RESEARCH ARTICLE

Clinical, Ultrasonographic and Haemato-Biochemical Assessment of Liver and Gall Bladder Affections in Dogs

Malhar D. Patel, Pinesh V. Parikh*, Hiren M. Barot

Abstract

A total of 109 dogs were screened, of which 30 dogs with hepatobiliary affections were included for this study. Common clinical findings in hepatobiliary disease were intermittent anorexia, lethargy, weight loss, and vomiting. Ultrasonographic examination showed reduced parenchymal echogenicity with prominent visualization of hepatic vasculature in acute hepatitis; hyperechoic bright liver with normal size in chronic hepatitis and bright and small liver with irregular margins in hepatic cirrhosis. Hepatomegaly on ultrasonography appeared as enlarged liver with rounded margins. Further ascites, hepatic cyst, hepatic neoplasia, cholelith, and gall bladder polyp/mass were diagnosed by using ultrasonography. Based on present findings, it was concluded that ultrasonography could be best exploited in hepatobiliary disorders.

Keywords: Dogs, Gall bladder affections, Haemat-biochemical alterations, Hepatic affections, Ultrasonography.

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Introduction

The liver is a key organ in the body's homeostasis. The liver also plays an important role in digestion, detoxification, and immune surveillance. Hepatic disorders are one of the top five causes of non-accidental deaths in dogs. It is associated with an enormous range of possible clinical signs that include intermittent anorexia, lethargy, polyuria/polydipsia, vomiting, and fatigue, all of which are vague and could be attributed to different physiological systems. Clinical symptoms such as jaundice and ascites usually appear later in illness, thus frequently presenting a diagnostic challenge to veterinary clinicians.

The laboratory evaluation includes complete blood count and serum biochemistry of hepatobiliary patients to determine/confirm the presence of hepatobiliary disease, assess hepatic function, aid in the differentiation of primary and secondary hepatic disease, and contribute to diagnostic planning and treatment response monitoring. Diagnostic ultrasonography is an important diagnostic tool and an outstanding non-invasive technique for an assessment of the liver and biliary system in dogs. Ultrasonography is useful for evaluating various hepatic and gall bladder abnormalities. It is also used to assess the liver size and identify changes in parenchymal echogenicity, mass lesions, distension, and wall thickness of the biliary system (Center, 2009). Hepatic abnormalities in dogs cause focal, multifocal, and diffuse parenchymal echogenicity on ultrasonographic examination. This study was aimed to assess liver and gall bladder affections through clinical, ultrasonographic, and haemato-biochemical analysis in dogs.

Materials and Methods

A total of 109 dogs of either sex and irrespective of age presented to the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Anand, Gujarat (India), from August 2020 to July 2021 were screened for hepatobiliary affections. Out of them, 30 dogs were diagnosed with different liver and gall bladder affections based on ultrasonography. In all dogs, detailed clinical examination was carried out and findings were noted for further evaluation.

Ultrasonography Examination

The ultrasonography was performed using ultrasound machine 'esaote MyLab Five' equipped with a micro convex probe (2.5–7.5 MHz) and 'esaote Mylab 40' equipped with a 4D volume probe (3.5–6.6 MHz). After being muzzled, the animals were placed in dorsal and gently restrained by an assistant holding the forelimbs and hind limbs. Abdominal hair was clipped by wetting the skin with water, soap, or by scrub solution. Shaving was done of the ventral abdominal starting from xiphoid cartilage up to the inguinal region of the animal. By placing an ample amount of acoustic coupling

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gel on the transducer, the probe was placed on the skin of the abdomen and moved across for examination of desired region/organ.

Haemato-Biochemical Parameters

Five-milliliter blood was collected from the cephalic or saphenous vein from each dog, and two milliliters of blood was transferred in K_3-ethylenediamine tetraacetic acid (K_3EDTA) vials for estimation of different haematological parameters, *viz.,* haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), and differential leukocyte counts (DLC). Another 3 mL blood was transferred in a clot activator vial for estimation of different serum biochemical parameters, *viz.,* alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine, and total protein (TP). The data were analyzed to determine the disease-wise mean ± SE values of these parameters and compared with physiological norms.

Results and Discussion

Through the assessment of the affected 30 cases, it was found that the most commonly encountered hepatobiliary affections in present study were hepatitis 13 cases (43.3%), followed by 4 cases of hepatic tumor (13.3 %), 4 cases of gall stone/choleliths (13.3%), 3 cases of hepatomegaly (10%), 2 cases of cholecystitis (6.6 %), 2 cases of cholangiohepatitis (6.6 %), 1 case of hepatic cirrhosis (3.3 %) and 1 case of gall bladder mass/ polyp (3.3%). One case of the hepatic cyst was also observed with hepatitis.

Hepatitis

Dogs with hepatitis showed the symptoms of prolonged partial anorexia, dehydration, pale mucous membrane, dullness, depression, weight loss, emaciation, abdominal pain or discomfort on palpation, chronic intermittent vomiting, and melena. Out of thirteen, two animals were extremely icteric to such an extent that their skin was yellowish. Similar observations were also reported by Chaudhary *et al.* (2008), Kumar *et al.* (2013), and Tantary *et al.* (2014). Pale mucous membrane observed in dogs suffering from hepatic disorders was attributed to the chronic nature of the disease due to increased transient time of erythrocytes through the spleen due to reduced portal blood flow and/or fragility of red blood cells due to high levels of bile acids (Bush, 2002).

In cases of acute hepatitis, reduced parenchymal echogenicity with enhanced visualization of portal vessels was noticed on ultrasonographic examination (Plate 1). Similar findings were also documented by Tantary *et al.* (2014). However, Marolf *et al.* (2012) stated that hepatic parenchyma may appear normal, hyperechoic, or heterogeneous in diffuse hepatic inflammatory processes. In cases of chronic hepatitis, hyperechoic bright liver with normal size was noticed (Plate 2). Tantary *et al.* (2013) found hepatomegaly associated with diffuse hyperechoic liver parenchyma with less distinct portal vessels and accumulation of peritoneal fluid in dogs affected with chronic hepatitis. Shin *et al.* (2007) also performed ultrasonography on 21 dogs and found 8 homogeneous, five hypoechoic, three hyperechoic hepatic echogenicities, and nine dogs had one or more nodules in the liver. They also observed abnormal liver in 6 dogs, normal in three and two dogs had an irregular border to their liver.

In the cases of hepatitis, the mean values of Hb, PCV, TEC, and total protein were markedly decreased with leucocytosis and neutrophilia, while the mean values of serum ALT, AST and creatinine were elevated (Table 1), which concurred with the observations of Shin *et al.* (2007), Chaudhary *et al.* (2008) and Tantary *et al.* (2014). However, contradictory to these findings, Tantary *et al.* (2013) observed unaltered leucocyte counts in chronic hepatitis. In dogs with hepatic dysfunctions,

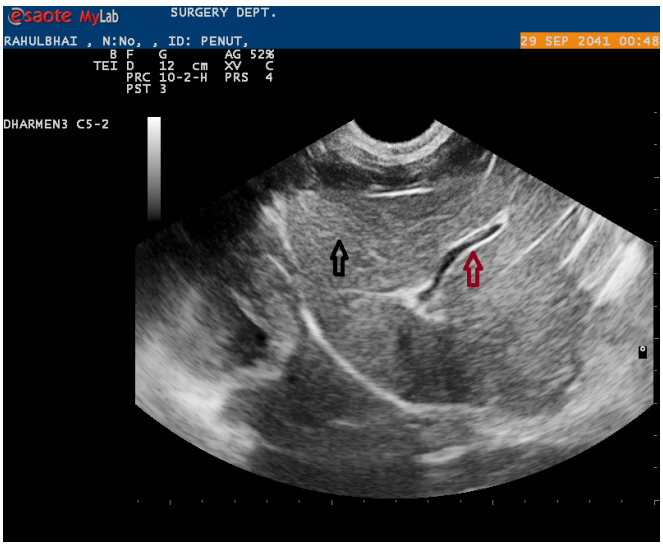

Plate 1: Sonograph showing reduced parenchymal echogenicity (black arrow) with enhanced visualization of portal vessels (red arrow) in transverse plane

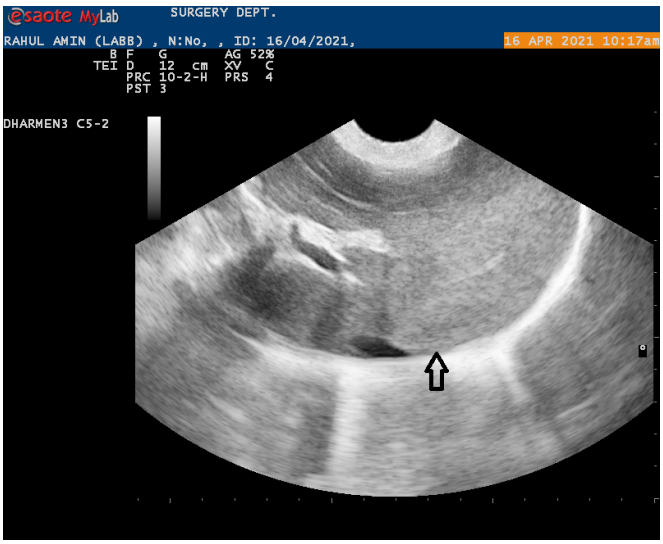

Plate 2: Sonograph showing hyperechoic bright liver with normal size in transverse plane

Table 1: Haemato-biochemical findings in dogs with hepatobilary disorders (mean ± SE)

Condition / Parameters	*Hepatitis (n = 13)*	*Hepatic tumours (n = 4)*	*Hepatic cirrhosis (n = 1)*	*Hepatomegaly with ascites (n = 3)*	*Gall bladder stone (n = 4)*	*Cholangio-hepatitis and cholecystitis (n = 2 each)*
Haemoglobin (g/dL)	9.63 ± 0.88	9.9 ± 0.20	9.6	10.21 ± 1.07	9.53 ± 0.96	9.56 ± 0.45
PCV (%)	32.66 ± 1.81	29.66 ± 2.60	28	30.66 ± 2.06	33 ± 3.21	31.50 ± 2.99
TEC (x10^6/µL)	4.56 ± 0.20	4.53 ± 0.20	4.6	4.44 ± 0.43	4.93 ± 0.24	4.88 ± 0.19
TLC (x10^3/µL)	18400 ± 2680	19000 ± 2271	19700	23650 ± 2238	20800 ± 1700	19500 ± 1793
Neutrophils (%)	83.33 ± 3.33	81.66 ± 2.18	85	84.33 ± 2.01	81 ± 4.16	80.55 ± 3.26
Lymphocytes (%)	13.16 ± 3.15	14.66 ± 2.02	10	11.66 ± 1.97	14.66 ± 3.84	13.70 ± 3.37
Monocytes (%)	2.5 ± 0.34	2.33 ± 0.88	3	2.5 ± 0.67	3 ± 1.15	2 ± 1.0
Eosinophils (%)	1.16 ± 0.16	1.33 ± 0.33	2	1.5 ± 022	1.33 ± 0.33	1.10 ± 0.45
Serum ALT (IU/L)	289.66 ± 46.48	121 ± 3.60	210	184.16 ± 26.44	85 ± 5.13	150 ± 4.13
Serum AST (IU/L)	77.33 ± 12.05	71 ± 2.30	80	70.83 ± 4.02	50 ± 3.21	75.55 ± 2.29
BUN (mg/dL)	21 ± 1.73	20 ± 1.73	21	25.33 ± 6.64	22.66 ± 1.45	-
Creatinine (mg/dL)	1.48 ± 0.23	1.06 ± 0.11	1.4	1.58 ± 0.35	1.13 ± 0.12	-
Total protein (g/dL)	4.46 ± 0.47	4.7 ± 0.26	3.1	4.67 ± 0.23	5.9 ± 0.15	-

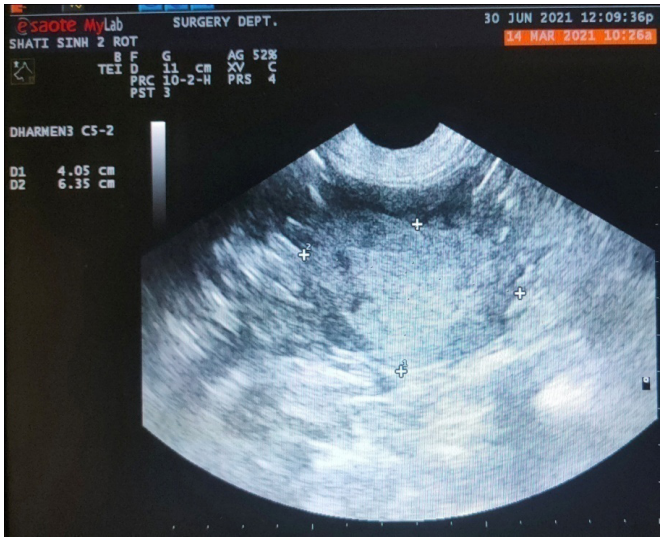

Plate 3: Sonograph showing large focal hyperechoic lesion with a clear border (black arrow) in sagittal plane

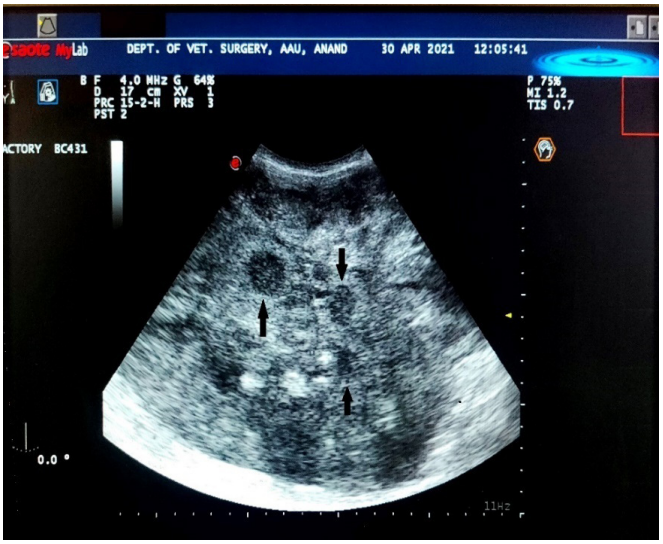

Plate 4: Sonograph showing hypoechoic nodules (black arrow) in hyperechoic liver parenchyma in sagittal plane

serum concentrations of AST and alkaline phosphatase were highly indicative of hepatocellular injury and monitoring clinical progress of the liver (Tennant and Center, 2008). Decrease in haemoglobin was attributed to increased degradation of erythrocytes due to increased transit time through spleen because of reduced portal blood flow or increased fragility of erythrocytes due to high levels of bile acids, besides impaired bone marrow responses, decreased erythrocyte survival time, decreased nutrient uptake due to inappetence or anorexia and reduced availability of micronutrients from liver (Bush, 2002).

Hepatic Tumors

Dogs with hepatic tumors showed palpable abdominal mass, weight loss, anaemia and nonspecific illness. Similar findings were observed by Liptak *et al.* (2004) and Hammond and Pesillo-Crosby (2008). The ultrasonographic examination revealed a large focal hyperechoic lesion with a size of 4.05 x 6.35 cm with clear borders (Plate 3) and hypoechoic nodules in hyperechoic parenchyma in other dogs (Plate 4). Present observations concurred with Cruz-Arambulo *et al.* (2004), who stated that histiocytic neoplasms are more commonly associated with hypoechoic nodules and masses. According to O'Brien *et al.* (2004), ultrasonographic findings of malignant liver nodules in dogs are variable. Masses may be focal or multifocal hypoechoic, focal hyperechoic or mixed echogenicity for hepatocellular carcinoma. Biliary carcinoma has been reported to have multifocal hyper-, hypo-, or mixed echogenicity of the masses. A multifocal hypoechoic or mixed echogenicity pattern has been observed for metastatic hemangiosarcoma. Lymphosarcoma shows diffuse-decreased or -increased echogenicity of the liver.

It may present as focal or multifocal poorly circumscribed hypoechoic areas or well-circumscribed hyperechoic nodules surrounded by areas of hypoechogenicity.

Mean values of Hb, PCV, TEC, and total protein were markedly decreased with leucocytosis and neutrophilia, while serum AST and ALT increased in dogs with hepatic tumors (Table 1), as were reported by Cruz-Arambulo *et al*. (2004) and Liptak *et al.* (2004). High serum levels of ALT and AST were associated with a poor prognosis of hepatocellular carcinoma as the degree of their change was proportional to the extent of hepatocellular injury (Liptak *et al*., 2004).

Hepatomegaly

Three dogs with hepatomegaly and ascites presented abnormally distended abdomen, anorexia, depression, dullness, groaning while lying down, melena, pale mucous membrane and abdominal discomfort. Similar observations were also reported by Saravanan *et al.* (2012) and Kumar *et al*. (2013). Pale mucous membrane observed in ascites dogs was due to anaemia which was associated with hepatic disease such as chronic, usually moderate, non-regenerative inflammatory reactions (defective iron utilization) (Tantary *et al.,* 2014). Out of three cases of hepatomegaly, two cases showed hepatomegaly with ascites, and one case showed hepatomegaly without ascites. In ascites, large anechoic spaces inside the abdominal cavity were identified moderate to massive accumulation of free fluid with remarkable visualization of visceral organs. In cases of hepatomegaly with ascites free-floating of visceral organs in abdominal fluids with coarsened hyperechoic liver, echo patterns with round margins were noticed. The liver had diffusely increased echogenicity with round margins indicative of hepatomegaly (Plate 5). According to Nyland *et al.* (2002), hepatomegaly was diagnosed when the liver extended well beyond the xiphoid cartilage, hepatic margins were rounded or blunt, and liver lobes extended well beyond the gastric fundus or more completely covered the cranial pole of the right kidney.

In cases of hepatomegaly with ascites, the mean values of Hb, PCV, TEC, and total protein were markedly decreased with leucocytosis and neutrophilia, while serum AST, ALT, and BUN were increased (Table 1). Similar findings were observed by Kumar *et al.* (2013), Tantary *et al.* (2013), Saravanan *et al.* (2014), and Tantary *et al.* (2014). According to Webster (2005) and Bigoniya *et al.* (2009), a high decrease in total protein and albumin levels were noticed in ascitic dogs, which could be due to the primary role of the liver in the synthesis of major plasma protein as well as the site of degradation and synthesis of many other proteins that was influenced by liver diseases in many ways. Additionally, hypoalbuminemia might occur in liver diseases caused by the significant destruction of hepatocytes. Ascites was a major clinical sign in chronic hepatitis which might be due to cirrhosis of the liver and was characterized by increased portal vein pressure and decreased production of albumin (Kaneko *et al.,* 2008).

Hepatic Cirrhosis

The clinico-physiological findings of the dogs with cirrhosis were anorexia, emaciation, pale mucous membrane, dullness, weight loss, depression, abdominal distention, and melena. Similar observations were also reported by Vijayanand and Nagarajan (2007) and Elhiblu *et al.* (2015). During ultrasonographic examinations of these cases, liver parenchyma appeared hypoechoic to hyperechoic in echotexture and showed irregular hyperechoic border margins and fibrotic hyperechoic liver lobules (Plate 6). The size of the liver was reduced, and abdominal effusion was also observed. These findings agreed with Elhiblu *et al.* (2015), who assessed the cirrhotic liver in dogs and found generalized and diffuse hyperechoic hepatic parenchyma,

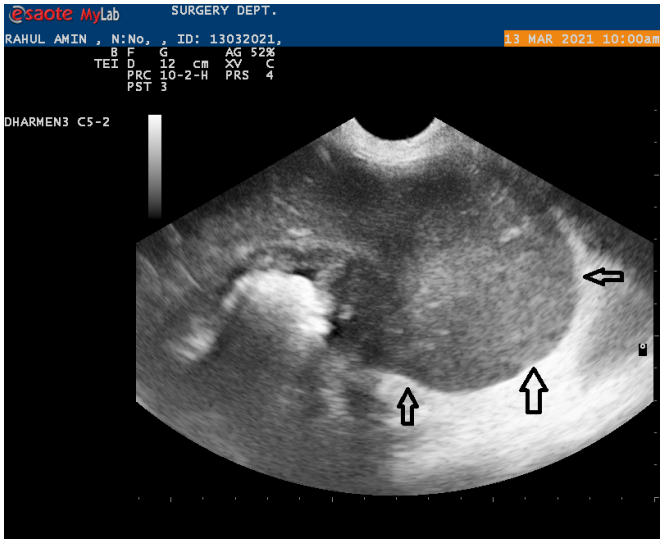

Plate 5: Sonograph showinghyperechoic smooth round liver margins in sagittal plane

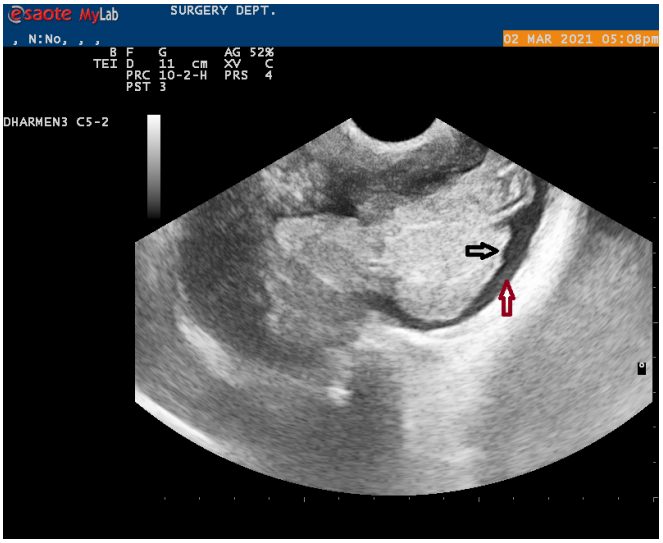

Plate 6: Sonograph showing hyperechoic liver parenchyma with irregular border (black arrow) and peritoneal effusion in sagittal plane

rounded and irregular liver margins, micro-hepatica and distended gall bladder and lots of free anechoic fluids in the abdominal cavity as consistent ultrasonographic features. Our findings were also consistent with Kumar *et al.* (2013) and Tantary *et al.* (2014).

In one cirrhosis case, decreased Hb, PCV, TEC and total protein values with neutrophilia and leucocytosis, and increased serum AST and ALT were observed (Table 1). Similar findings were observed by Vijayanand and Nagarajan (2007) and Chaudhary *et al.* (2008). Since liver cirrhosis was a consequence of chronic hepatitis, neutrophilic leukocytosis and left shift indicated inflammatory response of chronic hepatitis (Elhiblu *et al*., 2015). Hypoproteinemia was the most common finding in chronic disorders like cirrhosis and portosystemic vascular abnormalities (Tennant and Center, 2008). The liver is the main site for the synthesis and degradation of proteins.

Hepatic/Liver Cyst

Physical findings and haematological findings of the hepatic cyst were the same as hepatitis because the hepatic cyst was observed in one of the cases of hepatitis during an ultrasonographic examination. It was characterized by the presence of an anechoic round area with a size of 1.57 to 2.36 cm in the liver parenchyma with clear acoustic enhancement (Plate 7). These findings also supported the earlier report of Chaudhary *et al.* (2008).

Gall Stone/Choleliths

Dogs with stone in the gall bladder had intermittent vomiting, weight loss, dullness, and depression. The sonographic images of gall stone showed rounded hyperechoic structures (Plate 8) associated with distal acoustic shadowing in gall bladder. Similar clinco-sonographic findings were also observed by Ward (2006), and Kumar and Srikala (2014).

In cases of gall bladder stones, the mean values of Hb, PCV, and TEC were markedly decreased with leucocytosis and neutrophilia. Still, the mean values AST, ALT, BUN, creatinine, and total protein observed were within a normal range (Table 1). Similar haematological findings were observed by Ward (2006), Marchetti *et al.* (2007), and Tomar *et al.* (2011). However, biochemical findings were contradictory to the study of Ward (2006) and Marchetti *et al.* (2007).

Cholecystitis/Cholangiohepatitis

Clinical findings of cholecystitis/cholangiohepatitis in dogs were anorexia, lethargy, abdominal pain on palpation, and vomiting, which concurred with the report of Kumar and Srikala (2014). Ultrasonographically it was characterized by uniformly hyperechoic gall bladder wall thickness (0.45 cm and 0.39 cm) (Plate 9). The normal wall thickness of the gall bladder was approximately 1-mm thick in dogs (Hittmair *et al.,* 2001). According to Hittmair *et al.* (2001), inflammation of the gall bladder wall was often associated with gall stones,

Plate 7: Sonograph showing anechoic area (black arrow) with clear acoustic enhancement (red arrow) and right kidney (yellow arrow) in sagittal plane

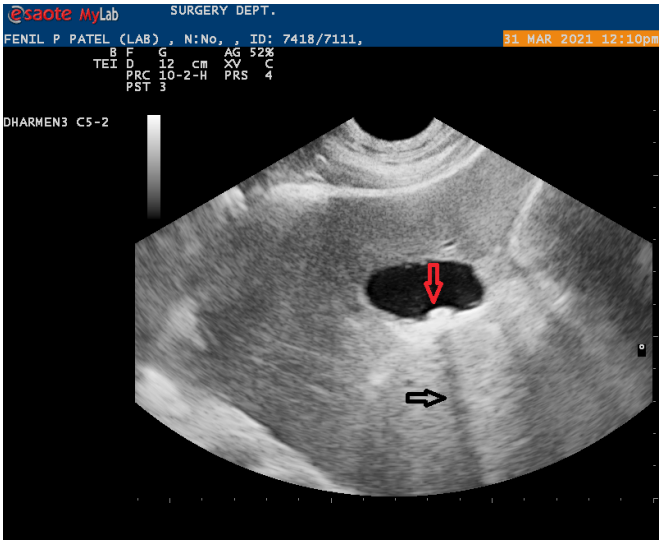

Plate 8: Sonograph showing rounded hyperechoic structure (red arrow) associated with acoustic shadowing (black arrow) in transverse plane

Plate 9: Sonograph showing hyperechoic gall bladder wall thickness

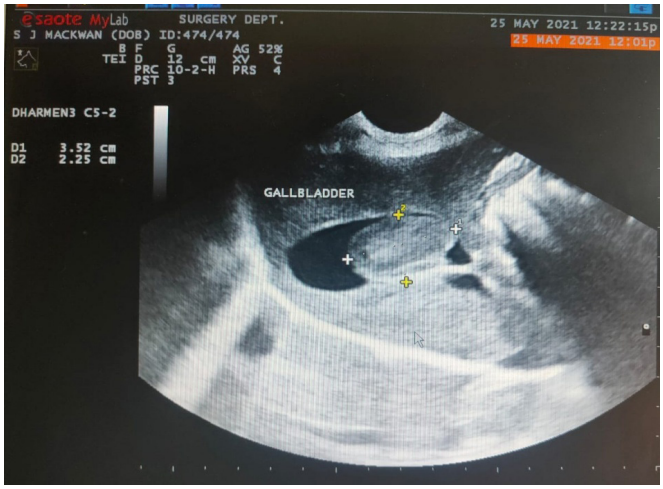

Plate 10: Sonograph showing round hyperechoic structure attached with gall bladder wall

edema, biliary obstruction, and cystic mucosal hyperplasia. Haemato-biochemical findings in cholecystitis/cholangio-hepatitis showed neutrophilia, leukocytosis, and decreased Hb, PCV, TEC. Mean values AST and ALT were mildly elevated. The result of the present study agreed with Kumar *et al.* (2012).

Gall Bladder Polyp/Mass

A dog with gall bladder mass of 2.25 to 3.52 cm large round hyperechoic structure attached within gall bladder lumen was observed during an ultrasonographic examination without distal acoustic shadowing (Plate 10), had symptoms of inappetence with anorexia, chronic vomition since 2 months, weight loss, dullness, depression and abdominal pain on deep palpation. Similar findings were also reported by Birettoni *et al.* (2008) in a dog with primary neuroendocrine carcinoma of the gall bladder. Surgical intervention and histopathology of the sample were indicated in the present study, but the owner refused. Samy *et al.* (2014) also reported similar observations.

Conclusion

In dogs with hepatobiliary disorders, clinico-physiological signs were often misleading. A haemato-biochemical profile indicated only the severity of ongoing diseases for the hepatobiliary system. So it can be concluded that abdominal ultrasonography is an important diagnostic modality for the diagnosis of hepatobiliary affections in dogs.

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