STUDY ON THE COMPARATIVE EFFICACY OF DIAGNOSTIC TESTS IN THE EARLY DIAGNOSIS OF CANINE LIVER DISEASES

SUMATHI, D.
DPV 02006 (CLM)

CENTRE OF ADVANCED FACULTY TRAINING IN VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE
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TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY
CHENNAI - 600 051

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Thesis submitted in part fulfillment of the requirements for the degree of

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In

CLINICAL MEDICINE AND THERAPEUTICS

to the

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CHENNAI - 600 051

2012
Dedication

To my husband and children for their love, patience and support...

To my parents for their love and care...

To my teachers for their guidance...

To all my friends & colleagues who stood by my side when I needed them...

To all pet owners for their co-operation...

Last but not the least to all the pets who had contributed to this study...
CURRICULUM VITAE

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Acknowledgement
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Special Thanks to my husband and kids for their patience and support.

And above all I chant the grandeur of my lord who had been with me in all my steps and gave me the courage and hope.

SUMATHI, D
ABSTRACT

STUDY ON THE COMPARATIVE EFFICACY OF DIAGNOSTIC TESTS IN THE EARLY DIAGNOSIS OF CANINE LIVER DISEASES

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The early diagnosis of liver disease remains a major challenge for the veterinary practitioners. In this backdrop, a study entitled “Study on the comparative efficacy of diagnostic tests in the early diagnosis of canine liver diseases” was planned and conducted with the objectives of evaluating the incidence, etiological pattern, clinico-pathological and ultrasonographic changes of canine liver diseases. The study consisted of 6 healthy dogs acting as controls and 100 cases of various liver diseases, which were divided into three groups viz. parenchymal disorders, biliary disorders and neoplastic disorders. The parameters studied consisted of incidence analysis, medical history, clinical and physical examination findings, hemato-biochemical parameters (Hemoglobin, Total erythrocyte count, PCV, MCV, MCH, MCHC, Total leucocyte count, Differential count, Cholesterol, ALP, ALT, AST, GGT, BUN, Creatinine, Glucose, Total protein, Albumin, Total Bilirubin, Direct bilirubin), Coagulation parameters (Platelet, PT and aPTT), Ultrasonography, cytology and histopathological findings.

The incidence of canine liver diseases was 0.15 per cent of total number of medical cases attended and 0.43 per cent of the gastrointestinal case loads of the Madras Veterinary College Teaching Hospital. The most
common liver disease was that of parenchymal disorders with 73 percent incidence (73/100), followed by biliary disorders 18 per cent (18/100) and neoplastic disorder 9 per cent (9/100). Non-descript dogs dominated the incidence in all the groups of liver diseases followed by Spitz in both neoplastic disorders and parenchymal disorders. In case of biliary disorders German shepherd followed the Non descript dogs in the incidence levels. Dogs aged above four years were most commonly affected and males dominated the incidence in all groups of liver diseases.

Common clinical signs observed in biliary disorders were vomiting followed by anorexia / decreased appetite and weight loss, jaundice and abdominal pain. The commonly observed clinical signs in neoplastic disorders were weakness and anaemia (Tachycardia/Tachypnoea) followed by anorexia / reduced appetite, palpably distended liver (Hepatomegaly), poor hair coat, vomiting, weight loss and ascites. The commonly observed clinical signs in parenchymal disorders were anorexia / reduced appetite followed by weight loss, vomiting, palpably distended liver (hepatomegaly), ascites, diarrhoea and abdominal pain.

Clinico-pathological changes such as erythrogram revealed a significant anaemia in all the three groups. Leucocytosis with neutrophilia was observed in all the three groups. Similarly a significant reduction in total protein and albumin levels was found to be consistent in all the three groups. The total bilirubin and direct bilirubin was significantly elevated in biliary disorders group. Though the elevation in liver enzyme activities was evident they were statistically non significant. Due to these limitations, ultrasound imaging was deployed and the same was found to have better diagnostic yield. Histopathology formed the gold standard in classification of type of liver diseases. 3D ultrasonography studies helped in better visualisation of lesions in liver diseases.

Key words: Canines - liver diseases - Ultrasonography - Diagnosis
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List of abbreviations
LIST OF ABBREVIATIONS

ALP - Alkaline Phosphatase
ALT - Alanine Amino Transferase
aPTT - Activated Partial Thromboplastin Time
AST - Aspartate Amino Transferase
BUN - Blood Urea Nitrogen
CH - Chronic Hepatitis
CAH - Chronic Active Hepatitis
CNS - Central Nervous System
CVC - Caudal Vena Cava
DLC - Differential Leukocyte Count
ECG - Electrocardiogram
Fig. - Figure
Hb - Haemoglobin
HV - Hepatic Vein
MCV - Mean Corpuscular Volume
MCH - Mean Corpuscular Haemoglobin
MCHC - Mean Corpuscular Haemoglobin Concentration
PCV - Packed Cell Volume
PT - Prothrombin Time
RBC - Red Blood Cell
TEC - Total Erythrocyte Count
TLC - Total leukocyte count
TP - Total protein
WBC - White Blood Cell
USG - Ultrasonography
CHAPTER - I

INTRODUCTION

In Veterinary Practice, liver disorders can be frustrating to diagnose. Although in the dog (in contrast to the cat), it is uncommon for a patient to have normal clinical pathology values in the presence of significant liver disease, enzymology and other clinical pathology tests rarely indicate the type of liver pathology present. In addition, even liver “specific” enzymes such as alanine amino transferase can be increased in non-primary hepatic disease and care must be taken in interpreting slight or even moderate increase. This underscores the need for much focus on the tests that may be utilised in the diagnosis of liver disease and the non-hepatic causes for changes in these tests that the clinician should be aware of when interpreting clinical pathology results (Meyer and Harvey, 1998).

When a jaundiced patient is presented for evaluation, it is one of the complicated investigatory tasks for the clinician, as he or she has to evaluate the cause, the severity and complications of the jaundice and try to correlate this with other physical and laboratory and other investigatory findings, before instituting a therapeutic plan.

Clinical signs associated with liver disease are wide-ranging and often non-specific, and consequently laboratory profiles are often run in patients that have a constellation of clinical signs that includes one or more of those seen in liver disease. It is against this background that one should assess the usefulness of laboratory tests.

In practice, the canine patients are presented invariably some time after the damage first occurred and a one-off serum activity is difficult to interpret in terms of severity. Furthermore, pathophysiological processes in diseases that are not primarily hepatic may generate fairly substantial serum
enzyme activity (secondary liver disease or induced enzyme activity). Even those primary diseases, such as parenchymal damage, cholangitis, cholangiohepatitis, chronic hepatitis and diffuse neoplasia, may be accompanied by negligible or no increases in serum enzyme activity. Hence, comparison of the diagnostic utility of liver enzymes and other diagnostic tests or procedures gains much clinical importance.

Several studies had assessed the utility and clinical value of different diagnostic tests for liver disease diagnosis. Assessing the small animal patients with suspected primary heptobiliary disease is rarely a simple process because no single diagnostic test currently available has perfect sensitivity and specificity (Hess and Bunch, 2000).

Radiography had demonstrated limitations with reduced abdominal contrast in presence of ascitic fluid and liver may appear normal even in severe diseased condition (Biller et al., 1992). Nyland and Park (1998) reported that hepatic ultrasonography was an extremely useful procedure for recognition and characterization of many types of disorders involving the liver.

Various diagnostic tests are employed to pinpoint diagnosis of liver diseases. Amongst these, the most easily accessible are pathological values, enzymology and other clinical pathological tests, but they rarely indicate the type of liver pathology present (Mathison, 2001). Serum activities of hepatic enzymes are analyzed as markers for hepatobiliary disease in both dogs and cats. Unfortunately, some of these enzymes are also synthesized in other tissues. Therefore, elevations of serum activities of hepatic enzymes outside the control range can be seen with many non-hepatic conditions (Steiner, 2010).

Salvekar et al. (2010) reported that ultrasonography is a valuable tool for correct diagnosis of liver disease in dogs with altered echotexture of liver parenchyma visualized confirmed various liver diseases like hepatic abscess,
cyst, fatty liver, cirrhosis and hepatic neoplasia and also suggested that best results were obtained by combination of both clinico-pathological and ultrasonographic evaluation for correct diagnosis.

When seeking advice from a veterinarian, pet owners expect accurate diagnosis and definitive therapy of the problem. In very few cases, the diagnosis can be made by history and physical examination alone, but in most cases the veterinarian has to utilize diagnostic tests to arrive at the diagnosis. The challenge for the veterinarian is to choose the most appropriate diagnostic test to arrive at the most accurate diagnosis (Webb et al, 2002).

Proceeding consecutively with the structure of query viz whether diagnosis can be made with preliminary findings, if so whether it is primary or secondary hepatobiliary disease, also the cause of it and understanding the pathophysiology of common liver disorders and characteristic clinical and laboratory abnormalities they may cause, can provide a coherent basis for test selection and interpretation, and most effective means of arriving at a early diagnosis appropriately.

Towards this goal, a study entitled “Study on the comparative efficacy of diagnostic tests in the early diagnosis of canine liver diseases” was planned and undertaken with the following objectives.

1. To study the incidence of liver diseases in dogs.
2. To evaluate the efficacy of the diagnostic tests in the early diagnosis of liver diseases in dogs.
3. To evaluate the efficacy of ultrasound in the diagnosis of liver diseases in comparison with available diagnostic tests.
4. To identify an effective and easy test for the early diagnosis of liver disease in dogs.
Review of Literature
CHAPTER - II

REVIEW OF LITERATURE

2.1. Canine liver disease in clinical practice

Liver disease in pets as well as people is very complex. The liver disease may be frustrating to diagnose and every practitioner feels it. Liver disease in dogs can develop as a result of many different insults (Rutgers, 1996).

Disease of the liver frequently present the small animal clinician with a diagnostic challenge; signs often varied and vague and despite a wide array of diagnostic tests of both hepatic damage and functions; there is rarely a single test that identifies the problem definitively (Hall, 1998).

Regularly encountered forms of primary hepatitis (PH) in dogs include acute hepatitis (AH) and chronic hepatitis (CH) with or without cirrhosis. The less frequently encountered were lobular dissecting hepatitis, granulomatous hepatitis (GH) and eosinophilic hepatitis (EH) (Poldervaart et al., 2009).

2.2. Categorization of canine liver disease

There are a large number of diseases that affect the liver and are generally categorized as primary and secondary diseases. Liver diseases have been classified into various categories on the basis of detailed investigations including hepatic biopsy by other workers (Jarret and O’Neil, 1985 and Reed, 1995).

Primary liver diseases are seen more often in cats than in other animals. They occurred when excess fat - triglycerides accumulates in hepatocytes and bile accumulates in hepatocytes (cholestasis). In Secondary disorders, the hepatic lipidosis occurs secondary to some other clinical
entities such as Diabetes Mellitus, Tetracycline antibiotics, Inflammatory Bowel Disease (IBD), Pancreatitis, Malnutrition or even starvation, Obesity, Hyperthyroidism, Kidney disease, chronic cystitis, Cancer and Cardiomyopathy.

Primary hepatitis (PH) is the most frequently occurring group of liver disease in dogs and comprises all inflammatory hepatic diseases that are not characterized by non-specific changes as observed in non-specific reactive hepatitis (Poldervaart et al., 2009).

Chronic liver disease can be divided anatomically into area of gross involvement (Vascular, Parenchymal and Biliary structures) and on basis of histological characteristics, including lobular distribution, the presence or absence of inflammation, type of inflammation (supportive, non-supportive or mixed infiltrate) and the presence localization and severity of fibrosis (Center et al., 1999).

In humans, chronic hepatitis has been divided into two groups. Chronic persistent hepatitis and chronic active hepatitis based on the microscopic pattern and relation to the inflammatory reaction of liver parenchyma where as the veterinary arbitrary classification has been as ascribed to classify chronic disease as chronic active, lobar dissecting and post necrotic cirrhosis (Fuentealba et al., 1997).

Rutgers and Haywood (1988) summarized the forms of chronic hepatitis that are currently recognized in dogs as hepatic copper accumulation, putative infectious causes and drug induced idiopathic.

WSAVA International Standardization group on liver diseases had divided the liver diseases into four groups viz (a) Vascular liver disorders (b) Biliary disorders (3) Parenchymal disorders including stellate and kupffer cells and (d) Neoplasia (Brovida and Rothuizen, 2010).
2.3. Incidence of canine liver diseases

It has been accounted that hepatic diseases account for three per cent of all diseases seen by veterinarians (Candlin, 1968). The increasing number of liver function tests, coupled with lack of knowledge concerning the pathogenesis of many liver diseases, has created new diagnostic difficulties for the practicing veterinarian (Comelius, 1971).

The percentage of hepatic disease in and around Madras city during 1993 was 0.42 per cent (Nambi, 1993). In the present scenario with the increasing number of pets in the cities, the owners themselves or the practitioners are forced for overzealous medication. Many drugs have been found to adversely affect the functioning of liver leading to signs of hepatopathy (Johnson, 1993).

Liver problems are relatively common in older animals but are by no means restricted to this age group. Anatomical abnormalities of the liver are rare but can cause very serious problems in young animals.

Chronic hepatitis in dogs has been reported with increasing frequency in the past several years (Hardy, 1985 and Rutgers and Haywood, 1988).

According to Fuentealba et al. (1997) among 4,675 cases, 47 cases were hepatitis of which 72.8 per cent was chronic hepatitis.

Boomkens et al. (2004) reported that approximately one per cent of the animals were having hepatitis in the referred canine population. They also stated that dogs were affected more frequently with hepatitis next to human beings and experimental animals.
The incidences of liver diseases, especially the primary liver disease are opined to be less than 10 per cent or even less (DeNicola, 2005). One of the Breed Club Survey put the incidence of liver diseases as 2% and opined that the incidences of liver diseases are on the increasing side in the recent years. (Halstead, 2007)

Poldervaart et al. (2009) observed that primary hepatitis occurred in 0.5% of dogs in a referral population of one-hundred and one dogs, which were examined histologically between 2002 and 2006. In the same study, acute and chronic hepatitis were diagnosed in 21 and 67 dogs, respectively.

The prevalence of chronic hepatitis was 12 per cent in a histopathological study of 200 unselected canine post-mortem examinations from first opinion practices. (Watson et al., 2010)

2.3.1. Breed predilections

Strombeck et al. (1988) reported that the Doberman pinschers had four times greater than the expected level of incidence, as observed from retrospective studies.

Anderson and Sevelius (1991) reported that the breed predisposition of CH was in the decreasing order among the breeds - American Cocker spaniel, Scottish terrier, West highland white terriers, English cocker spaniel and Labrador retrievers though Doberman pinschers showed elevated incidence.

Congenital Shunts had been reported in some breeds of dogs including Yorkshire terriers and Miniature Schnauzers with less than 5 per cent seen in mixed breed dogs (Martin, 1993).

Idiopathic hepatic fibrosis appeared to be a disease of young dogs with a predilection for German Shepherds (Rutgers et al., 1993).
Bedlington terriers developed CH due to an inherited metabolic defect and it had been estimated that 25 per cent and upto 50 per cent might be carriers (Twedt, 2004).

High incidence of CH was recorded in Doberman pinschers compared to other breeds by Mandique et al. (2004).

Shih et al. (2007) reported that Labrador retrievers were predisposed to develop chronic hepatitis that progress to hepatic failure.

Ranjith (2007) reported a higher incidence in mongrels (33.33 per cent), which was followed by Doberman and others breeds.

Poldervaart et al. (2009) observed an over representation from English and American Cocker spaniel, Labrador and Golden retrievers, West highland White and Jack Russell terriers and German pointers.

Pomeranians were over represented for primary or secondary hepatopathies as they were the predominant breed among the local canine population (Pooja et al., 2010).

2.3.2. Sex predilection

No sex predisposition was observed in dogs with primary hepatobiliary tumours (Trigo, 1982).

No significant difference was observed in the occurrence of CH males and females (Strombeck et al., 1988)

Mondelli et al. (1988) documented an increased portion of females as compared to males for CAH.

Anderson and Sevelius (1991) and Fuentealba et al. (1997) reported that males were over represented in American and English cocker spaniel, no sex difference was observed in West highland terriers and females were predominantly affected among Labradors.
Occurrence of chronic hepatitis is most common in Doberman bitches (Speeti et al., 1996).

Poldervaart et al. (2009) reported over representation of females than males among the cases with primary hepatitis in their study.

Hepatitis and cirrhosis were observed to be more prevalent in females and intrahepatic PSS in males (Pooja et al., 2010).

2.3.3. Age Predilection

Strombeck and Gribble (1978) observed that the mean age of all dogs with CAH was 5.3 years.

Primary hepatobiliary tumours were most reported in older dogs (average age = 10 to 11.1 years) (Patnaik et al., 1981).

Thormburg et al. (1983) reported a wide variation in the age of dogs, diagnosed with cirrhosis, which ranged from 8 months to 10 years.

Rutgers and Haywood (1988) stated that a hepatic copper accumulation has been reported in the form of chronic hepatitis in Doberman pinschers which occurred before 2 to 10 years of age.

Anderson and Selevius (1991) found that the age of dogs with histologically confirmed chronic (active / progressive) hepatitis, was five to seven years up on presentation.

The mean age of dogs with hepatic disease was higher than that of dogs showing hepatic reaction and responses to hepatic disease, whereas the mean age of dogs with intra hepatic PSS was lower (Pooja et al., 2010).

Mandigers et al. (2004) reported that liver disease was usually present between four and six years of age.
Shih et al. (2007) reported that the Labrador hepatopathy was observed in middle age to old age dogs with a median age of 7.3 years (range 3.9 to 14 years).

2.4. Etiology of canine liver diseases

A range of causes of hepatopathy have been documented in different publications and case reports, including micro organisms, toxins and drugs, immune mediated reactions and breed associated metabolic errors. Several hepatobiliary disorders have recently come under increased awareness in dogs. Understanding these specific conditions is essential in the diagnosis and management of canine liver disease.

In human, chronic active hepatitis most often occurs as a sequel to viral hepatitis or immune mediated diseases, such as auto immune haemolytic anaemia and systemic lupus erythematosus; In dogs, chronic hepatitis with fibrosis has been induced experimentally with infectious canine hepatitis virus. Additional inflammatory conditions of the pancreas and gastrointestinal tract and prolonged use of corticosteroids and Corynebacterium parvum immunotherapy had been implicated as the cause of chronic hepatic injury in dogs. Copper induced chronic hepatitis culminating in cirrhosis occurred in certain breeds of dogs such as Beddlington terrier as a genetic defect (Fuentealba et al., 1997).

Inherited disorders of copper metabolism had received particular attentions in the last few decades. In spite of significant research efforts, the cause of liver disease remained elusive (Poldervaart et al., 2009)

2.4.1. Vascular liver disorders

Primary vascular disorders are associated with portal vein obstruction due to inflammation, neoplasia, circumscribed fibrosis or after parasitic infections; primary hypoplasia of portal veins as congenital disorder and intrahepatic arteriovenous fistula (congenital); Chronic liver disease such as macro nodular and micro nodular cirrhosis lobar dissecting hepatitis and biliary tumours had led to portal hyper tension (Meyer, 1996 and Rothuizen and Brovida and 2010).
2.4.2. Biliary tract disorders

Bromel et al. (1998) reported 53, 62 and 48 per cent incidences of gall bladder sludge in healthy dogs, dogs with hepatobiliary and other diseases, respectively. Though, sludge is considered as precursor of gallstone in human beings, its clinical importance in canine is still not known (Bromel et al., 1998).

Mild degree of inflammation or biliary stasis was conjectured to be associated with gall bladder sludge (Voros et al., 2001). However, a close and careful investigation is required owing to its resemblance with tumour (Voros et al., 2001), mucocele (Pike et al., 2004) and cholelithiasis (Jensen et al., 1994; Ward, 2007).

Biliary tract disorders include biliary cystic disease and biliary atresia, cholestasis, cholangitis and disease of gall bladder (Brovida and Rothuizen, 2010).

2.4.3. Parenchymal disorders

Many causes of parenchymal disorders remain undetermined; but was associated with leptospirosis, CAV infection, anticonvulsant drugs and copper associated genetic disorder.

Toxic liver injuries were caused by plant and fungal products, drugs or chemicals; hepatic abscesses were caused by bacterial infection reaching the liver by several routes, as portal umbilical veins (particularly seen in new born animals), ascending infection of the biliary system and by direct contact and by penetration of liver capsule in adult animals; the infection may also be caused by Yersinia spp., Nocardia asteroids and Actinomyes spp. (Meyer, 1996 and Brovida and Rothuizen, 2010).
2.4.4. Neoplastic Disorders

Compared with metastatic or secondary cancerous processes involving the liver, primary hepatobiliary tumors were reported to be uncommon in companion animals, representing about 2.6% of canine tumors (Patnaik et al., 1981).

Metastatic, disseminated and locally infiltrating cancers including metastatic carcinoma, melanoma, lymphoma, haemangiosarcoma and histiocytic sarcoma had often affected hepatic parenchyma (Hammer and Sikkema, 1995).

2.5. Pathogenesis of canine liver disease

Hepatic disease may be primary and secondary. The secondary liver diseases were due to diabetes mellitus, pancreatitis, chronic bowel disease, bacterial infection, shock, anaemia and congestive heart failure (Sevelius and Jonsson, 1996).

Intrahepatic cholestasis and accumulations of excess hepatic copper were prominent features of canine active hepatitis in Doberman pinschers (Johnson, 1982).

Obstructive jaundice in dogs is commonly accompanied by neoplastic disease; In intrahepatic cholestasis, obstructive jaundice occurred due to accumulation of intra canalicular bile in the absence of any anatomical obstruction to bile flow through the biliary tract (Sherloch, 1968 and Meyer, 1996).

In human, CAH was a specific and potentially steroid responsive disease with a suspected immune pathogenesis, whereas the role of auto immunity in canine hepatitis was uncertain (Rutgers and Haywood, 1988).

In dogs, infections have been considered to be of less importance in pathogenesis of chronic hepatitis (Anderson and Sevelius, 1991). Canine copper toxicosis was an autosomal recessive disorder with a high frequency in Bedlington terriers (Brewer, 1998).
Hepatic encephalopathy was seen in a number of liver diseases. Clinical signs suggestive of hepatic encephalopathy included circling, head pressing, aimless wandering, weakness, ataxia, blindness, ptyalism, aggression, dementia, seizures, and coma. Although the pathophysiology was not completely understood, a synergistic effect between the failure of the liver to clear several neurotoxins (ammonia, mercaptans, and short-chain fatty acids) and an imbalance in plasma amino acids (β-aminobutyric acid [GABA], aromatic amino acids) along with an increased sensitivity of the brain to these changes were considered to be the major contributing factors (Hess and Bunch, 2000).

Currently, ammonia was considered as the primary neurotoxin contributing to the clinical signs of hepatic encephalopathy. Colonic bacteria metabolize proteins and urea into un-ionized ammonia, which was readily absorbed into the portal circulation. In animals with normal liver function, most of the ammonia was removed by hepatocytes and converted into amino acids or urea. However, with liver failure or in the case of portosystemic shunts, in which the portal blood bypasses the liver, blood ammonia levels remained high because of inadequate detoxification. Blood ammonia levels were also found to be increased during GI bleeding, azotemia, alkalosis, hypokalemia, and anorexia. Elevated ammonia levels has had an inhibitory effect on the CNS (Webb et al., 2002).

Clinical signs had also been attributed to an imbalance of the ratio of branched-chain amino acids to aromatic amino acids. In liver dysfunction, the ratio of branched-chain amino acids to aromatic amino acids was decreased because of increased utilization of branched-chain amino acids by myocytes and decreased hepatic clearance of aromatic amino acids. Increased CNS uptake of aromatic amino acids was favored due to the imbalance (Laflamme, 2000 and Webb et al., 2002).

Ascites in patients with liver disease was secondary to a combination of portal hypertension and an imbalance in sodium and water homeostasis. Portal hypertension could be hepatic, due to intrahepatic obstruction;
posthepatic, due to obstruction of the portal veins or increased portal blood volume; or prehepatic, due to obstruction or kinking of the caudal vena cava or secondary to right heart failure. Causes of hepatic portal hypertension included inflammation, fibrosis, necrosis, regenerative nodules, arteriovenous fistulas, or neoplastic masses. Imbalance in sodium and water homeostasis had either preceded or resulted from portal hypertension. Ascites could also be exacerbated by hypoalbuminemia. Cytologic evaluation of the ascitic fluid seen with hepatic failure was usually consistent with a modified transudate. (Hess and Bunch, 2000 and Webb et al., 2002)

GI bleeding could be seen in animals with liver disease due to ulceration or coagulation abnormalities. The cause of ulceration was multifactorial. Hypoalbuminemia could lead to decreased turnover of gastric mucosal cells. The integrity of the gastric mucosal layer could also be affected negatively by portal hypertension and increased levels of histamine, both of which could cause mucosal edema. Increased bile acids within the GI lumen can both decrease the effectiveness of the mucosal barrier and increase lumen pH (Laflamme, 2000 and Webb et al., 2002).

2.5. Clinical Presentation

The clinical signs in dogs with hepatic fibrosis revealed portal hypertension and acquired predominately rather resulting in ascites and hepatic encephalopathy; jaundice was also observed (Rutgers et al., 1993).

Clinical signs of chronic hepatitis were in general, non specific, and may be asymptomatic. It may have vague symptoms such as depression, anorexia, weight loss, vomiting and diarrhoea. In advanced disease, signs included icterus, ascites, polyuria and polydipsia as well as neurological signs suggestive of hepatic encephalopathy (Rutgers and Haywood, 1988).

Clinical signs were parallel to the extent of hepatic damage and the early signs were vomiting, diarrhoea and poor appetite or anorexia; whereas the disease progression resulted in ascites, jaundice and hepatic encephalopathy (Twedt, 1998; Rutgers and Haywood, 1998 and Sterczer et al., 2001).
According to Shih et al. (2007) most of the dogs had vague signs of decreased appetite, vomiting, lethargy, weight loss but some where asymptomatic except for increases in serum liver enzymes.

Clinical signs were vague and varying from decreased appetite, anorexia, nausea, vomiting, ascites, weakness, weight loss, pale mucosa, epigastric pain, pyrexia, bilateral hind limb edema, constipation, diarrhoea, melena, icterus, encephalopathy, polyuria, polydipsia to depression in different combination (Pooja et al., 2010).

2.7. Laboratory findings

2.7.1. Haematology

Non specific hematologic abnormalities were common and included mild to moderate anaemia and leukocytosis (Patnaik et al., 1981).

Fuentealba et al. (1997) stated that haematological values were unremarkable in case of chronic hepatitis.

Haematologic changes observed included the development of anaemia, abnormal erythrocyte morphology (acanthocytes, poikilocytosis) reduced platelet number or function and lipaemic plasma (Center, 1996).

Hepatobiliary disease was observed to be associated with a variety of CBC abnormalities; Congenital PSS in dogs and cats resulted in microcytosis without anaemia (Hess and Bunch, 2000).

Howe et al. (2000) reported in portosystemic shunts that there might be a mild, non-regenerative anaemia, microcythemia with normochromic erythrocytes and target cell formation.

Stockhan and Scott (2002) tabulated the CBC test results that suggested or indicated hepatic disease or dysfunction as following:
<table>
<thead>
<tr>
<th>Hematological findings</th>
<th>Hepatic lesion suggested</th>
<th>Pathogenesis of findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthocytosis</td>
<td>Haemangiosarcoma, lipid metabolism defect</td>
<td>Possible vascular trauma, altered lipid composition of erythrocyte membrane</td>
</tr>
<tr>
<td>Anaemia</td>
<td>Hepatitis, decreased functional mass</td>
<td>Anaemia of inflammatory disease possibly decrease EPO or abnormal proteins as amino acid metabolism</td>
</tr>
<tr>
<td>Codocytosis</td>
<td>Decreased functional mass</td>
<td>Altered lipid composition of erythrocyte membrane</td>
</tr>
<tr>
<td>Microcytosis</td>
<td>Decreased Functional mass, portosystemic shunt</td>
<td>Possibly decrease transferrin production and thus decrease delivery of iron to erythrocyte precursors</td>
</tr>
</tbody>
</table>

Chronicity of hepatic disease often led to non regenerative anaemia (Alvarez and Whitlemore, 2009).

### 2.7.2. Biochemistry

Various liver function tests which were well established in human diagnosis were applied to the sera of dogs. Stockhan and Scott (2002) tabulated the serum chemistry assay results that suggested or indicated hepatic disease or dysfunction as following:
<table>
<thead>
<tr>
<th>Biochemical findings</th>
<th>Hepatic lesion suggested</th>
<th>Pathogenesis of findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased Urea Nitrogen Hyperammonemia</td>
<td>Decreased functional mass</td>
<td>Decreased urea production</td>
</tr>
<tr>
<td></td>
<td>Decreased functional mass</td>
<td>Inadequate fixing of NH₄⁺ into urea</td>
</tr>
<tr>
<td></td>
<td>Portosystemic shunt</td>
<td></td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>Cholestasis</td>
<td>Inadequate biliary excretion of bilirubin</td>
</tr>
<tr>
<td></td>
<td>Decreased Bc transport</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Cholestasis</td>
<td>Increased production of cholesterol and decreased clearance of lipoproteins</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>Cirrhosis</td>
<td>Hyperglucagonemina or increased gluconeogenesis of hepatocutaneous syndrome</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>Decreased functional mass</td>
<td>Decreased albumin production</td>
</tr>
<tr>
<td>Hypocholesterolemia</td>
<td>Decreased functional mass</td>
<td>Decreased Cholesterol synthesis</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Decreased functional mass</td>
<td>Decreased gluconeogenesis</td>
</tr>
<tr>
<td>Hypoproteinemia</td>
<td>Decreased functional mass</td>
<td>Decreased production of albumin and globulin</td>
</tr>
<tr>
<td>Increased ALT, AST</td>
<td>Damaged hepatocytes</td>
<td>Release of cytoplasmic enzymes due to blebbing or necrosis</td>
</tr>
<tr>
<td>Increased ALP activity</td>
<td>Cholestasis</td>
<td>Increased production of ALP</td>
</tr>
<tr>
<td>Increased GGT activity</td>
<td>Biliary hyperplasia</td>
<td>Increased production of GGT</td>
</tr>
<tr>
<td>Lipemia (gross)</td>
<td>Decreased functional mass</td>
<td>Decreased clearance of lipoproteins</td>
</tr>
</tbody>
</table>
2.7.2.1. Alanine Amino Transferase (ALT)

Sevelius (1995) opined that serum ALT concentrations were significantly elevated in chronic non-specific hepatitis as compared with chronic progressive hepatitis.

High activity of serum ALT in Doberman pinchers with chronic active hepatitis was recorded (Crawford et al., 1985 and Fuentealba et al., 1997).

Sterczer et al. (2001) reported that serum biochemistry characterized by elevated serum alanine amino transferase (ALT) activity was consistent with the ongoing hepato cellular damage.

Center (2007) reported that the plasma ALT activity values achieved ten fold greater than normal in chronic hepatitis.

2.7.2.2. Aspartate Amino Transferase (AST)

AST elevation with elevated ALT suggested more severe damage than ALT alone (Mc Connell and Lumsden, 1983; Dunn, 1992; Center, 1996 and Sevelius Johnson, 1996). Dogs affected with hepatocellular carcinoma had elevated ALT, AST and ALP (Patnaik et al., 1981).

Hall (1996) stated that persistently elevated AST was a poor prognostic sign and smaller increases in AST were seen in chronic hepatitis and cholestasis disease.

Altered permeability of the hepatocellular membrane caused by injury or a metabolic disturbance result in a release of soluble enzymes. Subsequent to an acute, diffuse injury, the magnitude of ALT increase in the plasma crudely reflected the number of affected hepatocytes. In chronic inflammatory liver disease, the magnitude of ALT rise does not relate to the degree of pathology. (Hess and Bunch, 2000 and Webb et al., 2002).
A variety of tissues, notably skeletal muscle and liver, contained high aspartate aminotransferase activity (AST). Because myositis and myocarditis were uncommon diseases in the dog and cat, a rise in the plasma AST activity generally indicated hepatic pathology. (Meyer and Twedt, 2000 and Webb et al., 2002).

Past clinical experience suggested that interpretation of the both aminotransferases could provide an additional insight into the evaluation of certain types of hepatic pathology. Following an acute injury resulting in a moderate to marked increase in the serum ALT and AST activities, the serum AST activity would return to normal more rapidly (hours to days) than the serum ALT activity (days) due to their difference in plasma half-lives and cellular location (Hess and Bunch, 2000 and Webb et al., 2002).

It was opined that by determining these values, every 2 to 5 days, in the dog following an acute insult, a sequential "biochemical picture" indicative of resolution could be obtained; Persistent mild to moderately high serum ALT and AST values (documented multiple times over weeks to months) suggested a potentially serious, "smoldering" inflammatory process, chronic hepatitis. The persistent, often fluctuating abnormal aminotransferase values were probably a consequence of increased release subsequent to cell injury and on-going hepatocellular reparation or regeneration. (Meyer and Harvey, 1998 and Laflamme, 2000)

Serum AST was more sensitive than serum ALT in the detection of hepatobiliary disease, although it is considerably less specific because significant amounts of AST are also contained in muscle (Brovinda and Rothuizen, 2010).

2.7.2.3. Alkaline Phosphatase (ALP / SAP)

The sensitivity of ALP was high in chronic cholangio-hepatitis (Sevelius, 1995).
The increase in ALP with hepatic necrosis and chronic hepatitis were found to be usually three to six times the upper normal limit (Center, 1996).

The elevation of ALP reflected intra or extra hepatic cholestasis and was mainly due to the increased synthesis and decreased clearance (Selvelius and Johnson, 1996).

Minimal ALP increase was reported in the plasma, following an acute, severe insult in contrast to ALT and AST (Meyer and Harvey, 1998 and Meyer and Twedt, 2001).

It was opined that any initial rise was probably a reflection of enzyme activity on cell membrane fragments released to the plasma as a consequence of the damage and the initiation of increased synthesis due local cholestasis associated with damaged liver. During hepatic reparation following an injury, the serum aminotransferase activity slowly decreased while the serum ALP activity often increased until the intrahepatic architecture has recovered. Consequently, the serum ALP activity was usually the last serum hepatic enzyme test to return to the reference range following resolution of an acute insult. (Laflamme, 2000 and Webb et al., 2002).

Pathology that primarily obstructed the flow of bile, whether intrahepatic or extrahepatic, was initially associated with a rise in serum ALP activity followed by a raised serum total bile acid concentration and finally hyperbilirubinemia if the cholestatic process was protracted and sufficiently severe. None of these indicators of cholestasis alone were diagnostically reliable for differentiating extrahepatic and intrahepatic cholestatic disorders (Hess and Bunch, 2000 and Webb et al., 2002).

An increase in the serum ALP activity was often associated with the use of glucocorticoid medications and hypercortisolemia. The high frequency of a raised ALP value in association with corticosteroids was unique to the dog and there was considerable individual variation in the hepatic response and magnitude of ALP rise (Laflamme, 2000).
The corticosteroid-associated ALP isoenzyme could be distinguished from the cholestatic induced liver ALP isoenzyme by several procedures and had been proposed for the differential diagnosis of hyperadrenocorticism. Unfortunately, a raised value had been shown to be associated with hepatobiliary disease, diabetes mellitus, hypothyroidism, acute pancreatitis, and phenobarbital treatment (Laflamme, 2000 and Webb et al., 2002).

2.7.2.4. Gamma Glutamyl Transferase (GGT)

Guelfi (1982) reported that ALP was not very specific to the liver and serum GGT should therefore be used to confirm the cholestatic origins of increase in ALP.

Increases in GGT levels were noticed in chronic hepatitis; there were some correlation between ALP and GGT and GGT and Cholesterol (Fuentealba et al., 1997).

Center (2007) stated that ALP and GGT were membrane bound enzymes and located in biliary membrane and hence they were elevated in cholestatic disorders.

In dogs, moderate to marked increase in GGT were seen in intrahepatic and extrahepatic cholestasis, while mild elevations were seen with acute hepato cellular injury (Rothuizen and Brovinda, 2010).

2.7.2.5. Total and Direct Bilirubin

The serum bilirubin in dogs with biliary obstruction contained 60 to 90 per cent direct reacting (conjugated) bilirubin (Vleet and Alberts, 1968). The serum vanden bergh test was an extremely useful test because it measured the indirect reaction of the unconjugated pigment associated with haemolysis as well as conjugated, prominent in obstructions (Cornelius, 1979).
Elevation of bilirubin was reported in canine hepatocellular carcinoma, canine biliary carcinoma and canine neuroendocrine epithelial tumors (Patnaik et al., 1981).

Twedt (1998) found that 75 per cent of the cases with chronic hepatitis had abnormal bilirubin evaluations. Shih et al. (2007) found it abnormal in 45 per cent cases in a study of 21 Labrador dogs.

2.7.2.6. Total Protein and Albumin

Mwanza et al. (1998) found that a gradual decrease in A/G ratio in an experiment involving surgical occlusion of common bile duct.

Stein et al. (1989) observed normal levels of serum albumin and total proteins in both sudden and gradual common bile duct obstructions in canine models.

Colli et al. (1991) opined that thickening of gall bladder wall in patients with ascites was commonly related to hypoalbuminemia and/or portal hypertension.

Serum total albumin levels were markedly decreased in liver cirrhosis with high sensitivity and moderately decreased in chronic progressive hepatitis with fairly high sensitivity, indicating that hypoalbuminemia was an important marker of chronic inflammatory liver disease, when other causes of protein losing disorders were ruled out (Sevelius, 1995).

Twedt (1998) observed that albumin level falls as hepatic function declines in chronic hepatitis.

Bromel et al. (1998c) found an increased plasma protein concentration in case of canine porcelain gall bladder.

Besso et al. (2000) recorded a mild hypoalbuminemia in few dogs with gall bladder mucocele.
Hypoalbuminemia was observed in cases with chronic hepatitis (Twedt, 1998; Sterczer et al., 2001 and Shih et al., 2007).

Tiwari et al. (2001) recorded low levels of serum proteins and albumin in cases of biliary obstruction.

Sharma et al. (2001) believed that the decrease in albumin and increase in globulin levels in hepatobiliary disorders led to decrease in Albumin Globulin ratio.

Hypoalbuminemia and reduced total serum protein was observed in primary hepatitis in dogs (Poldervaart, 2009).

2.7.2.7. Blood Urea Nitrogen (BUN) and Creatinine

Cosenza (1984) observed of BUN levels of 9 mg / dl in obstructive jaundice. Moentk and Biller (1993) found an elevated level of creatinine in a cat having bilobed gall bladder.

Rutgers et al. (1993) reported a slightly high BUN concentration without an accompanying increase in creatinine in idiopathic hepatic fibrosis.

Sevelius (1995) observed a decreased BUN concentration in chronic liver disease as a consequence of impaired metabolism of ammonia. He further stated that low BUN levels were found in all types of chronic hepatitis and hence it could not be used for prognostic evaluation.

Laflamme (2000) observed that the hepatic insufficiency could result in reduced serum urea nitrogen (BUN) concentration relative to the serum creatinine concentration and a raised plasma ammonia concentration.

Tiwari et al. (2001) found higher levels of BUN in biliary obstruction and attributed it to anorexia.
Bexfield and Watson (2006) observed that the low blood urea nitrogen could occur in liver disease, reflecting a reduced ability to synthesis urea from ammonia in the hepatic urea cycle.

2.7.2.8. Glucose

Kimura et al. (1991) observed severe hypoglycemia with cholestasis caused by hypoplasia of the interlobular ducts in a premature infant. Younes et al., (1991) noted of significant decrease in the glucose levels in experimentally induced obstructive jaundice in rats which returned to normal following decompression.

Bromel et al. (1998a) found decreased glucose levels in a case of gall bladder perforation. Bromel et al. (1998d) reported hypoglycemia in a case of porcelain gall bladder, associated with adenocarcinoma.

Sevelius (1995) and Strombeck et al. (1988) observed that hypoglycemia due to impaired carbohydrate metabolism in the liver was a rare finding and indicated that glucose was a bad prognostic marker.

Center (1996) reported that abnormalities of the blood glucose concentration were not reliable indicators of chronic hepatitis unless end stage liver disease or systemic shunting has developed.

2.7.2.9. Cholesterol

Magne et al. (1985) and Matthiesen and Rosin (1986) reported of hypercholesterolemia in dogs with bile duct obstructions.

Mwanza et al. (1998) noticed of rapid increase in total cholesterol after bile duct ligation to peak values, which gradually decreased; but remained high throughout the experiment period.

Bromel et al. (1998d) reported of hypercholesterolemia (530 mg / dl) in a dog with porcelain gall bladder.
Center (1996) opined that hypercholesterolemia was associated with increased biliary excretion of cholesterol. There were no dependable trends in the total serum cholesterol concentration in canine and feline species unless chronic hepatitis is severe. In that circumstance of hepatic failure, hypocholesterolemia may develop in both dogs and cats (Center, 1996b).

Cholesterol was evaluated in 13 of the 22 dogs with chronic hepatitis; in more than 50 per cent cases, of chronic active hepatitis and in all cases of fibrosing hepatitis with cirrhosis (Fuentealba et al., 1997).

Meyer and Harvey (1998) were of view that hypercholesterolemia developed in cholestatic disease because it was eliminated from the body through both formation of bile acids and its dissolution in bile.

Hall (1998) concluded that the usefulness of serum cholesterol concentration as a marker of hepatobiliary disease was limited as its concentration might be decreased, normal or increased depending upon the type of disease and the dietary intake.

Banerjee (2003) reported that there was no significant difference in serum cholesterol levels among various groups of hepatitis.

Out of 24 labrador dogs affected with chronic hepatitis, 6 was reported to have increased cholesterol concentration (median 400 mg / dl, range 316-528 mg / dl) and 1 had low cholesterol levels (Shih et al., 2007).

2.7.3 Coagulation profile

Badylak (1988) observed that PT and APTT tests for detecting hepatic disease in the dog had proved useful in a clinical setting when adequate laboratory controls were established.

Screening tests evaluating the plasma coagulation pathways such as activated partial thromboplastin time (aPTT) and Prothrombin time (PT) were found to be useful tools in accurately identifying the abnormality (Giurgiu et al., 2009).
Prolonged PT and APTT were observed in dogs with chronic hepatitis (Poldervaart et al., 2009 and Prins et al., 2010).

2.8. Hepatic Ultrasonography in Small Animals

The first papers concerning the use of ultrasound to examine the abdominal organs of the dog and cat were published in 1981 (Cartee, 1981 and Nyland et al., 1981). Since then, ultrasonography has become an essential imaging tool for identifying abnormalities of the liver parenchyma, biliary tract and vascular system.

Initially papers described the positioning of the transducer, the topographical anatomy of the canine liver, the normal ultrasonographic appearance of the liver and cases of hepatic neoplasia, cirrhosis and cholelithiasis.

In 1985 ultrasound-guided liver biopsy was described (Hager et al., 1985), but most of the publications addressed methodology and the ultrasonographic appearance of liver diseases such as chronic hepatitis and cirrhosis.

In 1987 the ultrasonographic diagnosis of portosystemic shunts was reported by Wrigley et al. (1987). In 1989 Kantrowitz et al. described the measurement of portal blood flow velocity by means of pulsed Doppler (Kantrowitz et al., 1989).

Since 1990 the evolution of ultrasound machines and the wide availability of duplex Doppler allowed better description of the vascular anatomy of the liver (Carlisle et al., 1992). In those years the ultrasonographic diagnosis of portosystemic shunts replaced portography (Lamb, 1991).

At the same time, the diffusion of ultrasound-guided liver biopsy increased the knowledge of hepatic pathology (Biller et al., 1992).
The first report concerning the use of an ultrasound contrast medium was published in 2000 (Bahr et al., 2000), but most of the papers on contrast media have appeared since 2003, when specific software was developed to improve their use.

Today the liver ultrasonography of the dog and cat was the synthesis of these 25 years of history. The technological improvement of ultrasound machines had certainly conditioned either the diagnostic accuracy of this diagnostic technique, or our skill in small animal clinic. Now we were able to consider diseases such as cholecystitis or cholangitis in the differential diagnosis of jaundice, or we could make an early (sometimes serendipitous) diagnosis of a hepatic mass by examining the liver of a dog with biochemical abnormalities and no specific symptomatology.

Abdominal ultrasonography is routinely being used to identify hepatic parenchymal changes. Evaluation of the canine liver required a multi-faceted approach. This was because accurate assessment for liver disease requires consideration of both liver anatomy and liver function. Anatomic information needed includes gross evaluation of hepatic lobar architecture, histology, vascular anatomy, and estimated portal vein blood pressure. A complete examination of the liver includes the evaluation of the parenchyma, portal and hepatic veins and the biliary system.

2.8.1.1 Ultrasonographic Anatomy of Liver

The structures observed in the liver on ultrasound examination were the gall bladder, seen as a round or oval anechoic structure towards the midline, the CVC, the portal veins and the hepatic veins. With the transducer placed immediately caudal to the xiphoid process and directed cranially, the portal vein and the gall bladder were visualized. These two structures served as important land marks for further examination (Nyland and Park, 1983 and Mwanza et al., 1998).
By rotating the transducer into a transverse position and slightly caudal to the gall bladder neck, some hepatic veins were observed. The right and left branches of the portal vein were not clearly visualized in the same scan as their branching does not occur at the same place. The intrahepatic branches of the portal vein were differentiated from the hepatic veins by their echogenic walls (Mwanza et al., 1998).

Through the 11th or 12th right intercostal space, the portal vein and the CVC were easily visualized in transverse scans. From the left side, hepatic veins were recognized near the diaphragm. Here the veins from the left and medial lobes of the liver joined to form the main left hepatic vein, a major trunk draining into the CVC. The portal veins became smaller as they go deeper into the parenchyma while the hepatic veins became larger as they flow towards the CVC (Nyland and Park, 1983 and Mwanza et al., 1998).

2.8.2 Ultrasonography in Hepatic mass lesions

Ultrasonographic evaluation of hepatic masses consisted of quantification and localization of the masses, characterization of their echogenicity, identification of their distribution (solitary or diffuse), and observing for evidence of cystic or vascular components. However, the ultrasonographic appearance of benign lesions such as nodular hyperplasia and many malignant lesions may appear similar in dogs.

The difficulties in ultrasonographically differentiating between benign and malignant hepatic lesions have been reported (Voros et al., 1991). In this study, the ultrasonographic interpretation of 22 canine hepatic lesions resulted in a correct diagnosis in only 50% of cases, with more than 35% of focal benign lesions being ultrasonographically classified as neoplastic.

Although the ultrasonographic appearances of hepatic lesions were often nonspecific, some studies had attempted to characterize ultrasonography findings which were most consistent with liver cancer. Some lesions identified ultrasonographically within visceral organs may take on a
halo effect and were called target lesions because of a hypoechoic rim surrounding an isoechoic to hyperechoic center. Target lesions were associated with malignancy, with a positive predictive value of 74% for a focal lesion and 81% for multiple lesions (Cuccovillo and Lamb, 2002)

2.8.3 Ultrasonography in Parenchymal pathology

Nodular hyperplasia appeared as a homogenous or a diffuse mixed mass lesion with either increased or decreased echogenicity (Nyland, 1984).

Voros et al. (1991) correlated ultrasonography and pathomorphological findings in dogs with liver disease and suggested to combine clinico laboratory and ultrasonographic findings to achieve best possible diagnostic result.

Barr (1991) observed a diffuse, heterogenous disturbance in the normal even echotexture, irregularity of the margins of the liver lobes with free fluid and multiple tortuous blood vessels in the prehepatic region in cirrhosis.

Nodular hyperplasia was found to be present as a solitary finding or as multiple lesions. It was suspected that the presence of nodular hyperplasia might not be detected in many animals because the echogenecity of the lesion were not distinct (Center, 1996).

Sharma et al. (2001) described the merits of ultrasound as its ability to characterize internal parenchymal architecture, cost effectiveness and non radiation hazards.

Cuccovillo and Lamb (2001) reported that the target lesions were seen in ultrasound images of the liver or spleen as nodules or masses with a hypoechoic rim (or halo) and hyperechoic or isoechoic centre.

Banerjee (2003) found that in chronic hepatitis, the liver was bright with irregular border and histopathology revealed nodular hyperplasia and extensive fibrosis.
Bexfield and Watson (2006) observed that ultrasonography was an extremely useful tool in the investigation of most cases of hepatobiliary diseases and also helps to differentiate between focal and diffuse hepatic disease. It was stressed that an ultrasonographic diagnosis was not equivalent to a histological diagnosis and that histology was necessary to confirm the diagnosis.

2.8.4 Ultrasonography in Gallbladder and biliary disease

The biliary sludge was found to appear sonographically as a gravity dependent, echogenic material within the gall bladder or it mimicked a mass lesion within the lumen at which point it was called as tume failure sludge (Fakhery, 1982 and Newell et al., 1995).

The ultrasound finding of gall bladder wall thickening in patients with ascites was highly predictive of liver cirrhosis (Huang et al., 1989; Georgeier and Meechkov, 1991 and Brogna et al., 1996).

The use of ultrasonography for evaluation of gall bladder, identification of gall stones, assessment for extrahepatic biliary obstruction and other gall bladder abnormalities was advocated by several authors (Newell et al., 1995; Church and Matthiesen, 1988; Nyland and Gillett, 1982; Cartee, 1981; Cartee et al., 1993; Lamb, 1991, Finn Bodner et al. 1993 and Fahie and Martin, 1995, Kurtz and Middleton, 1996 and Sterczer et al., 2000).

The increased echogenicity of hyperechoic thickening of gall bladder wall with or without an irregular mucosal contour had been associated with cystic hypertrophy of the mucous producing glands of the gall bladder mucosa, a normal aging change in older dogs and biliary neoplasia (Lamb, 1991 and Burk and Ackerman, 1996).

Pandey et al. (1996) were of view that ultrasound could be used as a screening modality for the early detection of carcinoma of the gall bladder.
Kurtz and Middleton (1996) were of view that the normal gall bladder wall was sonographically visible as a thin echogenic line which typically measured about 2.03 mm in thickness and this varied with transducer type and placement or angulation of the sound beam relative to the organ insonated.

Besso et al. (2000) concluded that gall bladder wall discontinuity on ultrasound indicated rupture and pericholecystic hyperechoic fat or fluid were suggestive of but not diagnostic for a gall bladder rupture and described mucocele by the appearance of the stellate or finely striated bile patterns which differed from biliary sludge by absence of gravity dependent bile movement.

Hittmair et al. (2001) found that the ultrasound was accurate in measuring gall bladder wall thickness.

Hoque and Varshney (2001), Sharma et al. (2001) and Hittmair et al. (2001) concluded that the normal gall bladder was anechoic with smooth well defined margin like other fluid containing structures and produced enhancement in the tissues deep to it and bile duct was not visible in normal dogs.

Tiwari et al. (2001) recorded a highly distended gall bladder with blind end and hypoechoic liver with fluid within liver lobes in case of biliary obstruction.

2.8.5 Ultrasonography in Vascular abnormalities

Nyland et al. (1995) opined that the distended common duct was best visualized ventral to the portal vein in a transverse view at the right 11th or 12th intercostal space, which was usually not visible in that view in normal animals. The “shot gun” or “too many tubes” sign was sometimes recognized on sagittal scan of the liver after 5-7 days of obstruction which referred to visualization of dilated intra-hepatic ducts clustered around portal vessels.
Ultrasonography can identify the presence of shunt vessels whereas the accuracy of identification of PSS varies widely, and it is generally recognized that the diagnostic usefulness of ultrasonography is heavily dependent on the skill and experience of the operator (Pratschke, 2010).

2.8.6 Ultrasonically guided percutaneous liver biopsy

Nyland and Park (1983) observed that the ultrasonographically guided percutaneous liver biopsy had improved the success and safety of obtaining diagnostic cytologic material.

Interventional ultrasound is the most commonly used procedure in veterinary medicine to obtain hepatic, cytologic, or histologic biopsies (Lorenzi, 2010).

2.8.7 Quantitative Ultrasonic assessment of liver size

Godshalk et al. (1988) studied the quantification of liver size using Static and real-time B-mode hepatic ultrasound imaging in 16 anesthetized dogs. It was observed that only one of the static B-mode measurements had a significant correlation (p >0.05), and none of the real-time measurements was dependent on liver weight. They concluded that ultrasonographic assessment of canine liver size using these methods was of little value in predicting actual liver weight.

2.8.8 Color Doppler Ultrasonographic Studies on Canine Liver

Molazem et al. (2007) studied the canine liver with two-dimensional scan initially and then three dimensional power and color doppler scans. Image acquisition was performed with reconstruction and simultaneous display of sectional anatomy in three orthogonal planes or any arbitrary oblique plane and also a 360 degree rotating 3D plane. They had evaluated images for gross anatomical visualizations and characters of the portal vein,
caudal vena cava, hepatic vein and artery, and aorta in 10 standard planes. It was concluded that 3D ultrasound scans of the liver corresponding to the sagittal and transverse planes were found to be possible in 6 planes in dorsal recumbency and that 3D Color Doppler ultrasonography was seemed to have the potential to provide greater detail of the vascularity associated with abnormal lesion.

Sartor et al. (2010) observed that the Doppler ultrasonography for the evaluation of flow in the right branch of the portal vein was a viable alternative, or complement, to examining the main vessel segment. This was observed to be especially so in those animals in which an ideal insonation angle for examination of the main portal vein was hard to obtain.

2.8.9 Three-Dimensional Ultrasound and Contrast-Enhanced Ultrasound Studies on Canine Liver

Arita et al. (2007) described a novel technique for the 3D visualization of a liver segment on sonography, using a second-generation contrast agent in a dog that had applications for anatomic hepatic resection. After a mixture of YM454 and indigo carmine was injected into a portal vein branch, they have observed well-delineated 3D segmental staining, which was seen for approximately 10 minutes in harmonic mode sonography. The addition of indigo carmine significantly prolonged the contrast effect of YM454.

Nakamura et al. (2010) performed contrast-enhanced ultrasonography using Sonazoid® in six normal beagles and 27 dogs with spontaneous focal or multifocal liver lesions. Sonazoid® was a newly developed second-generation contrast agent with the ability to be used for real-time contrast imaging along with parenchymal imaging. They had developed an appropriate protocol for the evaluation of all three phases (arterial, portal, and parenchymal).
By evaluation of the echogenicity of hepatic nodules during the arterial and parenchymal phases it was possible to differentiate malignant tumors from benign nodules with very high accuracy. In 15 of 16 dogs diagnosed as malignant tumors, nodules were clearly hypoechoic to the surrounding normal liver during the parenchymal phase. Additionally, malignant tumors had different echogenicity compared with the surrounding normal liver during the arterial phase in 14 of 15 dogs. In the portal phase, there were no characteristic findings. Contrast-enhanced ultrasonography with Sonazoid® appeared to improve the characterization of canine focal and multifocal hepatic lesions (Nakamura et al., 2010)

2.9 Liver Biopsy, Cytology and Histopathology

Sevelius (1995) classified CH according to histopathological examination as chronic progressive hepatitis, chronic non specific hepatitis, chronic cholangio hepatitis and liver cirrhosis.

Center (1996) described of the liver biopsy and fine needle aspiration techniques. She also explained the various methods like blind percutaneous procedures, ultrasound assisted procedure, keyhole approach and laparoscopic methods.

Liver biopsy was required for definitive diagnosis and was indicated if the disease was chronic and / or signs were severe or progressive (Rutgers and Haywood, 1988 and Twedt, 1998).

The establishment of an accurate diagnosis was dependent on two important components sampling an adequate amount of tissue and interpretation of the histology by someone well-versed in liver pathology. The liver biopsy provides only a small "window" for viewing histopathologic changes (Meyer and Harvey, 1998).
Liver biopsy and histopathological examination were essential to determine the presence of CH and to identify any possible underlying cause. Further the histopathology was found to be helpful in establishing the chronicity, activity and prognosis (Sterczer et al., 2001).

Cole et al. (2002) stated that biopsy of the liver was important for formulation of reasonable management strategies and suggested that needle biopsy specimens of the liver from dogs and cats must be interpreted with caution.

Liver cytology is useful for the initial evaluation however, cytology is unable to distinguish between benign focal inflammatory disease and progressive chronic disease, and cannot establish the extent of lesion (Lorenzi, 2010).
Materials and Methods
Chapter III

Materials and Methods

The study entitled “Study on Comparative Efficacy of Diagnostic Tests in the Early Diagnosis of Canine Liver Diseases” was carried out at the Centre of Advanced Faculty Training in Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, TANUVAS, Chennai-7 during the period October 2011 - January 2012. The clinical study was conducted with the clinical cases presented to the Small Animal Medical Outpatient Unit of the Teaching Veterinary Hospital over a period of five semesters (two and half years).

3.1 Patient Selection

Apparently healthy male and female dogs aged between 4 and 8 years presented to the hospital for health check up and vaccination were taken for the study to act as control group for the assessment of baseline referral values of selected parameters.

Cases presented with signs such as anorexia, lethargy, ascites, icterus, pigmented urine and vomiting were chosen and screened for liver disorders. Selected cases were subjected to detailed assessment.

3.2 Design of the study

The study consisted of apparently healthy dogs and clinical cases of various liver diseases, which were presented to the Small Animal Medical Unit. The cases taken up for the study were divided into various groups as follows:
Groups of Clinical Study

Group I : Apparently Health Dogs acting as Control group

Group II : Liver Disease Group

Group II was further subdivided into three groups as

Group II A : Biliary tract disorders

Group II B : Parenchymal disorders and

Group II C : Neoplastic disorders.

All the animals were subjected to routine clinical examination comprising of physical examination as suggested by McCurin and Poffenbarger (1991). All the cases were subjected to routine laboratory investigations as suggested by Barger (2003) and Gunn and Alleman (2005) as per standard clinical and laboratory protocols.

3.3 Parameters of the Study

The following clinical and laboratory parameters were studied in the apparently healthy dogs and in the clinical cases of groups. Hematobiochemical and coagulation profile values of control group dogs were estimated for the establishment of reference normal values for the study.

3.3.1 Incidence Analysis

The data on breed, age, sex were collected for demographic studies.

3.3.2 Medical History

Chief complaints, age at onset, management practices, medication history and chronology of events were assessed.
Appetite, Physical activity, Weight gain / weight loss, Icterus, Abdominal enlargement and additional clinical findings were assessed.

3.3.3 Physical Examination Findings

Appetite, vomiting, icterus, abdominal pain, ascites, hepatomegaly, pigmented urine and vital signs were assessed.

3.3.4 Hematology Panel

Erythrogram: Hemoglobin (Hb), Packed Cell Volume (PCV), and Total Erythrocyte Count (TEC) were assessed. Erythrocyte indices viz. MCV, MCH and MCHC were derived.

Leucogram: Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC) were assessed.

3.3.5 Serum Biochemistry Panel

Alanine amino transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Gamma Gutamyl Transferase (GGT), Serum Glucose, Cholesterol, Total protein, Albumin, Blood Urea Nitrogen (BUN) and Creatinine were assessed.

3.3.6 Clotting panel

This included estimation of platelet count, Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

3.3.7 Ultrasonography (Hepatic and Gall Bladder) Instrumentation

Ultrasonography of the abdominal organs were performed using ESOATE, MyLab 20 ultrasound scanner with 3.5/5.0 Mhz mechanical transducer as per the standard imaging protocol described by Nyland et al. (2002) (Plate 1). Echogenicity and echotexture of the spleen, liver and kidneys were evaluated and was also performed using Larson and Toubro Symphony
ultrasound scanner with 3.5 / 5.0 MHz mechanical transducer and Aloka SDD500 ultrasound scanner with 3.5 MHz Curvilinear transducer (Plate 2). Frozen images were recorded.

**Preparation and restraining of the animal**

The animal was placed in supine position with one assistant securing the rear legs while another one assisted to maintain the required positioning by restraint of the front legs and head. The area between xiphisternum and the umbilicus, extending several centimeters on each side of the umbilicus was shaved. A liberal quantity of acoustic gel was applied for effective sound transmission.

**Imaging techniques**

The liver was imaged using 3.5 MHz or 5.0 MHz transducer. The selection of frequency was based on the body size of the animal i.e. lower frequency transducer was selected for bigger body size. Canine liver were imaged as per the procedure mentioned by Barr (1990) and Nyland *et al* (1995) (Plate 4). The transducer was placed directly under the sternum with firm but gentle pressure. In this position, the transducer was placed in the midline and angled craniodorsally to image a transverse section of the liver. The head of the transducer was then rotated through 90° to image a longitudinal section of the liver in the midline. The image was oriented with the cranial portion of the animal to the viewer’s left on sagittal scans and the right side of the animal to the viewer’s left on sagittal scans and the right side of the animal to the viewer’s left on transverse scan. To visualize all parts of the liver, multiple sweeps through the organ were made by directing the beam dorsally and ventrally in the sagittal plane and to the right of left in the transverse plane. Intercostal views at 11th or 12th intercostal space were used to supplement visualization of peripheral parts of the liver and viewing the major abdominal vessels. During the sweeps, ultrasonograms were evaluated for information on liver size, shape, contour and internal architecture including alternations in echogenicity (focal or diffuse), intensity
(an / hypo / normal / hyper echoic pattern), hepatic vessels and to that of the
surrounding organs as well as to the presence or absence of free peritoneal
fluid. The ultrasonograms obtained were recorded.

Right transverse oblique and left transverse oblique positions of the
transducer were used to examine the gall bladder and biliary system.
Transducer position for the right oblique was on the right side, approximately
6 to 8 centimeters cranial to the xiphoid and 4 to 5 centimeters dorsal to the
sternum. The transducer was angled towards the midline between costal
cartilages to produce a transverse oblique scan through the gall bladder.
Placing the transducer in a similar position on the left side of the animal
produced the left oblique position. Attention was paid to the ultrasonographic
appearance of the gall bladder size, shape, wall and contents.

**Ultrasonographic Hepatometry**

**Liver**

Liver size in dogs was assessed based on the procedure established
by Barr (1992a) and Nyland *et al* (1995). During scanning in the transverse
section of the liver, the head of the transducer was rotated through 90° to
image a longitudinal section of the liver in the midline. The dorso - ventral
angulation of the beam was adjusted until the caudal surface of the liver was
as near vertical as possible while retaining a clear image of the diaphragm
line and the liver parenchyma. The image was frozen at maximal expiration
and distance of vertical line taken from the skin surface to the diaphragm was
measured. Measurements were repeated for four frozen images in each dog.
The mean of the four measurements was substituted in the following formula
for obtaining liver weight in grams as advocated by Barr (1992a).

Liver weight (gm) = \{127 \times \text{Ultrasonographic measurement (cm)}\} - 348.68.
Gall bladder

Sagittal, longitudinal measurements were obtained by placing the transducer on or near the ventral sub-costal midline. Image was obtained at expiratory pause, to eliminate the apparent alternation in gall bladder shape and size during inspiration. Measurements were made by electronic cursor placement at the luminal mucosal interface. Four repeated measurements of length, width and height were made and the mean value \( (L_{\text{average}}, W_{\text{average}} \text{ and } H_{\text{average}}) \) were calculated. Gall bladder volume was determined using the formula advocated by Finn-Bordner et al. (1993).

\[
\text{Calculated Volume (ml)} = 0.52 \times (W_{\text{average}} \times H_{\text{average}} \times L_{\text{average}})
\]

\[
\text{Corrected Volume (ml)} = \{\text{Calculated Volume} \times a\} + b
\]

Where \( a \), regression co-efficient = 0.859; \( b \), regression constant = 0.358

Ultrasound Guided Interventional Techniques

A preliminary ultrasound scan was performed to visualize the hepatic parenchyma and appropriate site was selected for the biopsy. Standard surgical preparations of the biopsy site were performed. The skin near the sterile field was tensed and biopsy site was punctured using 16-G disposable needle. A sterile glove (containing gel to improve transmission) was used to cover the ultrasound transducer to maintain sterility. The needle was inserted at an angle guided by the ultrasound image and the director of the transducer. When the needle enters the liver, it would be displayed on the image by gentle movement of the needle. Once the needle is visualized as entering the liver at an appropriate site and depth, the cutting action of the needle was employed to remove a core of hepatic tissue. Care was taken to prevent penetration of diaphragm or entering into thoracic cavity. Once a core of hepatic tissue was obtained, the needle was removed. The core of liver resting within the specimen notch of the needle was transferred into 10% formalin. The biopsy sample was subjected to histopathological techniques as described by Lorenzi (2010). The animal was placed immediately in sternal
recumbency to control haemorrhage. After obtained biopsy, the patient was observed for evidence of any haemorrhage, by ultrasonography and clinical signs like mucous membrane color, capillary refill time, pulse rate and respiratory rate were recorded.

**Ultrasound guided aspiration**

For aspiration of cyst, the selected needle was advanced into the lesion. When the needle was seen in the middle of the lesion, moderate suction was applied to the syringe plunger while the needle was moved within the lesion. After complete release of the suction, the syringe with attached needle was removed from the abdominal cavity. The aspirated fluid was subjected to cytological examination (Lorenzi, 2010). Similarly, ultrasound guided aspiration of peritoneal fluid was done in animals with ascites. (Plate 6)

### 3.3.8 Liver aspiration cytology / Biopsy

Liver aspiration cytology and or biopsies were performed and samples were processed and examined as per Lorenzi (2010).

### 3.4 Collection of Clinical Materials

#### 3.4.1 Hematology Samples

Around 2.5 ml of blood was collected from either cephalic vein/recurrent tarsal vein in a dry vial containing 10 per cent EDTA for complete haematological studies.

#### 3.4.2 Biochemistry Samples

Three and a half milliliters of blood was collected from peripheral veins in serum tubes, taking all precautions for avoiding hemolysis as suggested by Meinkoth and Allison (2007).
3.4.3 Samples for Clotting Panel Assays

Mix gently 9 parts of blood collected non-traumatically from cephalic or saphenous vein was placed in a tube containing 1 part of 3.2 per cent trisodium citrate solution (0.109 M). Plasma was harvested by centrifugation at 3000 rpm for 15 min and plasma was separated. The harvested plasma was then used for the estimation of PT and APTT using Mispa\Clog which is an opto-mechanical coagulation analyser which applies the turbodensitometric measuring principle manufactured by Agappe Diagnostics LTD (Plate 2).

3.5 Examination of Clinical materials

3.5.1 Examination of Blood Samples

Haematological parameters such as Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and leucocyte Count were analysed using auto haematology analyzer BC-2800 Vet, Mindray. (Plate 3).

3.5.2 Examination of Biochemical samples

The serum was separated and analysed. Quantitative estimation of blood urea nitrogen (BUN), creatinine, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), Total bilirubin, direct bilirubin, total proteins, albumin, glucose and Cholesterol was carried out using diagnostic kits supplied by Agappe Diagnostics Private Limited, India, specific for each parameter. Quantitative estimation of lactate was done with LO-POD (Enzymatic calorimetric method), Chemlex, S.A., Canovelles, Barcelona. The estimations were carried out with automatic blood biochemistry analyzer, A15 random access analyzer, Biosystems, Barcelona, Spain (Plate 1).
3.5.3 Clotting Panel

3.5.3.1 Platelet count

Blood collected in 10 per cent anticoagulant EDTA was analysed for platelet count using auto-haematology analyzer BC-2800 Vet, Mindray.

3.5.3.2 Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT)

Prothrombin time (PT)

Test procedure:

a. Gently swirl the reagent PT Reagent R1 vials before use. Do not shake reagent.

b. Dispense from vial enough PT reagents for immediate use, into a thoroughly clean and dry test tube.

c. Prewarm the dispensed PT reagent to 37°C for 10 min.

d. Add forcibly 100 µl of plasma into a test cuvette at 37°C, and incubate for 3 minutes.

e. Add forcibly 200 µl of pre-warmed PT reagent.

f. Timer starts simultaneously and record the clotting time.

Activated Partial Thromboplastin Time (APTT)

Test procedure:

a. Gently swirl the reagent vials before use. Do not shake reagent.
b. Prewarm enough volume of reagent (CaCl$_2$) for immediate use in a clean dry test tube at 37$^\circ$C.

d. Add forcibly 100 µl of plasma into a test cuvette at 37$^\circ$C, and incubate for 3 minutes.

e. Add forcibly 100 µl of pre-warmed Reagent 2 (APTT reagent) into the test cuvette.

f. Mix well and incubate at 37$^\circ$C for 3 min.

g. Add forcibly 100 µl pre-warmed Reagent 1 (CaCl$_2$) into the test cuvette.

h. Timer starts simultaneously and record the clotting time.

3.6 Liver aspiration cytology / Biopsy

Pre-biopsy evaluation

Coagulation panels like platelet count, PT and APTT were determined before proceeding to biopsy.

Fine Needle aspiration is done blind or via ultrasound guidance. An area between 7$^{th}$ - 10$^{th}$ intercostal spaces was surgically prepared from sternum to mid thorax, disinfected and a 22-24 G needle inserted craniodorsally, just dorsal to costochondral junction. The needle contents are deposited carefully on a microscopic slide and smeared, ensuring that the smear is stopped before the end of the slide so as not to lose larger cell elements such as clusters of hepatocytes as they are very useful for diagnosis (Plate 5).
3.6.1 Histopathology

The tissue Specimen fixed in ten per cent formalin for 12 hrs were bisected at right angles to the surface with sharp razor blade. These specimen were dehydrated by graded alcohol, treated by graded xylol and mounted by paraffin wax. The mounted tissue was then sectioned into 4-6 µ thin section by using a microtome and fixed to microscopic slides. The section were stained with Haematoxylin and Eosin stain (Bancroft and Stevens, 1996) and subsequently examined under light microscope for detection of histopathologic changes.

3.7 Statistical analysis

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994). The statistical software package - SPSS 13.0 for Windows were utilized in this study and the results were analyzed based on one way ANOVA and Duncan’s technique. The results were presented in figures, tables and graphs and were discussed critically.
Results
CHAPTER - IV

RESULTS

The study entitled “Study on Comparative Efficacy of Diagnostic Tests in the Early Diagnosis of Canine Liver Diseases” was carried out at the Centre of Advanced Faculty Training in Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, TANUVAS, Chennai-7 during the period October 2011 to January 2012. The study was conducted with the clinical cases presented to the Small Animal Medical Outpatient Clinic of the Madras Veterinary College Teaching Veterinary Hospital over a period of five semesters. The selected dogs were subjected to detailed physical examination, clinico-pathological examinations and ultrasonographic examination. The results were given below.

4.1. Incidence of canine liver disease

The Incidence of canine liver disease was presented in Fig-1.

The overall incidence of canine liver disease in the present study was 0.15 per cent of the total number of cases (66,540) presented to the Small Animal Medical Unit of the Madras Veterinary College Teaching Hospital. The gastrointestinal case load during the study period was 23,289 and 0.43 per cent of this population was found to have liver diseases.

4.1.1 Etiological pattern of canine liver disease and their Incidence

The Incidence of various canine liver diseases was presented in Fig -2.

The commonly recognised canine liver diseases included parenchymal disorders 73 per cent (73/100), biliary disorders 18 per cent (18/100) and neoplastic disorders 9 per cent (9/100).
Canine liver diseases were 0.15 per cent of the total number of cases and 0.43 per cent of the gastrointestinal case load.

Etiological pattern of canine liver diseases

- Biliary Disorders: 18%
- Neoplastic Disorders: 9%
- Parenchymal Disorders: 73%
4.1.2 Breed wise incidence of canine liver diseases

The breed wise incidence was presented in Fig - 3 and 4.

Non-descript dogs dominated with an incidence of 26 per cent (26/100), followed by Spitz - 20 per cent (20/100), Labradors - 14 per cent (14/100), German shepherd - 12 per cent (12/100), Doberman - 7 per cent (7/100), mixed breed of dogs - 5 per cent (5/100), Great Dane - 4 per cent (4/100), Dachshund - 3 per cent (3/100), Pug and Golden retriever had an incidence of 2 per cent (2/100) each whereas cocker spaniel, Dalmatian, Shih Tzu, Lhasapso and Rajapalayam have an incidence of 1 per cent (1/100) each.

Biliary disorders was found to have higher incidence in non descript dogs - 38.89 per cent (7/18), followed by German Shepherd - 27.78 per cent (5/18), Spitz - 16.67 per cent (3/18), Labrador - 11.11 per cent (2/18) and mixed breed - 5.56 per cent (1/18).

Incidence of neoplastic disorders was highest in non descript and Spitz - 22.22 per cent (2/9) incidence in each, followed by Dachshund - 11.11 per cent (1/9), Labrador - 11.11 per cent (1/9), German Shepherd - 11.11 per cent (1/9), Great dane - 11.11 per cent (1/9) and Pug 11.11 per cent (1/9).

4.1.3 Age wise incidence of canine liver diseases

The age wise incidence was presented in Fig-5.

The overall incidence of canine liver diseases was high in dogs aged 4 to 8 years with an incidence of 36 per cent (36/100), which was followed by dogs aged above 8 years- 34 per cent (34/100). Dogs aged 1 to 4 years had an incidence of 24 per cent (24/100) and dogs less than one year had an incidence of 6 per cent (6/100).
Fig-3: BREED WISE INCIDENCE OF CANINE LIVER DISEASES

Breed wise incidence of canine liver diseases

Fig-4: BREED WISE INCIDENCE OF VARIOUS GROUPS OF CANINE LIVER DISEASES

Breedwise incidence of various groups of canine liver diseases
Parenchymal disorders were most common in dogs aged 4 to 8 years old - 34.25 per cent (25/73), followed by dogs aged above 8 years - 32.88 per cent, 1 to 4 year old dogs - 24.66 per cent (18/73) and less than 1 year old dogs - 8.22 per cent (6/73).

Dogs aged 4 to 8 years were most commonly affected with biliary disorders with an incidence of 38.89 per cent (7/18) which was followed by dogs aged above 8 years - 33.33 per cent (6/18), dogs aged 1 to 4 years - 22.22 per cent (4/18) and less than 1 year old - 5.56 per cent (1/18).

The incidence of neoplastic disorders was high in, dogs aged above 8 years and above and dogs aged 4 to 8 years with an incidence of 44.44 per cent (4/9) in each. While the incidence in dogs aged 1 to 4 years and less than 1 year had 11.11 per cent each (1/9).

4.1.4 Sex wise incidence of canine liver diseases

The sex wise incidence was presented in Fig-6.

Males dominated the overall incidence of canine liver diseases with 67 per cent (67/100) followed by females 33 per cent (33/100).

Parenchymal disorders were most commonly observed in male dogs - 60.27 per cent (44/73) and female dogs had an incidence of 39.73 per cent (29/73).

Male dogs were most commonly found to be affected with biliary disorders with an incidence of 88.89 per cent (16/18) and female 11.11 per cent (2/18).

Female dogs were less commonly affected by neoplastic disorders with an incidence of 22.22 per cent (2/9) while males dominated the study with an incidence of 77.78 per cent (7/9).
Fig-5: AGE WISE INCIDENCE OF VARIOUS GROUPS OF CANINE LIVER DISEASES

Fig-6: SEX WISE INCIDENCE OF VARIOUS GROUPS OF CANINE LIVER DISEASES
4.2 Medical history of canine liver diseases

The chief iatrophic stimuli of dogs suffering from various canine liver diseases were presented in Table - 2. All the dogs under study were primarily presented to the hospital with the chief complaint of anorexia and weight loss.

Dogs with parenchymal disorders were presented with predominant complaints of anorexia (100 per cent), weight loss - 75.34 per cent and vomiting- 46.58 per cent, diarrhoea - 31.51 per cent, pigmented urine and polyuria-polydipsia with 16.44 per cent each, icterus - 12.23 per cent and convulsions - 5.48 per cent.

Predominant complaints in dogs with biliary disorders were anorexia (100 per cent), high coloured urine (100 per cent) and icterus (77.78 per cent) less frequent complaints received included diarrhoea (33.33 per cent) and convulsions (11.11 per cent).

Dogs with neoplastic disorders found to have major complaints of anorexia (55.56 per cent), vomiting, weight loss and high coloured urine (33.33 per cent) each. Less frequent complaints included diarrhea, polyuria-polydipsia and icterus in 11.11 per cent each.

4.3 Clinical presentation of canine liver disease

The clinical findings observed in physical examination of dogs with liver diseases were presented in Table - 2 and Plates 7-17.

The most commonly observed clinical signs (Table-2) included gastro intestinal signs like anorexia to reduced appetite, vomiting, diarrhea, abdominal pain along with other signs like jaundice, ascites, hepatomegaly, anaemic changes, polyuria-polydipsia, bleeding tendencies, poor hair coat, weakness, behavioural changes / convulsions and elevated temperatures. All these signs were observed in various degrees.
**Table: 1. MEDICAL HISTORY OF CANINE LIVER DISEASES**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Medical history</th>
<th>Parenchymal disorders (73)</th>
<th>Biliary disorders (18)</th>
<th>Neoplastic disorders (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anorexia</td>
<td>100%</td>
<td>100%</td>
<td>55.56%</td>
</tr>
<tr>
<td>2</td>
<td>Vomiting</td>
<td>46.58%</td>
<td>100%</td>
<td>33.33%</td>
</tr>
<tr>
<td>3</td>
<td>Diarrhoea</td>
<td>31.51%</td>
<td>33.33%</td>
<td>11.11%</td>
</tr>
<tr>
<td>4</td>
<td>Weight loss</td>
<td>75.34%</td>
<td>100%</td>
<td>33.33%</td>
</tr>
<tr>
<td>5</td>
<td>Polyuria-Polydipsia</td>
<td>16.44%</td>
<td>-</td>
<td>11.11%</td>
</tr>
<tr>
<td>6</td>
<td>High coloured Urine</td>
<td>16.44%</td>
<td>100%</td>
<td>33.33%</td>
</tr>
<tr>
<td>7</td>
<td>Icterus</td>
<td>12.23%</td>
<td>77.78%</td>
<td>11.11%</td>
</tr>
<tr>
<td>8</td>
<td>Behavioural signs/convulsions</td>
<td>5.48%</td>
<td>11.11%</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table: 2. CLINICAL PRESENTATION OF CANINE LIVER DISEASES**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Clinical Presentation</th>
<th>Parenchymal disorders (73)</th>
<th>Biliary disorders (18)</th>
<th>Neoplastic disorders (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palpably distended liver (Hepatomegaly)</td>
<td>45.21%</td>
<td>11.11%</td>
<td>44.44%</td>
</tr>
<tr>
<td>2</td>
<td>Anorexia / Decreased appetite</td>
<td>90.41%</td>
<td>88.89%</td>
<td>55.56 %</td>
</tr>
<tr>
<td>3</td>
<td>Vomiting</td>
<td>46.58%</td>
<td>100%</td>
<td>33.33 %</td>
</tr>
<tr>
<td>4</td>
<td>Diarrhoea</td>
<td>31.51%</td>
<td>33.33%</td>
<td>(2 with haemorrhagic enteritis)</td>
</tr>
<tr>
<td>5</td>
<td>Weakness</td>
<td>9.59%</td>
<td>-</td>
<td>66.67%</td>
</tr>
<tr>
<td>6</td>
<td>Weight loss</td>
<td>75.34%</td>
<td>88.89%</td>
<td>33.33%</td>
</tr>
<tr>
<td>7</td>
<td>Elevated temperature</td>
<td>9.59%</td>
<td>16.67%</td>
<td>11.11%</td>
</tr>
<tr>
<td>8</td>
<td>Anaemia signs (Tachycardia/Tachypnoea)</td>
<td>16.45%</td>
<td>16.67%</td>
<td>66.67%</td>
</tr>
<tr>
<td>9</td>
<td>Ascites</td>
<td>34.25%</td>
<td>11.11%</td>
<td>33.33%</td>
</tr>
<tr>
<td>10</td>
<td>Abdominal pain</td>
<td>26.02%</td>
<td>66.67%</td>
<td>22.22%</td>
</tr>
<tr>
<td>11</td>
<td>Polyuria-polydipsia</td>
<td>16.44%</td>
<td>11.11%</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Jaundice</td>
<td>12.33%</td>
<td>77.78%</td>
<td>11.11%</td>
</tr>
<tr>
<td>13</td>
<td>Hepatic encephalopathy / Behavioural signs/ Convulsions</td>
<td>5.48% ( with convulsions)</td>
<td>11.11% ( with convulsions)</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Bleeding tendencies</td>
<td>12.32%</td>
<td>11.11%</td>
<td>11.11%</td>
</tr>
<tr>
<td>15</td>
<td>Poor hair Coat</td>
<td>41.10%</td>
<td>33.33%</td>
<td>44.44%</td>
</tr>
</tbody>
</table>
4.3.1 Parenchymal disorders

Parenchymal disorders had common clinical signs such as anorexia / reduced appetite - 90.41 per cent, weight loss - 75.34 per cent, vomiting and palpably distended liver (hepatomegaly) - 45.21 per cent each, poor hair coat - 41.10 per cent, ascites - 34.25 per cent, diarrhea - 31.51 per cent, abdominal pain - 26.02 per cent, anaemia signs (Tachycardia/ Tachypnoea) and polyuria-polydipsia - 16.44 per cent each, jaundice and bleeding tendencies - 12.32 per cent each, weakness - 9.59 per cent, elevated temperature - 9.59 per cent and convulsions - 5.48 per cent.

Except for anorexia (90.41 per cent) and weight loss (75.34 per cent) all the other signs were found to be less predominant and were not much useful to call it as characteristic clinical presentation.

4.3.2 Biliary disorders

The most commonly observed clinical signs included vomiting - 100 per cent, anorexia / decreased appetite and weight loss - 88.89 per cent each, jaundice - 77.78 per cent, abdominal pain - 66.67 per cent, diarrhea (2 dogs with haemorrhagic enteritis) and poor hair coat has been observed 33.33 per cent in each, elevated temperature and anaemic signs (tachycardia/ tachypnoea) - 16.67 per cent each, ascites, palpably distended liver (hepatomegaly), convulsions and bleeding tendencies - 11.11 per cent each.

The characteristic clinical presentation of biliary disorder was evidenced by predominance of signs like vomiting (100 per cent), weight loss (88.89 per cent) anorexia (88.89 per cent) and icterus (77.78 per cent). When compared with parenchymal and neoplastic disorders, icterus was in high proportion in dog with biliary disorders.

4.3.3 Neoplastic disorders

Dogs with neoplastic disorders had clinical signs such as weakness and anaemic signs (Tachycardia/Tachypnoea) - 66.67 per cent each,
anorexia / reduced appetite - 55.56 per cent, palpably distended liver (Hepatomegaly) and poor hair coat - 44.44 per cent each as a major sign. Less commonly observed signs include vomiting, weight loss and ascites - 33.33 per cent each, abdominal pain - 22.22 per cent and polyuria/polydipsia, jaundice, bleeding tendencies, diarrhea and elevated temperature - 11.11 per cent each.

The characteristic clinical presentation of neoplastic liver disorders in the study included weakness and anaemic signs (tachycardia/tachypnoea) which were not observed to be the predominant signs in biliary or parenchymal disorders.

4.4 Clinico-pathological findings in canine liver diseases

The hematology, serum biochemical and coagulation parameters findings of the control group and various groups of liver diseases were presented in Table 3-6. Comparisons between the control and a particular group of liver diseases and comparison between different groups of liver diseases were given in the tables.

The mean erythrogram values of control group animals in this study were Haemoglobin -14.10 ± 0.66 g/dl, Packed Cell Volume - 45.20 ± 2.79 %, RBC count - 7.37 ± 0.33 $10^6$/cu.m.m, MCV- 61.20 ± 1.84 fl, MCH-19.13 ± 0.32 pg and MCHC-31.34 ± 0.64 g /dL.

The mean leucogram values of control group animals in this study had total WBC count - 8683.33 ± 470.76 cells / mm$^3$, Neutrophils - 6,181.50 ± 347.80 cells / mm$^3$, Lymphocytes - 1897.33 ± 132.68 cells / mm$^3$, Monocytes - 442.17 ± 12.51 in cells / mm$^3$ and Eosinophils - 162.33 ± 56.16 cells / mm$^3$.

The healthy dogs in the present study had mean values of Platelet - 2, 36,000 ± 52,496.98 cell / µl, PT - 9.3333 ± 0.63 sec and aPTT - 38.8667 ± 2.10 sec.
Table: 3. MEAN ± SE VALUES OF ERYTHROGRAM IN CANINE LIVER DISEASES

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Haemoglobin (g/dl)</th>
<th>Packed Cell Volume (%)</th>
<th>RBC count (10^6 cells / mm^3)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n=6)</td>
<td>14.10±0.66a</td>
<td>45.20±2.79a</td>
<td>7.37±0.33a</td>
<td>61.20±1.84</td>
<td>19.13±0.32</td>
<td>31.34±0.64</td>
</tr>
<tr>
<td>2</td>
<td>Parenchymal Disorders (n=73)</td>
<td>9.43±0.44b</td>
<td>26.02±1.35b</td>
<td>3.97±0.21b</td>
<td>66.69±1.46</td>
<td>28.16±3.22</td>
<td>43.92±5.84</td>
</tr>
<tr>
<td>3</td>
<td>Biliary Disorders (n=18)</td>
<td>9.36±0.58b</td>
<td>29.39±1.65b</td>
<td>4.66±0.35b</td>
<td>65.50±2.85</td>
<td>20.72±0.80</td>
<td>32.04±1.11</td>
</tr>
<tr>
<td>4</td>
<td>Neoplastic Disorders (n=9)</td>
<td>9.89±1.12b</td>
<td>25.76±3.00b</td>
<td>4.14±0.57b</td>
<td>64.45±5.55</td>
<td>24.79±2.07</td>
<td>38.68±1.43</td>
</tr>
<tr>
<td>5</td>
<td>F Value</td>
<td>3.457</td>
<td>6.450</td>
<td>8.009</td>
<td>0.422</td>
<td>0.706</td>
<td>0.505</td>
</tr>
</tbody>
</table>

Table: 4. MEAN ± SE VALUES OF LEUCOGRAM IN CANINE LIVER DISEASES

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Total WBC count (cells / mm^3)</th>
<th>Neutrophils (cells / mm^3)</th>
<th>Lymphocytes (cells / mm^3)</th>
<th>Monocytes (cells / mm^3)</th>
<th>Eosinophils (cells / mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n=6)</td>
<td>8683.33±470.76a</td>
<td>6,181.50±347.80a</td>
<td>1897.33±132.68</td>
<td>442.17±12.51</td>
<td>162.33±56.16a</td>
</tr>
<tr>
<td>2</td>
<td>Parenchymal Disorders (n=73)</td>
<td>18,220.55±895.36b</td>
<td>14,610.89±779.03b</td>
<td>2733.01±171.77</td>
<td>528.11±52.91</td>
<td>325.16±27.70b</td>
</tr>
<tr>
<td>3</td>
<td>Biliary Disorders (n=18)</td>
<td>21,094.44±1,660.84b</td>
<td>17,218.67±1,480.80b</td>
<td>2813.83±222.31</td>
<td>510.50±62.26</td>
<td>356.44±47.02b</td>
</tr>
<tr>
<td>4</td>
<td>Neoplastic Disorders (n=9)</td>
<td>15,088.89±2,079.29b</td>
<td>11,974.22±1,681.97b</td>
<td>2204.22±339.84</td>
<td>486.67±101.11</td>
<td>394.00±89.21b</td>
</tr>
<tr>
<td>5</td>
<td>F Value</td>
<td>4.883</td>
<td>5.050</td>
<td>1.172</td>
<td>0.106</td>
<td>1.385</td>
</tr>
</tbody>
</table>
### Table: 5. MEAN ± SE VALUES OF COAGULATION PARAMETERS IN CANINE LIVER DISEASES

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Group</th>
<th>Platelet (cells/ µl)</th>
<th>PT (sec)</th>
<th>aPTT (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n=6)</td>
<td>236,000±52,496.98</td>
<td>9.33±0.63</td>
<td>38.87±2.10</td>
</tr>
<tr>
<td>2</td>
<td>Parenchymal Disorders (n=73)</td>
<td>174,263.01±12,037.44</td>
<td>9.88±0.22</td>
<td>40.93±0.98</td>
</tr>
<tr>
<td>3</td>
<td>Biliary Disorders (n=18)</td>
<td>133,422.22±12,913.81</td>
<td>9.16±0.31</td>
<td>45.47±2.39</td>
</tr>
<tr>
<td>4</td>
<td>Neoplastic Disorders (n=9)</td>
<td>115,544.44±17,351.33</td>
<td>9.24±0.44</td>
<td>44.66±2.07</td>
</tr>
<tr>
<td>5</td>
<td>F Value</td>
<td>2.833</td>
<td>1.144</td>
<td>1.997</td>
</tr>
</tbody>
</table>

### Table: 6 a. MEAN ± SE VALUES OF SERUM BIOCHEMICAL PARAMETERS IN CANINE LIVER DISEASES

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Group</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>SAP (IU/L)</th>
<th>GGT (IU/L)</th>
<th>BUN (mg / dl)</th>
<th>Creatinine (mg / dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n=6)</td>
<td>64.57±7.18</td>
<td>45.15±5.01</td>
<td>87.78±14.73</td>
<td>3.73±0.28</td>
<td>20.00±3.86</td>
<td>0.89±0.10</td>
</tr>
<tr>
<td>2</td>
<td>Parenchymal Disorders (n=73)</td>
<td>180.77±27.67</td>
<td>121.78±22.91</td>
<td>365.58±47.34</td>
<td>4.28±0.26</td>
<td>29.05±2.83</td>
<td>2.37±0.52</td>
</tr>
<tr>
<td>3</td>
<td>Biliary Disorders (n=18)</td>
<td>78.53±6.34</td>
<td>41.06±3.05</td>
<td>237.03±39.43</td>
<td>15.07±2.01</td>
<td>25.14±3.17</td>
<td>1.36±0.58</td>
</tr>
<tr>
<td>4</td>
<td>Neoplastic Disorders (n=9)</td>
<td>134.40±85.17</td>
<td>31.52±7.36</td>
<td>125.67±29.21</td>
<td>4.98±0.40</td>
<td>20.13±2.49</td>
<td>0.79±0.15</td>
</tr>
<tr>
<td>5</td>
<td>F Value</td>
<td>1.537</td>
<td>1.914</td>
<td>2.546</td>
<td>36.825</td>
<td>0.802</td>
<td>0.850</td>
</tr>
</tbody>
</table>
Table: 6 b. MEAN ± SE VALUES OF SERUM BIOCHEMICAL PARAMETERS IN CANINE LIVER DISEASES

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Group</th>
<th>Total Protein (g / dl)</th>
<th>Albumin (g / dl)</th>
<th>Globulin (g / dl)</th>
<th>Total Bilirubin (mg / dl)</th>
<th>Direct Bilirubin (mg / dl)</th>
<th>Glucose (mg / dl)</th>
<th>Cholesterol (mg / dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n=6)</td>
<td>8.13±0.21 a</td>
<td>3.65±0.15 a</td>
<td>4.48±0.15 a</td>
<td>0.44±0.026 a</td>
<td>0.12±0.01 a</td>
<td>97.50±2.29</td>
<td>105.65±4.48 a</td>
</tr>
<tr>
<td>2</td>
<td>Parenchymal Disorders (n=73)</td>
<td>6.57±0.20 b</td>
<td>2.02±0.10 b</td>
<td>4.55±1.70</td>
<td>1.20±0.11 ab</td>
<td>1.0949±0.24 ab</td>
<td>88.93±2.60</td>
<td>127.90±5.50 ab</td>
</tr>
<tr>
<td>3</td>
<td>Biliary Disorders (n=18)</td>
<td>6.42±0.32 b</td>
<td>2.52±0.228 b</td>
<td>3.90±0.22</td>
<td>1.55±0.25 b</td>
<td>0.5056±0.73 b</td>
<td>87.06±3.93</td>
<td>169.57±21.12 b</td>
</tr>
<tr>
<td>4</td>
<td>Neoplastic Disorders (n=9)</td>
<td>6.59±0.22 b</td>
<td>2.42±0.15 b</td>
<td>4.17±0.18</td>
<td>0.66±0.17 a</td>
<td>0.4100±0.24 a</td>
<td>95.22±2.99</td>
<td>151.01±23.48 ab</td>
</tr>
<tr>
<td>5</td>
<td>F Value</td>
<td>2.003</td>
<td>8.378</td>
<td>1.011</td>
<td>3.099</td>
<td>2.954</td>
<td>0.669</td>
<td>3.300</td>
</tr>
</tbody>
</table>
The biochemical parameter of healthy dogs in the present study had mean values of ALT - 64.55 ± 7.18 IU/L, AST - 45.15 ± 5.01 IU/L, SAP - 87.78 ± 14.73 IU/L, GGT - 3.73 ± 0.28 IU/L, BUN - 20.00 ± 3.86 mg / dl, Creatinine - 0.89 ± 0.10 mg / dl, Total Protein - 8.13 ± 0.21 g / dl, Albumin - 3.65 ± 0.15 g / dl, Globulin - 4.48 ± 0.026 g / dl, Total Bilirubin - 0.44 ± 0.026 mg / dl, Direct Bilirubin - 0.12 ± 0.014 mg / dl, Glucose - 97.50 ± 2.29 mg / dl and Cholesterol - 105.65 ± 4.48 mg / dl.

4.4.1 Parenchymal disorders

The erythrogram (Table-3) revealed a significant reduction in the levels of hemoglobin (9.43 ± 0.44 g/dL), total erythrocytic count (3.97 ± 0.21 ×10^6 /cumm) and a non significant reduction in PCV (26.02 ± 1.35 %).

The erythrocytic indices, only MCV had a significant elevation (66.69 ± 1.46 fl) with non significant changes in MCH and MCHC.

The total leucocyte count (Table-4) was significantly elevated to 18,220.55 ± 895.36 cells /cumm. There was also a significant elevation of neutrophil count (14,610.89 ± 779.03 cells /cumm), while there were no significant difference in the lymphocyte and monocyte count.

The platelet count (Table-5) was significantly reduced (174,263.01 ± 12,037.44 cells/µL). There was a significantly elevated activated partial thromboplastin time (40.93 ± 0.98 sec). No significant change in prothrombin time was noticed.

Serum biochemical studies (Table-6a) revealed elevations in alkaline phosphatase (365.58 ± 47.39 IU/L), alanine aminotransferase (180.77 ± 27.67 IU/L) and gamma gutamyl transferase (4.275 ± 0.261 IU/L) and aspartate amino transferase (121.78 ± 22.11 IU/L) levels. However, these elevations were statistically non significant.

The levels of blood urea nitrogen and creatinine varied non significantly from the control. However, there was a significant reduction in the total protein (6.57 ± 0.20 mg/dL), a non significant reduction in albumin (2.018 ± 0.10 mg/dL) and non significantly elevated globulin levels (4.99 ± 1.70 mg/dL) was noticed.
Elevations in total bilirubin (1.30 ± 0.24 mg/dL) (Table-6b) and direct bilirubin values (1.30 ± 0.24 mg/dL) were though observed, were statistically non significant.

There was no significant change in the serum cholesterol level (127.90 ± 5.50 mg/dL) and glucose level (88.93 ± 2.60 mg/dl).

4.4.2 Biliary disorders

Erythrogram (Table-3) revealed a significant reduction in the values of hemoglobin (9.356 ± 0.58 g/dl), total erythrocytic count (4.66 ± 0.35 x10^6/cumm) and packed cell volume (29.39 ± 1.65 %). Erythrocytic indices revealed non significant changes in MCV, MCH and MCHC values.

The total leucocyte count (Table-4) was significantly elevated (21,094.44 ± 1,660.84 cells / cumm) while the neutrophil counts (17,218.67 ± 1,480.80 cells / mm^3) increased significantly whereas there was no significant difference in the lymphocyte and monocyte count.

The platelet count (Table-5) was found to be reduced significantly (133,422.22 ± 12,913.81 cells / µL). There was a non significant elevation in activated partial thromboplastin time (45.47 ± 2.39 sec). The prothrombin time was found to be in the normal range (9.1611 ± 0.31 sec).

Serum biochemical studies (Table-6a) revealed non significant elevation in alkaline phosphatase (237.03 ± 39.43IU/L) and alanine aminotransferase (78.53 ± 6.34 IU/L) levels, non significantly reduced aspartate amino transferase (41.06 ± 3.05 IU/L) levels and significantly elevated gamma glutamyl transferase (15.07 ± 2.01IU/L) levels when compared with the control group.

The levels of blood urea nitrogen and creatinine values were non significantly elevated from the control. However, there was a significant
reduction in the total protein level (6.42 ± 0.32 mg/dL) and significant reduction in albumin (2.52 ± 0.22 mg/dL) and globulin levels (3.8967 ± 0.22403 mg/dL)

The total bilirubin (Table-6b) (1.55 ± 0.247 mg/dl) and direct bilirubin (2.51 ± 0.73 mg/dl) were significantly elevated from the control group.

The serum cholesterol level (169.57 ± 21.12 mg/dL) was also significantly elevated and glucose level (87.06 ± 3.926 mg/dL) was non significantly decreased but it was within physiological range when compared with the control group.

4.4.3 Neoplastic disorders

Erythrogram (Table-3) revealed a significant reduction in the values of hemoglobin (9.89 ± 1.12 g/dl), total erythrocytic count (4.14 ± 0.57 x10^6/cumm) and packed cell volume (25.76 ± 3.00 per cent). Erythrocytic indices revealed non significant changes in MCV, MCH and MCHC values.

The total leucocyte count (Table-4) was significantly elevated (15,088.89 ± 2,079.29 cells / cumm). The neutrophil count (11,974.22 ± 1,681.97 cells / mm^3) was increased significantly whereas there was no significant difference in the lymphocyte and monocyte count.

The platelet count (Table-5) were significantly reduced (115,544.44 ± 17,351.33 cells/ µL).There was a non significant elevation in activated partial thromboplastin time (44.66 ± 2.07 sec) and prothrombin time was found to be in the normal range (9.24 ± 0.44 sec).

Serum biochemical studies (Table-6a) revealed a non significantly elevated alkaline phosphatase (125.67 ± 29.21IU/L), alanine aminotransferase (134.40 ± 85.17 IU/L) and gamma gutamyltransferase (4.98 ± 0.40 IU/L) levels. Aspartate amino transferase (31.52 ± 7.36 IU/L) levels was reduced but it was not significant.
The level of blood urea nitrogen and creatinine was found to be non significantly increased from that of the control. However, there was a significant reduction in the total protein level (6.59 ± 0.22 mg/dL) and a non significant reduction in albumin (2.42 ± 0.15 mg/dL) level. No significant change in globulin level was observed.

Total bilirubin (Table-6b) and direct bilirubin values had showed non significant elevations. There was also no significant change in serum cholesterol levels (151.01 ± 23.48 mg/dL) and glucose level (95.22 ± 2.99 mg/dL).

4.5 Ultrasonographic findings

The sonographic findings of the control and various groups of canine liver diseases are presented in Table-7 and Plates 18-29, 33.

In the control group there were no abnormal changes in size, shape and echogenicity visualized.

4.5.1 Parenchymal disorders

Out of 73 dogs with parenchymal disorders (Table-7 and Plate 21,23,33) had normal hepatic volume in 18 dogs, increased volume in 37 dogs and decreased in 8 dogs, changes in hepatic shape included rounded in 33 dogs and irregular in 14 dogs (cirrhosis/fibrosis), parenchymal changes were noticed as homogeneous parenchyma in 67 dogs and heterogeneous parenchyma in 6 dogs, hepatic echogenicity was decreased in 16 dogs, increased in 18(2 lipidosis) dogs and mixed pattern in 3 dogs, hypoechoic masses and mixed masses were visualized in one dog each, gall bladder was distended in 11 dogs, spleen was normal in 62 dogs and splenomegaly was found in 9 dogs (mild to moderate splenomegaly) & 2 dogs (splenectomized), ascites was visualised in 25 dogs, there was prominent hepatic veins in 22 dogs and portal veins in 27 dogs.
Table: 7. SONOGRAPHIC FINDINGS IN CANINE LIVER DISEASES

<table>
<thead>
<tr>
<th>SONOGRAPHIC FINDINGS IN CANINE LIVER DISEASES</th>
<th>Normal (6)</th>
<th>Parenchymal Disorders (73)</th>
<th>Bilary Disorders (18)</th>
<th>Neoplastic Disorders (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatic volume</strong></td>
<td>Normal</td>
<td>6</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>0</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td><strong>Hepatic shape</strong></td>
<td>Normal</td>
<td>5</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Round</td>
<td>1</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Irregular</td>
<td>0</td>
<td>14 (cirrhosis/fibrosis)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Hepatic parenchyma</strong></td>
<td>Homogeneous</td>
<td>6</td>
<td>67</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Heterogeneous</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Hepatic echogenicity</strong></td>
<td>Normal</td>
<td>6</td>
<td>36</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Decreased</td>
<td>0</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>0</td>
<td>18 (lipidosis)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>Hepatic focal lesions</strong></td>
<td>Hypoechoic nodules</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hyperechoic nodules</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hypoechoic masses</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hyperechoic masses</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mixed masses</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cysts</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Not detected</td>
<td>6</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td><strong>Biliary tract</strong></td>
<td>Normal</td>
<td>4</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>1. Distended</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Wall thickened</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Cholecystolith</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Sludge</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Spleen</strong></td>
<td>Normal</td>
<td>6</td>
<td>62</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>6</td>
<td>9 (mild to moderate spleenomegaly &amp; 2 spleenectomized)</td>
<td>4 (mild spleenomegaly)</td>
</tr>
<tr>
<td><strong>Ascites</strong></td>
<td>Not detected</td>
<td>6</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Detected</td>
<td>0</td>
<td>20 (prominent)+5 (scanty)</td>
<td>2 (prominent) &amp; 1 (scanty)</td>
</tr>
<tr>
<td><strong>Hepatic Vein</strong></td>
<td>Normal</td>
<td>6</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>0</td>
<td>15 (indistinct), 7 (prominent approaching CVC)</td>
<td>3 (engorged but less than CVC)</td>
</tr>
<tr>
<td><strong>Portal veins</strong></td>
<td>Normal</td>
<td>6</td>
<td>46</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>0</td>
<td>15 (indistinct)+12 (prominent)</td>
<td>4 (prominent)</td>
</tr>
</tbody>
</table>
4.5.2 Biliary disorders

Dogs that were subjected for sonographic evaluation in the biliary disorder group (Table-7 and Plate 24-29) had normal hepatic volume in 15 dogs whereas the volume was found increased in 3 dogs, hepatic shape was rounded in 5 dogs and 13 dogs had normal shape, the hepatic parenchyma was homogeneous in all the dogs with normal echogenicity in 14 dogs and decreased echogenicity in 4 dogs, there were no focal lesions visualized in parenchyma in all the dogs, gall bladder was distended in 9 dogs, thickened gall bladder wall was visualized in 7 dogs. Two dogs had cholecystolith and 8 dogs had sludge. Spleen was normal in 14 dogs and 4 dogs had mild splenomegaly. Ascites was visualized in 3 dogs. Engorged hepatic veins was recorded in 3 dogs (engorged but less than CVC) and prominent portal veins in 4 dogs.

4.5.3 Neoplastic disorders

In the present study out of 9 dogs with neoplastic disorders (Table-7 and Plate 18-20, 22) the hepatic volume was increased in 7 dogs, changes in shape included: rounded - 3 dogs, irregular - 4 dogs and normal shape- 2 dogs. Hepatic parenchyma was homogeneous in 3 dogs and heterogeneous in 6 dogs, hepatic echogenicity was normal in 2 dogs while mixed pattern was observed in 7 dogs. Hepatic focal lesions included hypoechoic nodules in 2 dogs, hyperechoic nodulesin 2 dogs, hyperechoic masses in 2 dogs, mixed masses in 5 dogs, gall bladder was found to be distended in 4 dogs, wall thickening and sludge was visualized in 2 dogs. Spleen was normal in 5 dogs while tumour and splenomegaly was associated in 4 dogs, ascites was visualized in 3 dogs, hepatic vein engorgement was observed in 3 dogs and 4 dogs had prominent portal veins.
4.5.4 3D Ultrasonographic studies

In the present study 3 dimensional ultrasound studies were attempted in 6 dogs which were normal and those with ascites, cirrhosis and neoplasia (Plates 30-32). Three-dimensional US in this study provided a more accurate anatomic structure of liver with complete visualization of lobes and in disease entities like cirrhosis and tumour gave precise location of nodules and even those areas where 2D gray scale ultrasound cannot display were readily visualized.

4.6 Cytological and histopathological findings

Out of 100 dogs cytology samples was attempted in 12 dogs with owners consent. All the samples revealed neutrophilic in filtration, while one sample revealed numerous lymphocytic cells.

Histopathological studies were performed in 8 dogs that were sent for post-mortem. The histopathological changes were focal necrosis of hepatocytes with neutrophilic infiltration, hydropic degeneration, cirrhotic nodules contained dilated sinusoids, thinning of the hepatic cords and enclosed by fibrous tissue (Plate 39) in dogs with parenchymal disorder. In dogs with biliary disorder the histopathological changes included cellular infiltration into lamina propria, cystic distension of surface mucosal epithelium of gall bladder and biliary hyperplasia with bile duct epithelium showing hyperplastic changes (Plate 38). In dogs with hepatic neoplasia the changes included poly hydral shaped neoplastic cells arranged in ductular and glandular pattern (Plate 37-38). The gross lesions of hepatic cirrhosis, cholangiocellular carcinoma with multiple irregularly formed tumours showing umbilications and macronodular cirrhosis are shown in Plate 34-36.
Discussion
CHAPTER - V

DISCUSSION

In small animal practice liver disorders remained an important part in day to day patient care. Liver disease remains a frustrating clinical entity in both diagnostic and therapeutic aspects. Although it was uncommon for a canine patient to have normal clinical pathology values in the presence of significant liver disease, enzymology and other clinical pathology test rarely indicated the type of hepatic pathology present. Many of the so called liver specific enzymes were found increased even in non primary hepatic disease. The challenge for the veterinary practitioners is to choose the most appropriate test to arrive at the most accurate diagnosis. In this back drop the “Study on comparative efficacy of diagnostic tests in the early diagnosis of canine liver diseases” was undertaken to address these challenges and to evolve a suitable diagnostic protocol for early diagnosis of liver disease.

5.1 Incidence of liver disease in canines

Studies concerning small animal liver disorders were limited in veterinary literature when compared to those in human medicine. There were not many studies on the incidence of liver diseases in India. This necessitated establishment of data related to prevalence of liver disorders in the area of practice. Various studies had documented the liver disease incidence at various levels. Candlin (1968) reported that liver disease accounted for 3 per cent of all diseases seen by veterinarians. Nambi (1993) reported of 0.42 per cent incidence of hepatic diseases in and around Chennai. Boomhens et al. (2004) reported one per cent incidence of hepatitis in a referred canine population. De Nicola (2005) observed that the incidence of liver disease, especially that of primary liver disease was less than 10 percent. The incidence of primary hepatitis was reported to be 0.5 per cent of dogs in a referral population of 101 dogs (Poldervaart et al., 2009). The prevalence of chronic hepatitis was found to be 12 per cent in a histopathological study of 200 unselected canine post mortem examinations (Watson et al., 2010).
In the present study the incidence of gastrointestinal disorders were observed to be 35 per cent (23,289 dogs) out of 66,450 dogs attended the Small Animal Medical Out-Patient Unit. Out of these 23,289 dogs, 100 dogs were found to have liver diseases of different kinds and accounted for 0.43 per cent. Out of the total population of 66,450 dogs the incidence was found to be 0.15 per cent (Fig. 1). The current incidence level was in concurred with the reports of the previous authors Nambi (1993), Boomhens et al. (2004) and Poldervaart et al. (2009).

5.1.1 Etiological pattern of canine liver disorders and their incidence

There are large numbers of diseases that affects the liver, and were generally categorized as primary and secondary disorders. Canine liver diseases included neoplastic and non neoplastic disorders. Many authors had classified liver diseases based on clinical; clinicopathological and ultrasound imaging (Tarret and O’Neil, 1985 and Reed, 1985). Primary liver disorders were most often reported in cats than in other animals. Secondary disorders of liver occurred due to hepatic lipidosis that occurred secondary to clinical entities such as Diabetes mellitus, IBD, Pancreatitis, Malnutrition etc.,

Chronic liver disease was categorized into vascular, parenchymal and biliary on the basis of gross and histopathological findings by Center et al. (1989). Presently the board of the WSAVA guided the meetings of an international Liver Standardization Group of internationally recognized clinicians and pathologists with specific expertise on liver diseases and based on these guidelines the liver disease was divided into four groups viz. (a) Vascular disorders (b) Biliary disorders (c) Parenchymal disorders including stellate and kupffer cells and (d) Neoplasia (Rothuizen and Brovida and 2010). In the current study liver diseases were categorized based on the clinical, clinicopathological and imaging findings into, (a) Parenchymal (b) Biliary and (c) Neoplastic disorders. Chronic hepatitis in the dog was reported with increasing frequency in the past few years (Hendy, 1985 and Rutgers and Haywood, 1988). An incidence of 72.8 per cent of chronic hepatitis was reported among 47 cases of hepatitis (Fuentealba, 1997). In another study
chronic hepatitis was reported in 67 out of 101 liver histopathological examinations (Poldervaart et al., 2009). Recently an incidence of 12 per cent of chronic hepatitis was reported in a study of 200 histopathological examinations (Watson, 2010). All of these previous reports and the current study highlighted the increasing incidence of parenchymal liver disorder.

The incidence of parenchymal disorders was found to be 73 per cent (73/100), biliary disorders-18 per cent (18/100) and neoplastic disorders-9 per cent (9/100) (Fig. 2). In the present study this increased incidence of parenchymal disorders was in concurrence with the previous reports.

Biliary disorders were found to affect 18 per cent (18/100) of dogs in the current study and ranked second. The reports on incidences of biliary disorders in small animals were very few (Bromel et al., 1998, Besso et al., 2000 and Voros et al., 2001). In one study an incidence of 41.53 per cent of cholecystic disorders were reported in a biliary ultrasonographic assessment of 130 dogs, where in the mere presence of sludge was taken as biliary disorder (Bandyopadyay et al., 2007). This increased incidence levels may be attributed to factors like urbanization, environmental pollution and unscientific feeding practices, inappropriate use of drugs and stress levels as well as increased diagnostic abilities with modern diagnostic protocols involving ultrasound imaging of liver and biliary tree.

Primary hepatobiliary tumours were reported to be uncommon in companion animals, with 2.6 per cent prevalent among the canine tumours studied (Patnaik et al., 1981). The estimated prevalence of liver neoplasms in canine necropsies had been estimated to be 0.6 to 2.6 per cent (Pastor and Bachs, 2010). The liver metastases were found to be much more frequent than the primary hepatic tumours and were estimated to affect 30.6 to 36.8 per cent of dogs with non-hepatic neoplasms (Pastor and Bachs, 2010). In the present study the incidence of liver tumours were found to be 9 per cent. In the present study, tumours were found to be metastatic tumours (lymphoma) in 3 dogs, two dogs were found to have hepatocellular carcinoma and cholangiocellular carcinoma which were primary liver tumours and remaining
four dogs had tumour which were unclassified. As Veterinary Oncology is a rapidly developing field, focussed research in hepatic oncology and related molecular studies will throw more light in this area and there by improving the quality of life of pet animals.

5.1.2 Breed Predilections

Doberman pinchers were documented had four times greater than the expected level of incidence of liver diseases as observed from retrospective studies (Stombeck et al., 1988 and Mandique et al., 2004). Idiopathic hepatitic fibrosis was reported to be a disease of young dogs with a predilection for German shepherds (Rutgers et al., 1993). Labrador retriever was found to be predisposed to develop chronic hepatitis that progressed to hepatic failure (Shih et al., 2007).

Labrador and Pomeranian were found to be over represented by Poldervaart et al. (2009) and Pooja et al. 2010 respectively. In the present study non-descript dogs were found to have a higher incidence of 26 per cent, followed by 20 per cent incidence in Spitz and 14 per cent incidence in Labrador (Fig. 3 and 4). Incidence in German shepherd was 12 per cent and that of Doberman were 7 per cent. The higher incidence observed in non-descript dogs were possibly due to overpopulation of non-descript dogs in the study area. However, further detailed studies could identify predilection of non-descript dogs for liver disorders and possible etiological role such as inappropriate drug usage, infectious and toxic etiologies. The incidence levels observed in Labradors, German shepherds and Dobermanns were comparable to the incidence levels in the previous reports (Mandique et al., 2004, Shih et al., 2007 and Poldervaart et al., 2009).

5.1.3 Age and Sex Predilections

Studies had documented that the liver disorders generally occurred in high frequency in dogs aged above 4 years. The mean age of dogs with chronic hepatitis was reported to be 5.3 years (Stombeck and Gribble, 1978).
A wide variation (8 month to 10 years) was reported in the age of dogs diagnosed with cirrhosis (Thormburg et al., 1983). Similarly copper related liver diseases in Dobermann were reported to occur in 2 to 10 years of age (Rutgers and Haywood, 1985). Mandigers et al. (2004) reported that liver disease was present between 4 and 6 years of age. In the present study dogs aged 4 to 8 years had a higher incidence (36 per cent) of liver disorders followed by 34 per cent in dogs aged 8 years and above. In the dogs aged less than 4 years the incidence was 24 per cent. The observed incidence levels in the current study were in accordance with the earlier reports. However the incidence level of 24 per cent observed in dogs aged less than 4 years in the study requires further investigations to elucidate possible etiological agent such as infectious or toxic.

No sex predisposition was identified in dogs for primary hepatobiliary tumours (Trigo, 1982) and chronic hepatitis (Strombeck et al., 1988). Females were found to have more commonly affected with chronic hepatitis (Speeti et al., 1996). However Poldervaart et al. (2009) opined of over representation of females with primary hepatitis. In the present study (Fig. 6) male dogs dominated the incidence (67 per cent) over female dogs (33 per cent). This could possibly due to the over representation of male dogs or the preference for the male dogs by the companion animal owners in this study area.

5.2 Medical history of canine liver disease

The medical history in dogs with hepatic disease was usually vague and non-specific. The medical history often consisted of anorexia, vomiting, diarrhea and constipation. Jaundice and other signs were rarely observed by client but may become the sole complaint on hospital admission and questioning by the physician (Rutgers and Haywood, 1988; Rutgers et al., 1993; Shih et al., 2007 and Pooja et al., 2007).

While parenchymal and neoplastic disorders in this study had non specific complaints like anorexia, weight loss and vomiting, biliary disorders had some specific complaints like high coloured urine (100 per cent) and
icterus (77.78 per cent) besides non specific signs like anorexia, vomiting and weight loss (Table 1). Alvarez et al. (2009) observed that lethargy, inappetance, vomiting, diarrhea was common factors in the assessment of medical history. The same was observed in this study also. However the reporting of high coloured urine and icterus with other non-specific history such as anorexia, vomiting and weight loss shall sensitize the clinician and he/she has to maintain a high suspicion for biliary disorders if such medical history is presented.

5.3 Clinical Presentation of canine liver disease

The physical examination of small animal patient with hepatic disease was often no more rewarding than the history. Many times physical examination was reported to be unremarkable or may reveal abnormalities consistant with an extrahepatic disorder (Webster, 2006). It was important to ascertain the other signs and their chronology for determining the appropriate diagnostic protocol. Dogs with liver disease were shown to have vague symptoms such as depression, anorexia, weight loss, vomiting and diarrhea. In advanced disease, the reported signs included icterus, ascites, polyuria and polydipsia as well as neurological signs suggestive of hepatic encephalopathy (Rutgers and Haywood, 1988; Webster, 2006 and Alwaraz, 2009).

In the present study parenchymal disorders were characterized by (Table 2) anorexia/decreased appetite (90.41 per cent), weight loss (75.34 per cent), vomiting (46.48 per cent), hepatomegaly (45.21 per cent) and ascites (34.25 per cent). These signs were almost non-specific as observed by Pooja et al. (2010) who reported of vague and varying degree of signs ranging from decreased appetite, anorexia, nausea, vomiting, ascites, weakness, weight loss, pale mucosa, epigastric pain, pyrexia, bilateral limb oedema, constipation, diarrhea, melena, icterus, encephalopathy, polyuria, polydipsia to depression occurring in different combinations. Such a non specific findings underscored a need for further diagnostic investigations for appropriate and early diagnosis of liver disorders.
Biliary disorders in this study were characterized by (Table 2) vomiting (100 per cent), anorexia and weight loss (88.89 per cent each), jaundice (77.78 per cent), abdominal pain (66.67 per cent), diarrhoea and poor hair coat 33.3 per cent each. The signs like vomiting, jaundice and abdominal pain was found to be predominant signs in biliary disorders whereas it was not observed in such predominant levels in parenchymal and neoplastic disorders. Hence these signs could be taken as clinical indicators for the presence of biliary disorders. Lecoindre (2010) reported of signs such as localized cranial abdominal pain in cases of gall bladder or pancreatic diseases. Nausea and vomiting were commonly observed in inflammatory diseases of the biliary tract, especially when the gall bladder and common bile duct were involved. In the present study vomiting, jaundice and abdominal pain were predominant signs in the biliary disorders.

Most animals with hepatic neoplasia had non specific clinical signs such as anorexia and weight loss. These two signs were observed in 75 per cent of canines with hepatic neoplasia. The other reported less frequent signs included vomiting and diarrhoea. It was also observed that approximately 50 per cent of the animals had polydipsia/polyuria whilst others had anaemia and hypovolemic shock, secondary to tumour rupture (Thamm, 2001). Thirty per cent of cases were reported to had palpable mass in the cranial abdomen and an abdominal bloating. Jaundice was reported only in 18 per cent of cases with liver tumors and the same was observed to vary in frequency in dogs with metastases (Thamm, 2001). In the present study the predominant signs observed included anaemia and weakness (66.6 per cent each), anorexia (55.56 per cent), palpable distension of liver and poor haircoat (44.44 per cent each), weight loss and ascites (33.33 per cent each). These observed signs were in accordance with the findings of the previous study by Thamm (2001). Interestingly weakness and anaemia was observed as predominant signs (66.67 per cent), only in dogs with neoplastic disorders and their presence was much less in parenchymal and biliary disorders.
5.4. Clinico-pathological findings in canine liver disease.

The mean value of haematological parameters of control group dogs presented in table 3 & 4 were within the physiological normal values as reported by (Jain, 1986 and Villiers, 2005). The mean values of biochemical parameters of control group dogs were presented in table 6a & b and the observed values of ALT, AST, SAP, GGT, BUN, Creatinine, Total Protein, Albumin, Globulin, Total bilirubin and Direct Bilirubin, Glucose and Cholesterol were also within the physiological normal range as reported by Viliers (2005). The mean values of coagulation parameters of control group dogs were presented in table 6 and were within the physiological range as reported by Ettinger and Feldman (2010).

5.4.1 Parenchymal disorders

Many studies had observed that the haematological changes noticed in parenchymal disorders were non-specific or unremarkable (Patnaik et al., 1981 and Fuentealba, 1997). Haematological changes such as anaemia, abnormal erythrocyte morphology, reduced platelet number and function and lipaemic plasma were reported by Center (1986). Chronicity of hepatic disease was observed to be characterized by non regenerative anemia (Alvarez and Whittemore, 2009). In the present study significant anaemia was evident (Table 3). Significant reductions were observed in Haemoglobin (9.43 ± 0.44 g/dl), PCV (26.02 ± 1.35 %) and RBC count (3.97 ± 0.21 x 10⁶ cells/mm³). However there were no significant changes in erythrocytic indices. However the observed anaemia was in accordance with the reports of Center (1996) and Alvarez and Whittemore (2009). This anemia could be due to the chronicity of liver disease as well as inefficient iron utilization observed in chronic liver diseases.

Rothuizen and Brovida (2010) observed that the anaemia present in dogs with hepatobiliary diseases was of non regenerative anaemia and attributed this anaemia to the inefficient utilization of systemic iron stores. Lecoindre and Arpaillange (2010) also observed that anaemia in hepatic
disease was associated with chronic inflammatory reactions and related
defective iron utilization. Significantly elevated leucocyte count (18,220.55 ±
895.36 cells / mm$^3$) and neutrophil count (14,610.89 ± 779.03 cells / mm$^3$)
observed in this study indicated the ongoing inflammatory process in the
parenchymal disorders of liver. This inflammatory process could have caused
the anemia as observed in previous studies (Brovida and Rothuizen, 2010
and Lecoindre and Arpaillange, 2010)

Elevations in the values (Table 5a&b) of liver enzymes such as ALT,
AST, ALP, GGT was observed in parenchymal disorders, they were above the
normal range. These increases in liver enzymes indicated the hepatobiliary
injury. The observed increases in ALT, AST and ALP indicated a mixed
pattern of increased liver enzyme activity. Such a mixed pattern of liver
enzyme activity was generally due to concurrent hepatocellular injury and
cholestasis as well as other concurrent disease processes or progressive
disorders as opined by Alvarez and Whittemore (2009). Possible causes
attributed to this mixed pattern of injury were hepato toxins, drugs, heavy
metals, aflatoxins, other fungal and bacterial toxins (Alvarez and Whittemore,
2009). In the present study there were significant reductions in total protein
and albumin (Table 6 b) as well as non significant increases in the total and
direct bilirubin. All of them indicated the parenchymal pathology. The
observed hypoalbuminemia was result of disruption of hepatic protein
metabolism, as observed by previous studies (Sevelius, 1995 and Shih et al.,
2007). While mixed pattern of elevated liver enzyme activity and
hypoproteinemina indicated the liver disease, further diagnostic tests such as
ultrasound imaging and fine needle aspiration biopsy studies were undertaken
for diagnostic confirmation of liver disease in this study. Thus for diagnosis of
parenchymal liver disorders a combinatory protocol consisting of clinical
pathology, imaging and cytology is essential.

5.4.2 Biliary disorders

Significant anaemia was characteristic in dogs with biliary disorders.
Significant decreases in haemoglobin (14.10 ± 0.66 g/dl), PCV (29.39 ± 1.65
%) and RBC (4.66 ± 0.35 x 10^6 cells / mm^3) were observed in dogs along with non-significant changes in erythrocytic indices. The significantly increased levels of WBC count (21,094.44 ± 1.660.84 cells / mm^3) and neutrophil count (17,218.67 ± 1,480.80 cells / mm^3) evidenced in this study indicated the inflammatory process involved in the biliary disorders and that could be the possible cause for observed anaemia. These chronic inflammatory processes and the related inefficient iron utilization could have caused the anaemia as reported in previous studies (Brovida and Rothuizen, 2010 and Lecoinder and Arpallange, 2010).

The biochemical changes observed in biliary disorders (Table 5 a&b) included a significant rise in GGT (15.07 ± 2.01 IU/L), total bilirubin (1.55 ± 0.25 mg/dl) and direct bilirubin (0.5056 ± 0.73 mg/ dl). There was significant decrease in total protein and albumin and all of these changes indicated the presence of biliary pathology. The hypoproteinemia could be due to disruption in hepatic protein metabolism. Similar findings were reported in previous studies also (Sevelius, 1995, Shin et al., 2007, Poldervaart et al 2009, Brovida and Rothuizen, 2010). Besides these changes, non-significant elevations were also observed in liver enzymes such as ALT, AST, ALP as well as metabolites like BUN, Creatinine and Cholesterol. In these dogs the liver enzyme elevations revealed a cholestatic or inducible pattern of activity as evidenced from the predominant increases noticed in GGT and ALP values. Such a significant cholestatic pattern of liver enzyme activity coupled with elevations in total bilirubin and direct bilirubin indicated the biliary pathology involved. It was observed that the specificity of concurrently increased activities of ALP and GGT for hepatobiliary disease was more than 90 per cent (Center et al., 1992). This increased cholestatic pattern was also observed in cases with intra or extra hepatic cholestasis, extra hepatic neoplasia, endocrine disorders or breed related or drug induced. Since such numerous causes were causing cholestatic pattern of enzyme activity, the ultrasonographic assessment undertaken in this study helped in the diagnostic confirmation and localization of biliary pathologies.
One notable observation in this study was that the dogs with biliary disorders had significantly decreased platelet count (1.33, 422.22 ±12.913.81 cells /µL) with non-significant changes in PT and aPTT. Recent studies have documented that there is quantitative and qualitative platelet defects accompanying hepatobiliary disease (Prins et al., 2010 and Brovida and Rothuizen, 2010).

In the present study elevation of GGT was significant in dogs with biliary disorders, whereas only non-significant changes observed in dogs with parenchymal and neoplastic liver disorders. This emphasized the importance of GGT as preliminary screening test for dogs with suspected biliary disorders. Brovida and Rothuizen (2010) observed that serum elevations in GGT were most common in cholestatic disorders and were associated with increased de nova synthesis as well as membrane elution. Serum GGT activity has less influence by non hepatic disease process or enzyme inducing drugs compared with serum ALP activity hence GGT is considered of more diagnostic value in diagnosing biliary disorders (Center et al., 1992).

5.4.3 Neoplastic disorders

Dogs with neoplastic disorders of liver had a significant anaemia as evidenced from significant reductions in haemoglobin (9.89 ±1.12 g/dl), PCV (25.76 ± 3.00 %), RBC (4.14 ± 0.57 x 110\(^6\) cells / mm\(^3\)). The platelet count was significantly reduced (115,544.44 ± 17,351.33 cells /µL) with non significant changes in PT and aPTT. The observed anaemia was also clinically evident through the signs such as tachycardia/tachypnoea and anemia (66.67 per cent) observed in dogs with hepatic neoplasia. Such a predominant anaemic signs were not evident in parenchymal disorders (16.45 per cent) and biliary disorders (16.67 per cent)

This anemia observed in dogs with neoplastic disorders could be due to chronic illness or inflammation or iron deficiency as observed by Pastor and Bachs (2010). In animals with hepatocellular carcinoma 50 per cent of them had thrombocytosis and the same was attributed to the paraneoplastic
syndrome which was characterized by thrombopoietin production, iron deficiency or anaemia (Pastor and Bachs, 2010). In contrast to this a significant thrombocytopenia was observed in this study and the mechanism by which the thrombocytopenia evolves in these neoplastic dogs could not be elucidated at the moment. It requires further detailed studies on platelets and the entire coagulation cascade to understand the precise mechanism involved. There was a significant leucocytosis (15,088.89 ± 2,079.29 cells / mm$^3$) and neutrophilia (11,974 ± 1,681.97 cells / mm$^3$) in dogs with neoplastic disorders.

This significant leucocytosis could be the result of inflammation and necrosis associated with large tumours as observed by Pastor and Bachs (2010). Center et al. (1992) reported that animals with primary liver tumours had showed marked increase in ALT and ALP activities. ALT, ALP was found to be non-significantly increased in dogs with neoplastic disorders. No major changes were noticed in AST and GGT in these dogs. This revealed a mixed pattern of liver enzyme activity which was observed to occur in progressive hepatic disease, concurrent disorders, hepatotoxicity, hepatocellular inflammation and necrosis with secondary cholestasis or drug induced (Center et al., 1992). Therefore other better diagnostic protocols has to be deployed for identification of neoplasia and ultrasound imaging in the present study helped for diagnostic confirmation of hepatic tumours.

Liver enzyme elevation was a frequent but not universal finding in animals with liver neoplasms and the degree of enzyme elevation was found not to correlate with the degree of hepatic involvement or the severity of the disease (Pastor and Bachs, 2010). This necessitated further diagnostic testing and ultrasound imaging proved to be better protocol as observed in this study.

Dogs with neoplastic disorders in this study had non-significant changes in the metabolites such as total and direct bilirubin, glucose and cholesterol and BUN and Creatinine (Table 6 a & b). However there were
significant decreases in total protein and albumin levels in this study. Center et al. (1992) reported of, hypo or hyper albuminemia in dogs with liver tumours. In this study hypoalbuminemia was observed. This observed hypoalbuminemia could be the clinical consequence of disruption in hepatic protein metabolism noticed in chronic hepatic disorders (Brovida and Rothuizen, 2010).

The enzyme ALT and ALP elevation in all the groups were found to above the physiological normal range but they were not statistically significant this may be due to the short half life the enzyme and single insult to liver had resulted in high values that soon returned to normal. Moreover, being referral hospital the time delay in presentation of case could be a cause for the increased levels that was not statistically significant. Haematobiochemical results when used in correlation with the results of further diagnostic tests and ultrasound to image liver formed to be a better diagnostic protocol.

5.5. Hepatic ultrasonographic findings

The sonographic findings of the hepatobiliary system control dogs were well in agreement with the ultrasonographic features of normal liver and associated structures described by Nyland (1984) and Anre d Ajou (2008) (Table 7 & Plate 18-29,33).

5.5.1 Sonographic findings in parenchymal disorders

Out of the 73 dogs with parenchymal disorders, hepatic volume was found to be normal in 18 dogs, increased in 37 dogs and decreased in 8 dogs. Changes in hepatic shape include rounded borders in 33 dogs and irregular borders in 14 dogs (indicative of cirrhosis/fibrosis). Hepatic parenchymal changes included homogeneous parenchyma in 67 dogs and heterogeneous parenchyma in 6 dogs. Hepatic echogenicity was found to be decreased in 16 dogs, increased in 18 dogs (2 lipidosis) and mixed pattern in 3 dogs. Hypoechoic masses and mixed masses were visualized in one dog each. Gall
bladder was distended in 11 dogs. Spleen was normal in 62 dogs and spleenomegaly was found in 9 dogs (mild to moderate splenomegaly) & 2 dogs (splenectomized). Ascites was recorded in 25 dogs. Prominent hepatic veins were visualised in 22 dogs and prominent portal veins were visualized in 27 dogs. All these ultrasonographic findings were in concurrence with the previous reports (Nyland and Park, 1998).

In an ultrasonographic study of 21 dogs, Shih et al. (2007) reported changes in echogenicity which included - inhomogeneous-8 dogs, hypoechoic-5 dogs and hyperechoic-3 dogs. Nine of the dogs had one or more nodules. Abnormal liver size was reported in 6 dogs and ascites in 2 dogs. They also observed splenomegally, hypo/hyper echoic spleen in 9 dogs. Nyland and Park (1998) in their study with 11 case history reports described hepatic abscess appearing as echogenic, cyst as echoic and diffuse hepatic disease with changes in echogenicity and increase or decrease in size. These sonographic findings in the parenchymal disorder group were in agreement with the reports of Nyland and Park (1998) and Shih et al. (2007).

5.5.2 Sonographic findings in biliary disorders

Dogs with biliary disorder in this study had increased hepatic volume in 3 dogs while the volume was normal in 15 dogs. Hepatic border was rounded in 5 dogs and 13 dogs had normal shape. The hepatic parenchyma was homogeneous in all the dogs. Normal echogenicity was noticed in 14 dogs and decreased echogenicity observed in 4 dogs. There were no focal lesions visualized in parenchyma in all these dogs. Gall bladder was distended in 9 dogs. Thickened gall bladder wall was observed in 7 dogs. Two dogs had cholecystolith and 8 dogs had sludge. Spleen was normal in 14 dogs and 4 dogs had mild splenomegaly. Ascites was visualized in 3 dogs and hepatic veins was engorged in 3 dogs (engorged but less than caudal vena cava) and Portal veins were prominently visualised in 4 dogs. These ultrasonographic changes were similar to that of previous reports.
Spaulding (1993), Lamb (1995) and Burk and Ackerman (1996) reported that the gall bladder wall thickening had an appearance as hyper echoic wall with or without irregular muscular contour. Hypoechoic thickening was reported to be associated with gall bladder mucocele, cholangiohepatitis and hypoproteinemia (Newell et al. 1995; Nyland and Hager, 1985; Barr, 1990; Reed, 1995 and Sceler, 1995). Gall bladder size was reported to be variable depending on animals feeding status (Barr, 1990). The presence of sludge was reported in dogs even with normal liver functions; presence of cranial abdominal pain correlated with pathology of gall bladder (Jennings et al., 1992). Such an abdominal pain was evident in this study too. Thus the ultrasonographic findings in this study were in accordance with the findings of the previous studies.

5.5.3 Sonographic findings in neoplastic disorders

Diffuse or multifocal liver neoplasms presented variable ultrasonographic characteristics. Lymphomas were visualized as hypo/hyper/mixed echogenicity with or without nodules. Histiocytic neoplasms were associated with multiple nodules and hypoecic masses. Mast cell infiltration presented a diffuse hyperechogenicity. Nodular patterns were manifested as focal masses of variable size and normally hyperechoic characteristics. Primary liver neoplasms were reported to have a focal hypoechoic lesion with central hyperechoic areas known as target / bull’s eye lesions (Barr, 1990; Sceler, 1995; Nyland and Park, 1998 and Pastor and Bachs, 2010).

In the present study out of 9 dogs with neoplastic disorders, the hepatic volume was increased in 7 dogs. The observed changes in shape included rounded borders in 3 dogs, irregular borders in 4 dogs and other 2 dogs had normal shape. Hepatic parenchyma was found to be homogeneous in 3 dogs and heterogenous in 6 dogs. Hepatic echogenicity was normal in 2 dogs while mixed pattern was observed in 7 dogs. Hepatic focal lesions observed included hypoechoic nodules in 2 dogs, hyperechoic nodules in 2 dogs, hyperechoic masses in 2 dogs and mixed masses in 5 dogs. Gall bladder was found to be distended in 4 dogs. Gall bladder wall thickening and sludge was
visualized in 2 dogs. Spleen was found to be normal in 5 dogs while tumour mass and splenomegaly was observed in 4 dogs. Ascites was visualized in 3 dogs. Hepatic vein engorgement was observed in 3 dogs and 4 dogs had prominent portal veins. These sonographic findings in the present study were in agreement with the reports of the previous studies (Barr, 1990; Sceler, 1995; Nyland and Park, 1998 and Pastor and Bachs, 2010).

5.5.4 3D Ultrasonographic Studies of liver

In the present study 3 dimensional ultrasound studies were attempted in 6 dogs which were normal and those with ascites, cirrhosis and neoplasia (Plates 30-32). Three-dimensional US in this study provided a more accurate anatomic structure of liver with complete visualization of lobes and in disease entities like cirrhosis and tumour gave precise location of nodules and even those areas where 2 D gray scale ultrasound cannot display were readily visualized. With 2D ultrasonography, a “flat” anatomic section is displayed on a video monitor or on film 3D ultrasonography allows the obtained data to be displayed with a variety of techniques, including surface rendering, volume rendering, and multiplanar reformatting. Such a 3D ultrasonography had been shown to provide a more accurate and repeatable method of evaluating anatomic structures and disease entities (Downey et al., 2000). Compared to CT scan or MRI, ultrasonography has an advantage that the scanning plane can be selected more freely than with the other modalities. 3D ultrasonography could display information in a manner that was not previously been possible with conventional techniques (Kim et al., 2009). Towards this better visualization, an attempt was made in the present study to assess the canine liver with 3D ultrasonography and the results were found to be promising. In the present study the imaging of dogs with liver cirrhosis and tumour had been visualized (Plate 30-32) in a better way. This was in agreement with the above authors who reported that that the anatomic visualization and localization 3D in comparison with 2D imaging was more accurate. Further studies on volumetric assessment of liver, irregular shaped structures like gall bladder would enable to evolve a standard sonographic
procedure and help make accurate volume assessments in dogs with hepatobiliary diseases. Since 3D ultrasonography has the advanced capacity to demonstrate lesion margins and topography, it could help in differentiating benign from malignant masses. It can also help to determine the need for biopsy and help facilitate needle localization and guidance during biopsy. Further exclusive studies in this area are essential, as they would help to develop precise and accurate diagnostic protocols for hepatobiliary disorders.

Three-dimensional US are rapidly gaining popularity as it moves out of the research environment and into the clinical setting this modality offers several distinct advantages over conventional US, including 3D image reconstruction with a single pass of the US beam, virtually unlimited viewing perspectives; accurate assessment of long-term effects of treatment; and more accurate, repeatable evaluation of anatomic structures and disease entities. 3D ultrasonography can be very useful in determining the likelihood of malignancy and therefore the need for biopsy. In addition, 3D ultrasonography can help facilitate needle localization and guidance during biopsy. Compared with other imaging modalities, CT is associated with a significant dose of ionizing radiation depending on patient age and exam protocol, CT may cause a non-negligible increase in lifetime cancer risk (Downey et al., 2000). CT scan exposes patients to 500 times the radiation that of X-ray, causing changes at genetic levels and triggering for cancers. 3D and 4D USG is risk free and frames the future.

5.6. Cytology and histopathology

In the present study the cytology in 11 dogs revealed neutrophilic infiltration, while one sample revealed numerous lymphocytic cells which revealed a diffuse hepatic tumor mass on ultrasonographic examination. The liver is highly vascular organ, all cytologic specimens contain blood and, techniques using negative pressure to collect liver specimen frequently produce slides resembling blood smear, hence the leukocytes are compared with the peripheral blood smear and if found in excess then it can be taken as associated with neutrophilic infiltration of liver otherwise its mere a blood
contamination. The frequencies of diagnosis of canine liver cytology were classified into seven viz. normal (9 to 10 per cent), inflammation (22 - 56 per cent), malignant neoplasms (19-23 per cent), extramedullary hematopoiesis (5 -6 per cent, metabolic / degenerative (16 - 32 per cent), pigmented abnormalities (7 per cent) and non diagnostic (4 - 5 per cent) (Moritz, 2002). Neutrophilic infiltration is associated with necrosis and bacterial infection or with suppurative hepatitis or cholangitis more neutrophils were present in liver tissue than peripheral blood (Raskin, 2000). The cytological findings in the present study with high number of neutrophilic infiltration concurred with reports of previous authors.

Histopathological studies were performed in 8 dogs that were sent for post-mortem. The histopathological changes were focal necrosis of hepatocytes with neutrophilic infiltration, hydropic degeneration, cirrhotic nodules contained dilated sinusoids, thinning of the hepatic cords and enclosed by fibrous tissue (Plate 39) in dogs with parenchymal disorder. These concurred with the reports of Cullen et al. (2006); Van den Ingh et al. (2006) and Winkle et al. (2006). In dogs with biliary disorder the histopathological changes included cellular infiltration into lamina propria, cystic distension of surface mucosal epithelium of gall bladder and biliary hyperplasia with bile duct epithelium showing hyperplastic changes (Plate 38). Similar changes have been reported by Van den Ingh et al. (2006). In dogs with hepatic neoplasia the changes included poly hydral shaped neoplastic cells arranged in ductular and glandular pattern (Plate 37) these were in concurrence with the reports of Charles et al. (2006). This suggests that along with routine clinical, haematobiochemical, ultrasonographic imaging histopathology has to be taken in order to establish a confirmative early diagnosis.
Summary and Conclusions
CHAPTER - VI

SUMMARY AND CONCLUSIONS

The study entitled “Study on Comparative Efficacy of Diagnostic Tests in the Early Diagnosis of Canine Liver Diseases” was carried out at the Centre of Advanced Faculty Training in Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, TANUVAS, Chennai-7 during the period October 2011 to January 2012. The study was conducted with the clinical cases presented to the Small Animal Medical Outpatient Clinic of the Madras Veterinary College Teaching Veterinary Hospital over a period of five semesters.

The incidence of liver disease was found to be 0.15 per cent of dogs in the study population of 66,540 dogs, while it formed 0.43 per cent incidence in the gastrointestinal caseload.

Non-descript dogs were found to be more commonly affected and the age groups of 4-8 years were commonly affected. However dogs less than 4 years also found to be affected with second highest incidence and it requires further studies to ascertain the breed predisposition of nondescripts and etiopathophysiological studies to identify the cause or risk factors such as infectious or toxic agents.

Parenchymal liver disorders were found to be highest in occurrence 73 per cent (73/100), followed by biliary disorders 18 per cent (18/100) and neoplastic disorders 9 per cent (9/100).

Iatrotrophic stimulus for biliary disorders included high coloured urine, icterus, vomiting, weight loss and anorexia. Out of these complaints, high coloured urine and icterus was much more the commonest complaint. They were reported with less frequency in dogs with parenchymal and neoplastic liver disorders.
While the clinical presentations for parenchymal disorders were almost non specific, biliary disorders had a characteristic clinical presentation with signs such as vomiting, jaundice, and abdominal pain. These clinical findings were reported with less frequency in dogs with parenchymal and neoplastic liver disorders.

Dogs with neoplastic liver disorders showed clinical signs such as weakness, anaemic signs like tachycardia /tachypnoea as predominant signs, while such signs were reported in less frequency in parenchymal and biliary disorders.

Significant anemia was evident in all three kinds of liver disorders viz. parenchymal, biliary and neoplastic disorders and the cause of which was the chronic inflammatory process involved in hepatic diseases and the associated inefficient iron utilization by these patients. The chronic inflammatory process was evidenced by significant leucocytosis and neutrophilia, observed in all the three kind of liver diseases.

Platelet counts were found to be significantly reduced in all the three kind of liver disorders, and non significant changes were observed in PT and aPTT. Neoplastic disorders had shown a thrombocytopenia in contrast to previously reported thrombocytosis in dogs with hepatic tumours. This necessitated further studies on platelet function and quantum and coagulation profile in dogs with hepatobiliary diseases.

Liver enzyme elevations were though documented, they were statistically non significant in this study. This necessitates the need for further diagnostic testing such as ultrasound imaging for early and precise diagnosis and appropriate interventions.
Cholestatic or inducible pattern of liver enzyme elevations was evident in dogs with biliary disorders. Though parenchymal and neoplastic disorders showed a mixed pattern of liver enzyme elevations, these elevations were statistically non significant. It highlights the need for further diagnostic testing for appropriate diagnosis of liver diseases.

Hypoalbuminemia and reduced serum protein concentrations were significant in all the three groups of liver disorders and this could be due to the disruption of protein metabolism observed in chronic inflammatory process encountered in liver disorders.

Due to limitations in liver enzyme elevations and their associated causes of varied nature, ultrasound imaging was deployed for diagnostic confirmation and the same was found to have better diagnostic yield. Besides helping in diagnosis, Ultrasound imaging facilitated biopsy of liver tissues and subsequent cytological / histopathological assessment of liver diseases. 3D ultrasound imaging was found to have superior diagnostic yield, especially to visualize the exact location of the lesions/ changes involved in the liver diseases.

The following conclusions were derived from this study

1. The incidence of canine liver disease was found to be 0.15 per cent of dogs in the study population of 66,540 dogs and 0.43 per cent of the gastrointestinal case load of 23,289 dogs. Out of which parenchymal disorders were the commonest - 73 percent (73/100), followed by biliary disorders - 18 per cent (18/100) and neoplastic disorders - 9 per cent (9/100).

2. Gamma glutamyl transferase was found to be significantly elevated in biliary disorders. The other enzyme levels were found to be elevated in
all the groups beyond the physiological normal range but the increase was non significant and their increase cannot be correlated with the type of canine liver diseases.

3. Ultrasound was found to be very useful in the diagnosis of canine liver disease and strategic interpretation of the results can be effectively used for identifying the canine liver disease and type of liver disease in majority of the cases.

4. Hepatic histopathology is gold standard test for the diagnosis of various liver disorders. In comparison, ultrasound is a non invasive technique found to be effective in the early diagnosis of canine liver diseases.
REFERENCES


