Ascites and hepato-renal syndrome in cirrhosis in Dogs


Abstract
Ascites is a common problem in dogs but due to the involvement of different etiological factors, the clinicians are facing lots of difficulty in therapeutic management. There is no detailed investigation carried out in Mizoram till today. Given this background and facts, the present study has been undertaken to know the role of the hepato-renal problem on ascites and its pathological changes in dogs. Study revealed that the incidence of ascites in Mizoram is 1.9% and 6 cases were confirmed as ascites due to the hepato-renal problem. The haemato-biochemical analysis revealed anemia, neutrophil leukocytosis, Hypoproteinemia, Hypoalbuminemia, hypocholesterolemia, hyperbilirubinemia, increased alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine level in all the ascitic dogs. Ultrasonography (USG) examination revealed anechoic areas mild to extensive suggestive of fluid accumulation along with floating of intestines and internal viscerum, is echo kidney cortex and medulla; enlarged renal pyramids with anechoic echo texture, focal hyperechoic and loss of echogenicity of hepatic parenchyma with increased size and distended gall bladder. Radiographic examination showed ground glass appearance with increased in vertical heart size (VHS). Electrocardiogram (ECG) showed sinus tachycardia, atrial standstill and prolonged QRS duration. Echocardiography examination revealed increased left ventricular internal diameter at diastole (LVDD), Left ventricular internal diameter at systole (LVDS), left atrial to aortic ratio (LA: AO) and e-point to septal separation (EPSS) values and decreased interventricular septum thickness at diastole (IVSd), interventricular septum thickness at systole (IVSs), ejection fraction (EF) and fractional shortening (FS). Histopathological examination revealed coagulative necrosis of liver, kidney and heart. From the study, it was confirmed that the involvement of hepato-renal dysfunction may responsible for the critical condition of ascitic patients.

Keywords: Ascites, Hepato-renal dysfunction, Dog, Cirrhosis

1. Introduction
Etiology. Literatures on ascites in dogs are readily available; however, published reports on its association with different etiology in Indians. There is no detailed investigation carried out in Mizoram till today. Given this background and facts, the present study has been undertaken to know the role of the hepato-renal problem on ascites and its pathological changes in dogs.

2. Materials and Methods
2.1. Study area
The study was conducted in Teaching Veterinary Clinical Complex (T.V.C.C), College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram (Fig.1)

2.2. Selection of animals
A total of 1066 dogs were brought to the Teaching Veterinary Clinical Complex, Selesih, Aizawl, Mizoram, during the study period i.e.April, 2018 to April 2019 for various diseases. All the dogs were put through preliminary screening for the presence of ascites. It consisted of history taking, recording of temperature, pulse, respiratory rate, heart rate, STT, CRT, the colour of the mucous membrane and body condition score. Dogs showing clinical signs of the distended abdomen, decreased appetite, lethargy, vomiting, diarrhea, melena, weight loss and
lethargy, vomiting, diarrhoea, melena, weight loss and mild jaundice, exercise intolerance were subjected to thorough clinical examination.

2.3. Collection of samples
Blood was collected from a cephalic or saphenous vein in a sterile K3EDTA containing vial for estimation of haematological parameters and about 5 ml of blood was collected in a sterile test tube without an anticoagulant for serum separation to the analysis of biochemical and oxidant-antioxidant status.

2.4. Measurements
Hemoglobin (HB), Packed cell volume (PCV), Total leucocyte count (TLC), Differential Leucocyte count (DLC), Erythrocytic Indices (MCV, MCH, MCHC) and Platelet Count was analysed by automated blood analyzer (Melet Schlosinge, France).

Total protein (TP), Albumin, Globulin, A:G ratio, Bilirubin, Blood Urea Nitrogen (BUN), Creatinine, Alanine Amino Transferees (ALT), Alkaline Phosphatase (ALP), Triglycerides, Total Cholesterol, Creatine kinase MB (CK-MB), Sodium and Potassium was evaluated by using Fuji Film Drichem 4000i biochemistry analyser.

Among the various antioxidants level indicators, lipid hydroperoxide (LPO) and superoxide dismutase (SOD) were analysed by using commercial kit (Cayman Chemicals, USA). The parameters were estimated in serum as per manufacturer’s instructions.

2.5. Ascitic fluid analysis
Abdominal paracentesis was performed to obtain the fluid for the biochemical and cytological analyses. The fluid collected from the peritoneal cavity was evaluated for colour, turbidity, specific gravity and total protein. The serum ascites albumin gradient (SAAG) was calculated by subtracting the albumin concentration of the ascitic fluid from the albumin concentration of a serum specimen obtained the same day.

2.6. USG Examination
Ultrasonography study in ascitic dogs was performed as per the procedures described by Nyland et al. with My Lab 40 Vet Esaote (Netherlands) and Curvilinear probe (5-8 MHz). The images were recorded on a thermal printing paper using a black and white thermal printer.

2.7. Radiography
Thoracic and abdominal radiographs were obtained by using Heliphos-D X-Ray generator (Siemens Healthcare Pvt. Ltd., India). The digital X-ray cassettes were read using FCR Prima T2 (Fujifilm, India) and when required, prints were obtained using Dry Ptx Lite (Fujifilm, India). Thoracic radiography was done in lateral and dorsoventral views.

2.8. Electrocardiography
ECG was done in right lateral recumbence using standard bipolar limb lead II which is the most commonly used lead for diagnosing different cardiac abnormalities in dogs.

2.9. Echocardiography
Echocardiography was performed 2D, M-mode and Doppler mode. Suspected cardiac involvement was further confirmed by using cardiac troponin-I, a double antibody lateral flow immunoassay kit with a sensitivity of detecting > 1.5 ng/L (Alfa Scientific, USA).

2.10 Post-mortem and Histology
Those animals expired during the study period, were post-mortem and collected liver, heart, spleen for histopathological examination which was carried out as per standard procedures described by Bancroft and Gamble respectively to find out the etiological factors of ascites. Histopathology examination of tissue processing was carried out as per the method described by Luna.

Six healthy dogs brought for routine general checkup and vaccination were selected as control groups for obtaining normal data for comparison of parameters understudy.

2.11 Statistical analysis
The results were analyzed using paired ‘t’ test for comparing healthy and diseased groups.

3. Results and Discussion
3.1 Incidence of Ascites due to hepato-renal involvement
Out of 1066 cases, 21 cases were suspected for ascites by physical and clinical observation and 1.96% (21/1066) cases were confirmed as ascites by non-investigative and investigative techniques. All the positive cases of ascites were confirmed according to their clinical signs, ascitic fluid analysis result, haemato-biochemical changes and imaging
techniques results and out of 21 cases, 28.6% (6/21) cases were confirmed as hepato-renal dysfunction. The present findings were in agreement with other workers [16,17] who reported that the prevalence of ascites in canines was from 0.59% to 2.5% in Uttarakhand, Nigeria, and Bareilly. Major clinical signs observed in ascites cases were abdominal distensions (95.23%), fluid wave test (95.23%), inappetence (90.47%), dyspnoea (80.95%), exercise intolerance (52.38%), pale mucous membrane (42.85%), icterus (28.57%), melena (23.80%) vomition (19.04%), peripheral edema (14.28%) and tachycardia (9.52%) and these findings were in accordance with the work of Saravanan et al. [16]. Varied signs observed in ascites cases might be due to reduced or failure function of different vital organs viz. liver, heart, kidney etc. Anaemic animals have decreased the ability of blood supply to tissues with adequate oxygen for proper metabolic functions. [17] As a result, there will be lethargy, weakness, exercise intolerance, anorexia, heart murmurs, dyspnoea and pale mucous membranes [18].

3.2 Haemato-biochemical and oxidative stress indices changes

The mean values of Hb (6.60±1.80 g/dl), PCV (25.83±5.94 %), TEC (3.93±0.68 X 10⁹/µl), THR (1.70±0.52 X 10³/µl), MCV (57.72±1.62 fl) were significantly decreased as compared to healthy control (14.55±0.76 g/dl, 46.61±1.61%, 6.82±0.20 X 10⁹/µl, 3.82±0.35 X 10³/µl, 68.45±0.78 fl respectively). Similarly, MCH (17.10±0.85 pg), MCHC (27.53±0.99 g/dl) and lymphocytes (15.00±2.10 %) were also significantly (p<0.05) decreased as compared to healthy dogs (22.05±1.17 pg, 32.26±0.69 g/dl and 24.00±2.06 % respectively). On the other hand, the mean values of TLC (14.12±1.36 X 10⁹/µl) and neutrophils (80.00±0.60%) were significantly (p<0.05) increased as compared to healthy control (14.12±1.36 X 10⁹/µl and 73.66±0.95 % respectively). The mean values of TP (3.90±0.26 g/dl), ALB (1.70±0.20 g/dl), A/G Ratio (0.87±0.27) and Na (114.66±7.83 mEq/L) were significantly (p<0.01) decreased as compared to healthy control (6.53±0.19 g/dl), 3.41±0.20 g/dl, 1.10±0.08, 146.83±0.97mEq/L and 103.00±1.82mEq/L respectively). Similarly, Cl values (87.00±1.52 mEq/L) of multi-organ dysfunction was also significantly (p<0.05) decreased as compared to healthy dogs (103.00±1.82mEq/L). On the other hand the mean values of TBIL, ALT, ALP and BUN were significantly (p<0.05) decreased as compared to healthy control. A similar result also observed by Shaden M Hanafy et al. [27] Our study showed decreased levels of SOD and significantly (p<0.05) increased LPO level in ascitic dogs as compared to healthy control. A similar result also observed by Shaden M Hanafy et al. [27] Our study showed decreased levels of SOD in serum and increased levels of LPO in ascitic patients with liver cirrhosis might be due to more oxidative stress results from liver failure or inflammation as liver disease progresses. In liver disorders, the liver is primarily affected by reactive oxygen species and their natural structures are altered [28]. The onset of cirrhotic cardiomyopathy triggered by an increase in free radicals and abnormality in lipid profile doubles this problem.

3.3 Ascitic fluid analysis

The ascitic fluid analysis revealed that the colour of the ascitic fluid was exuded ascites fluid. In exuded, the values of specific gravity, total leukocyte count (cells/ml), total protein(g/dl), albumin(g/dl) and SAA G(d) were 1.027, 8054, 3.5 0.1 and 0.60 respectively. Cytological ascitic fluid revealed the presence of few mesothelial cells, lymphocytes, monocytes and neutrophils in most of the ascitic dogs, Bacterial culture of the ascitic fluid showed positive for gram-positive cocci (E. faecium) and gram-negative cocci (E. coli). This is in agreement with other studies that revealed the monomicrobial infection [29]. Analysis of ascitic fluid is an important component of diagnosis. It can either assist the pathological process responsible for the fluid accumulation or
help in diagnosing the etiological factors of ascites[30].

3.4 Ultrasonography examination

USG examination revealed isoechoic kidney cortex and medulla; renal pyramids enlarged with anechoic echotexture and reduced blood circulation to the kidney (Fig. 2 E & F). One dog showed focal hyperechoic and loss of echocentricity of hepatic parenchyma (Fig.2A), one dog showed hyperechoic hepatic parenchyma with irregular sharp edges and one dog showed hyperechoic hepatic parenchyma with increased size, distended gall bladder filled with echogenic sludge and hyperechoic wall, hyperechoic spleen with slightly irregular margin and dense ascitic fluid containing numerous echogenic particles (Fig.2 B, C, D). A similar result also observed by Vijaykumar et al. [3] USG examination revealed that ascites was observed in this study due to hepato-renal with cardiac dysfunction but which organ was responsible for the initial cause of ascites did not find out in this study. But as per the previous report, it was informed that cardiac and renal failure is a common complication of patients with advanced liver cirrhosis[31].

3.5 Radiographic examination

Radiographic examination showed ground-glass appearance due to the presence of ascitic fluid (Fig 2 A) and thoracic radiographic examination showed increased in VHS (13.25±0.35), consolidation of the lung, displacement of trachea dorsally, cardiac silhouette and aorta were not clear. One dog showed kinking of posterior vena cava (Fig. 3). Vertebral heart size (VHS) is useful for the diagnosis of different heart problems with variable accuracy. [32] VHS has been found as the most accurate radiographic index for identifying dogs with pericardial effusion (PE) and also to differentiate it from other cardiac diseases[33]. It was observed that VHS increases significantly in DCM which is similar to the other reports [33]. The VHS was high (13.25±0.35) that suggest cardiac pathology such as dilated cardiomyopathy, agreeing with the findings of Marin et al. [34] However, breed and body conformation can influence VHS.

3.6 Electrocardiographic examination

ECG was carried out in all the cases to find out cardiac involvement. In the case of multiple organs disorder, one animal showed sinus tachycardia, atrial standstill (absence of P wave) and prolonged QRS duration (Fig.4A). One case showed the alternating amplitude of R wave and decreased QRS amplitude, notching of R waves (Fig.4B) and one dog showed fragmented QRS (f QRS) and Osborn wave (Fig.4C). Electrocardiogram (ECG) provides critical information on several changes in the electrophysiological function, in particular, cardiac rhythmicity, conduction, depolarization and repolarization, which cannot be assessed by other methods. Sinus tachycardia might be due to anaemia or congestive heart failure which was also reported by Edwards [35]. Atrial standstill is characterized by the absence of atrial activity on the surface and intracavity electrograms [36]. The condition has been documented in dog and cat with hyperkalaemia [37] which is disagreement with the present study as potassium level in the present study was found normal. Atrial standstill is a serious condition which leads to heart failure observed in the present study. A prolonged QRS duration and a low R-wave score observed in the present study were independent predictors of an abnormal EF. The presence of fQRS on conventional ECG in this study might be due to cardiac involvement. In cardiac diseases, it correlates with subclinical left ventricular dysfunction or scarring and predicts a higher incidence of ventricular arrhythmias [38]. The presence of J waves or Osborn wave can represent a “current of injury” and seem to be indicative of severe ischaemia due to anaemia.

3.7 Echocardiographic examination

Echocardiographic examination revealed dilated cardiomyopathy, pleural effusion, increased EPPS and increased LA: Ao ratio (Fig.5 & 5a). The value of LVDd (mm) for Grane Dane (n=4), GSD and Mixed was increased (63.23±2.40, 60.1 and 61 respectively) as compared to normal reference range (44-59, 36-53 and 33-42 respectively). The value of LVDs (mm) was also increased (56.2±4.32, 52.4 and 52 respectively) as compared to normal reference range (34-45, 22-39 and 20-35 respectively). The values of IVSd (mm) and IVSs (mm) in all the dogs were within normal reference range except in Grane Dane (n=4) where the values of IVSd (mm) and IVSs (mm) were decreased (11.53±1.96 and10.53±1.82 respectively) as compared to normal reference range (14-19 and 12-16 respectively). The value of PWd (mm) in Grane Dane (n=3) and GSD were decreased (8.26±2.67 and 5.7 respectively) as compared to normal reference range (10-16 and 7-14 respectively). In Mixed breed the value of PWd (mm) was within the normal reference range. The value of PWs (mm) in all the dogs were decreased (9.30±1.2, 8.5 and 7.2 respectively) as compared to normal reference range (11-19 and 10-18 respectively). The values of EF (34.20±12.87%) and FS (15.60±8.98%) were decreased as compared to normal reference range (50-65 and 33-46 respectively) and the values of LA: Ao (1.88±0.39) and EPSS (16.76±2.93 mm) were increased as compared to normal reference range (0.83-1.13 and <7.7). The increase in LA/Ao ratio (cm) suggested atrial dilatation [39]. Echocardiography revealed severe dilatation of right ventricle and atrium with right ventricular hypo contractility leads to right heart systolic ejection failure which was also observed in this study. Decreased EF and FS, especially in the presence of a large volume of pericardial effusion were noted as indicators of decreased cardiac output in this study. [40]

3.8 Post-mortem and histopathological examination

The post-mortem examination revealed that the peritoneal cavity was filled with ascitic fluid (Fig. 6A). The liver showed swollen, congested, and mildly cirrhotic (Fig. 6B). The heart was enlarged and dilated (Fig. 6C &D). Lung was edematous and focal anthracosis was observed (Fig.6E). Kidney showed congestion of corticomедullary junction (Fig.6F) and spleen was small and wrinkled (Fig. 6G). The intestine was congested (Fig.6H).

On histopathological examination, it was observed that liver sectioned showed coagulative necrosis and dilatation of sinusoids (Fig. 7A). Kidney showed coagulative necrosis in some tubules, presence of proteinaceous cast in some tubules, acute nephrosis in some tubules. Glomeruli showed dilatation of Bowman's space, and vacuolations in the glomerular tuft indicating loss of cells (Fig. 7B). Heart revealed coagulative necrosis indicated by loss of nucleus & loss of cross striations in cardiac myocytes and the presence of micro haemorrhages between myocytes (Fig.7C). So, from post-mortem and histopathology, it could be concluded that ascites was developed due to multi-organ dysfunction which leads to death of the animal. A similar result also observed by Hiblu [41].
A. Hyperechoic foci with loss of echogenicity of hepatic parenchyma.
B. Dense ascitic fluid containing numerous echogenic particles
C. Distended gall bladder filled with echogenic content and hyperechoic wall
D. Hyperechoic hepatic parenchyma and enlarged liver size
E. Isoechoic kidney cortex and medulla, enlarged renal pyramids with anechoic echotexture (Hydronephrosis)
F. Reduced blood circulation to the kidney (using CFM)

Fig 2: USG examination of ascitic affected dogs caused by hepato-renal dysfunction

A. Showing ground glass appearance of abdomen due to ascites
B. Cardiomegaly (VHS=13), consolidation of lung, displacement of trachea dorsally, cardiac silhouette, aorta and posterior vena cava not visible
C. Cardiomegaly (VHS=13.5), consolidation of lung, displacement of trachea dorsally and kinking of posterior vena cava

Fig 3: Radiographic examination of ascitic dogs caused by multi organ dysfunction
a) Sinus tachycardia, atrial standstill (absence of P wave) and prolonged QRS duration
b) Alternating amplitude of R wave and decreased QRS amplitude, notching of R wave P-mitrale (a prolonged P wave duration and notched P wave) and alternating amplitude of the R wave
c) Fragmented QRS (f QRS) and osborn wave

Fig 4: Electrocardiographic examination of ascitic dogs caused by Multi organ dysfunction

Fig 5: Echocardiographic examination of Thoracic cavity
A. Accumulation of fluid in the abdomen
B. Swollen, congested, mildly cirrhotic
C. C & D. Enlarged, dilated and flabby heart
D. Edematous and focal anthracosis of lung
E. Small and wrinkled spleen
F. Congestion of corticomedullary junction of kidney
G. Congested intestine

**Fig 6:** Post-mortem examination

A. Liver: Coagulative necrosis, dilatation of sinusoids H&E X100
B. Kidneys: Coagulative necrosis in some tubules, presence of proteinaceous cast in some tubules, acute nephrosis in some tubul tubules. Glomeruli show dilatation of bowmans’ space, and vaculatiouns in the glomerular tuft indicating loss of cells H&E X100
C. Heart: Coagulative necrosis indicated by loss of nucleus & loss of cross striations in cardiac myocytes. Presence of micrhemorrhages between myocytes, H&E X400

**Fig 7:** Histopathological examination
4. Conclusion
From the above haemato-biochemical changes and applying different imaging diagnostic techniques and post-mortem with histological study, it was confirmed that the ascites in the present study were caused by hepato-renal with cardiac involvement. The prognosis of ascites in dogs is influenced by the clinical signs of disease at the time the dog is diagnosed, as well as by the extent of hepato-renal damage that then exists. Early diagnosed and properly treated may be associated with prolonged survival times. Unfortunately, most of the dogs with the hepatic-renal problem are presented in the quite advanced stage after the development of noticeable clinical signs where most of the hepatic, as well as kidney function, have been lost. The prognosis for long-term survival in such cases is guarded to grave.

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6. References


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