandial bile acids concentration (µmol/l)</b>	38.8	40.1	42.3	41.1
<b>Total Protein (fluid) (g/dl)</b>	1.0	1.1	0.9	0.1
<b>Fluid Cytology and Microbiological Culture</b>	No bacteria. Hypocellularity. Negative Microbiological culture.			

Reference Ranges: Glucose: 65-118mg/dl; Urea: 15-59.9mg/dl; Creatinine:0.5-1.5mg/dl; Total Protein: 5.4-7.1g/dl; Albumin: 3.1-4g/dl; Total Bilirubin: 0.1-0.3mg/dl; Cholesterol: 92-324mg/dl; Triglycerides: 29-291mg/dl; ALP: 20-156U/L; ALT: 21-102U/L; AST: 23-66U/L; GGT: 6-28U/L; Creatine Kinase: 0-200U/L; LDH: 45-233U/L; TIBC (Total iron binding capacity): 235-495µg/dl), Total Calcium: 9-11.3mg/dl; Copper: 0.1-0.2mg/dl; Ammonia: 0-0.68µmol/l; Pre-prandial Bile Acid Concentration: 0-0.5µmol/l; Post-prandial Bile Acid Concentration: <12µmol/l.

**Table 4.** Detailed ingredients of the dry pet food

Ingredients	Adult Dry Pet Food	Puppy Dry Pet Food
<b>Moisture (%)</b>	7,00	6,70
<b>Crude Protein (%)</b>	26,00	25,00
<b>Crude Fiber (%)</b>	1,90	2,00
<b>Ether Extract (%)</b>	17,50	18,20
<b>Crude Ash (%)</b>	7,05	7,30
<b>Metabolic Energy (Kcal/kg)</b>	3570	3590
<b>Microbiology Lab.</b>	No growth of any pathogenic bacteria or fungi. No evidence of Mycotoxins or aflatoxins exposure	

## DISCUSSION and CONCLUSION

Fibrosis, generally indicates irreversible liver damage. Chronic hepatopathies in human and veterinary patients ultimately lead to fibrosis. End-

stage liver disease is associated with nodule formation, organ contraction and fibrosis. The complications of this stage include metabolic dysfunction, ascites, portal hypertension and hepatic encephalopathy (Brown et al. 2010, Eulenberg and Lidbury 2018). Histopathologically, hepatic fibrosis (HF) has been known as an increase in ECM in liver tissue. Under chronic or repetitive injuries, ECM is produced by hepatocytes, sinusoidal endothelial cells, kupffer cells and stellate cells (Freidman 2000). Lipocytes (Ito cells or stellate cells) located in dissepimental space cause a decrease in ultrafiltration between the sinusoidal blood and hepatocytes by ECM, cytokines and collagen production (Center 1999). Progression of ECM pathology causes also hypertension and ascites. Therefore, anorexia and severe lethargy in dogs result from the progression of ascites and dehydration.

In these cases presented here German Shepherd dogs referred to hospital with the progressive ascites and portal hypertension. Making the diagnosis before 2 years of age showed that hepatic fibrosis in these dogs would be congenital and juvenile. Histopathologically, the substantial compensatory fibrous connective tissue bundles bridging between two adjacent triad and formed parenchymal nodules were reported in all dogs. Rutger et al. 1993 diagnosed idiopathic hepatic fibrosis by liver biopsy in 15 young dogs, of which 9 were German Shepherd Dogs. In the study previously described (Rutger et al. 1993), clinical signs including progressive ascites, dehydration, anorexia and weight loss (except hepatic encephalopathy) in cases with idiopathic hepatic fibrosis were consistent with the report presented here.

Microcytosis, hypoproteinaemia, increased serum activities of ALP and ALT have been described in dogs with hepatic fibrosis (Rutger et al. 1993). In the study of Rutger et al. 1993, fasting blood ammonia and serum bile acid concentrations were increased in most dogs examined. In a study reported by Thornburg 2000, the serum copper levels in dogs were within normal reference range. In this study, the results of complete blood count revealed microcytosis in all dogs and neutrophilic leukocytosis in some cases (case 3 and 4). Fever in case 3 and 4 with cholecystitis were taken under control with antibiotic therapy. Hypoproteinemia, hyperbilirubinemia, increased ammonia, pre and post prandial bile acids concentrations and ALT,

ALP, AST and GGT levels were consistent with the report previously described (Rutger et al. 1993). In the study reported by Desmet 1998, refractory hyperbilirubinemia in hepatic fibrosis was strongly dependent on some stages of biliary fibrosis. In the cases presented here (in generally young dogs) cholecystitis and hyperbilirubinemia was clearly dependent on fibrous connective tissue bundles bridging between two adjacent triad.

Analysis of intraabdominal fluid in all dogs revealed low protein levels (less than 2.5 mg/dl) and hypocellularity (some neutrophils and lymphocytes) without any bacteria. This result was the same with the reports of Owczarczak 2010, Rutger et al. 1993 and James et al. 2008.

The prognosis of Hepatic Fibrosis is controversial. According to Favier 2009 etiology-based treatments for canine (chronic) hepatitis can provide a better prognosis. In other study (James et al. 2008), poor prognosis in dogs with idiopathic hepatic fibrosis has been reported. In the same study to use of prognostic indicators such as histological, imaging and biochemical profiles were not useful (James et al. 2008). In the study of Rutger et al. 1993, while seven dogs died or euthanized after diagnosis, only one dog were alive during two years. Three dogs were also alive more than four years after the initial diagnosis (Rutger et al. 1993). All the dogs died after 63 days in spite of aggressive and supportive treatment. Surgical cholecystectomy did not also improve the survival in case 2.

In conclusion, several publications and case reports were discussed about congenital predisposition or possibility of idiopathic condition in canine hepatic fibrosis. The cases presented here reflect the congenital condition of idiopathic hepatic fibrosis in young German Shepherd Dogs with histopathological evidence. In the study of Rutger et al. 1993, the dogs of idiopathic hepatic fibrosis were also young and prognosis of the dogs were similar to our cases. However, there was evidence of familial relationship in the cases presented here. Two generation of the same parents showed clinical signs of idiopathic hepatic fibrosis in the age of below 2 year-old. Although the study reflects the possibility of congenital condition of the disease further research about familial relationship in German Shepherd Dogs is required.

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## REFERENCES

- Brown DL, Winkle TV, Cecere T, Rushton S, Brachelente C, Cullen JM.** Congenital hepatic fibrosis in 5 Dogs. *Vet Pathol.* 2010; 47: 102-107.
- Desmet V.** What is congenital hepatic fibrosis? *Histopathol.* 1998; 20: 465–477.
- Center SA.** Chronic liver disease: Current concepts of disease mechanisms. *JSAP.* 1999; 40: 106-114.
- Eulenberg VM, Lidbury JA.** Hepatic fibrosis in dogs. *J Vet Intern Med.* 2018; 32: 26-41.
- Favier RP.** Idiopathic hepatitis and cirrhosis in dogs. *Vet Clin North Am: Smal An Pract.* 2009; 39; 481-488.
- Friedman S L.** Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem,* 2000: 275(4); 2247-2250.
- James FE, Knowles GW, Mansfield CS, Robertson ID.** Ascites due to pre-sinusoidal portal hypertension in dogs: a retrospective analysis of 17 cases. *AVJ,* 2008; 86: 180-186.
- Owczarczak S.** Chronic Hepatic Fibrosis in an 8 year old French Bulldog. Seminar Paper, Cornell University College of Veterinary Medicine, USA, 2010.
- Rutger HC, Haywood S, Kelly DF.** Idiopathic hepatic fibrosis in 15 dogs. *Vet Record.* 1993; 133; 115-118.
- Thornburg LP.** A perspective on copper and liver disease in the dog. *J Vet Diagnostic Invest.* 2000; 12: 101–110.