www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 742-746

www.thepharmajournal.com
Received: 21-09-2022
Accepted: 23-10-2022

Padhi Pratibha
Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Patra RC
Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Senapati SK
Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Panda Sonali
Department of Veterinary Clinical Medicine College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Das DP
Department of Veterinary Pathology College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Biswal Sangram
Department of Preventive Medicine College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Corresponding Author:
Padhi Pratibha
Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Prevalence, clinicpathological changes and therapeutic management of ascites in dogs

Padhi Pratibha, Patra RC, Senapati SK, Panda Sonali, Das DP and Biswal Sangram

Abstract
Ascites, a multi-etiological disease, is characterized by distension of abdomen, poor appetite and lethargy. Lack of adequate literature on etiopathology and clinico-pathological alterations in ascites in dogs possesses serious problem for management of the disease. The present study was conducted on owned dogs with distension of abdomen to study the prevalence of the disease with respect to primary organ involvement in the pathogenesis of ascites, and hematological and biochemical changes and the efficacy of therapeutic management. The ascetic dogs were grouped into causative types; heart-related, liver-related, or dogs with both heart and liver involvement, kidney-related and others on the basis of clinical symptoms, physical examination findings, and radiography and haemato-biochemical studies. The affected dogs were treated with drugs depending on the cause of the disease along with supportive drugs. Twelve apparently healthy dogs were recruited for the study to serve as controls. The blood samples collected before (day 0) and 15 days following treatment and from control dogs were subjected to haemato-biochemical analysis. Ascetic fluid was collected by abdominocentesis for gross, cytological and clinicopathological examinations. The collected faecal samples were examined for the presence of parasitic ova, Estimation of serum ascetic albumin gradient (SAAG), thoracic and abdominal radiography and electrocardiography were performed. Out of the total 3541 dogs examined during the period from January to June 2016, 12 dogs (0.34%) had ascites, consisting of four (33.33%) Labradors, 3 (25%) Spitz, 2 (16.67%) German shepherd, one (8.33%) mixed breed dog, one (8.33%) Boxer and one (8.33%) Golden Retriever. Ascitic dogs were grouped into 4 causative types depending on the primary organ involvement, and appropriate therapy was provided depending on the cause of the disease. The ascetic dogs with kidney involvement had significant decrease in mean haemoglobin level and Packed Cell Volume. There was significant increase in neutrophil percentage in all groups as compared to control, but no significant difference was recorded in eosinophil percentage among the groups. The mean serum total bilirubin & direct bilirubin of group with primary liver affection was significantly higher than other groups and control. The mean serum AST activity of all the groups was significantly higher than control. Post treatment haematological examination on day 15 revealed increase in Hb and PCV in all groups, Serum ascetic albumin gradient (SAAG) of two cases were more than 1.1 g / dl suggestive of presence of portal hypertension. It was concluded that specific and supportive treatment after identification of primary organ involvement in the pathogenesis of ascites restored clinic-pathological alterations.

Keywords: Ascites, dogs, hematology, biochemical changes, SAAG

Introduction

Ascites, also known as hydro peritoneum, is defined as an accumulation of excess fluid in the peritoneal cavity. The cavitary liquids are produced by filtration in capillaries of the parietal layer of the pleura or the peritoneum. Normally, a small amount of lubricating fluid (Less than 1 ml/kg body weight) is found in healthy animals and their function is to lubricate and prevent friction between organs [1, 2, 3]. The fluid accumulation within a body cavity results when the rate of filtration of fluid into space is greater than the rate of fluid resorption from the space. It is technically grouped into three grades based on the amount of accumulated fluid. Grade 1 ascites is a mild form associated with accumulation of a small amount of excess fluid that can only be detected by ultrasound examination. Grade 2 ascites is a moderate form, manifested by moderate symmetrical distension of the abdomen, while grade 3 ascites is the clinically observable gross and large accumulation of fluid, manifested as marked abdominal distension. Grade 3 ascites cases are normally presented at the clinic for medical or veterinary attention [4].
Ascites is a multi-factorial disease. The common cardiac problems resulting in ascites include heartworms infestation, congestive cardiomyopathy, right side heart failure and congenital pulmonary stenosis. Hepatic diseases leading to ascites are liver insufficiency, chronic active

hepatitis, cirrhosis and cholangitis, most of which are associated with portal hypertension. Other causes are hypoproteinaemia resulting from renal loss of protein in disease like glomerulonephritis, amyloidosis, neoplasm, ruptured urinary bladder and haemorrhage from trauma [5]. Ascites is also a rare complication in post laparoscopic cholecystectomy, the most common general surgery in medical practice in the United States that might be due to biliary leak, lymph duct injuries, infections, peritoneal reaction bowel injuries, malignancies, etc [6].

Another system of classifying ascitic fluid proposed by) is based on the amount of albumin in the ascetic fluid compared to the serum albumin; this is known as the serum–ascites albumin gradient (SAAG) [7]. This method of classification is considered clinically useful especially in humans where liver cirrhosis due to alcoholism accounts for about 80% of ascites cases [8, 9]. In humans, ascitic fluid with high SAAG (>1.1) was found to accumulate in conditions of liver cirrhosis (81%), heart failure (3%), hepatic venous occlusion, constrictive pericarditis and kwashiorkor or, while fluid with low SAAG (<1.1) accumulates in conditions of primary or metastatic peritoneal carcinomas (10%), infections like tuberculosis and spontaneous bacterial peritonitis (2%), pancreatitis (1%), serositis, nephrotic syndrome and hereditary angioedema [7, 10]. In cattle, cardiac disease is the major cause of ascites, accounting for about 66% of cases, followed by obstruction of venous drainage and malignancies, liver damage, hypoproteinaemia and kidney damage [11]. The classification of ascitic fluid as transudate or exudate may aid in the diagnosis of the effusion, reducing the range of differential diagnoses [12]. In addition, cytological evaluation of the fluid can identify the presence and morphology of bacteria or other infectious organisms [13], as well as neoplastic cells [14], which along with the anamnesis of the animal and the clinical signs may direct the veterinarian to the therapeutic approach.

Except for few documented individual case reports of ascites in dogs [15, 16], there is paucity of information on the specific causes of ascites in dogs and the varied contributions of the possible causes. Yet, cases of ascites in dogs continue to present diagnostic enigmas to the average veterinary clinician and are one of the most commonly referred case types in veterinary clinical practice. The limited knowledge of the specific causes and clinico-pathological alterations leads to usual difficulty in management of cases of ascites in dogs. Therefore, this study investigated the prevalence of and clinicopathological changes in ascites in dogs and its therapeutic management.

Materials and Methods

The study was carried out in dogs presented in the Teaching Veterinary Clinical Complex, Bhubaneswar in Odisha State, India. The dogs with the complaint of abdominal distensions were recruited for the study after ruling out other causes of the abdominal distension by physical, radiographic and haemato-biochemical examinations. The prevalence of the ascites in dogs with respect to age, gender, breed and cause was analyzed. Palpation, percussion and auscultation were performed to know the cause of abdominal distension such as gross obesity, gas, liquid, organomegaly, bowel obstruction, intussusceptions, hernia etc. Fluid thrill test and auscultation of heart were performed for any abnormality in heart rate and rhythm.

Experimental design

The ascitic dogs were grouped into causative types; heart-related, liver-related, or dogs with both heart and liver involvement, kidney-related and others on the basis of clinical symptoms, physical examination findings, and radiography and haemato-biochemical studies. The affected dogs were treated with drugs as given in Table 1 along with vitamin B complex, iron supplementation, ranitidine, metoclopramide and prednisolone and antibiotics like ceftriaxone, enrofloxacin, amoxicillin, clavulinic acid, or oxytetracycline as required. Twelve apparently healthy dogs were recruited for the study to serve as controls.

Sampling

Five ml of blood was collected aseptically from recurrent tarsal vein of affected dogs using sterilized syringe and needle, in the morning hours before and 15 days after treatment. Blood samples were also collected from the control dogs. Out of five ml blood collected, one ml was dispensed into EDTA (ethylene diamine tetra acetic acid) vial for hematology and remaining was transferred to clot activator vial for serum biochemistry. Blood samples were put in an ice box and brought to the laboratory for analysis. The tests were performed on the same day of collection. Faecal sample was collected either from the dogs directly from the rectum or plastic specimen container were given to the dog owner with instruction for collecting the faeces during morning hours. For the collection of peritoneal fluid, bladder was emptied before sampling. Five ml ascetic fluid was collected by abdominocentesis by inserting an 18 gauze needle, 2-3 cm caudal to the umbilicus and 2-3 cm left of the midline at 30 40 degree angle [2] and then dispensed into EDTA vial, clots activator vial and sterile tube as per the requirement.

Haematological study

Different blood parameters such as Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were estimated (Coles,1986).

Biochemical study

The blood sample collected in clot activator vial was allowed to stand for 20 minutes in slanting position at room temperature. Then, the tubes were centrifuged at 2500-3000 rpm for 10 minutes for serum separation. The biochemical tests indicative of liver function such as alanine aminotransferase (ALT), serum glutamate pyruvate transaminase (S.G.P.T.). Aspartate aminotransferase (AST), serum glutamate oxaloacetate transaminase (S.G.O.T.), alkaline phosphatase (ALP), bilirubin, glucose, serum total protein, serum albumin were estimated before and 15days after treatment. Serum globulin and albumin/globulin ratio was also calculated. Kidney function tests such as blood urea nitrogen (BUN), serum creatinine, serum urea were analyzed. All these biochemical parameters were carried out spectrophotometrically using standard protocol as described in the reagent kits.

Faecal sample examination

The collected faecal samples from dogs were examined for the presence of parasitic ova, particularly for *Ancylostoma spp*. by direct smear method and/or floatation technique method by using saturated NaCl solution of specific gravity 1.18 to 1.19.

Peritoneal fluid examination

Gross examination of ascitic fluid was performed for appearance (transparency, turbidity), colour, and clot formation. Smear made from sediment of centrifuged sample after staining with Giemsa stain was used for cytological examination for the predominant cell types as well as other cells including malignant cells. Smears were stained with Z-N stain when suspected for tuberculosis. The culture of ascitic fluid was also carried out for isolation of organism.

SAAG

Serum ascetic albumin gradient (SAAG) was calculated by subtracting the albumin concentration of ascetic fluid from that of serum obtained on the same day [7].

Radiology and Electrocardiography**:** Thoracic and abdominal radiography was conducted to visualize heart, urinary bladder, liver and other viscera to know their size and relative position. Electrocardiogram (25 mm/sec and 10 mm/mV) was done to determine any cardiac dysfunction.

Statistical analysis

The data collected during the present study in respect of different parameters were statistically analyzed for calculation of mean, standard error of mean and two-way analysis of variance to compare the means of different treatment groups over different periods (Day 0 and 15) using SPSS version 16.0 (SPSS Inc, IBM, USA).

Results

Prevalence

Out of the total 3541 dogs examined during the period from January to June 2016, 12 dogs (0.34%) had ascites, consisting of four (33.33%) Labradors, 3 (25%) Spitz, 2 (16.67%) German shepherd, one (8.33%) mixed breed dog, one (8.33%) Boxer and one (8.33%) Golden retriever. There were nine females (75%) and three males (25%). The age distribution of the confirmed cases of ascites was one case < 1 year (8.33%), 5 cases in age group between 1 to 3 years (41.67%) and 6 cases in age group above 3 years (50%). All the 12 cases of ascites were grouped into 4 causative types depending on the primary organ involvement, diagnosed on the basis of clinical symptoms, physical examination findings, radiography and haemato-biochemical analysis; 3 (25%) cases had ascites of cardiac origin, 2 (16.67%) had liver damage, 2 (16.67%) had both heart and liver problem, 2 (16.67%) had kidney damage, while 3 (25%) cases of ascites were due to other causes (Table 1).

Clinical and Physical Examination

All the 12 dogs (100%) had abdominal distension. Out of 12 affected dogs, 3 (25%) were anorectic, 6 (50%) had inappetence, while 2 (16.67%) were having normal appetite. Five dogs (41.67%) had pale mucous membrane and 6 (50%) were having higher body temperature above 102 ºF. The dogs exhibiting the above clinical signs were subjected to abdominal palpation for confirmatory diagnosis of ascites. All the dogs were found positive in fluid thrill test by feeling the fluid thrill on opposite side with assisted abdominal pressure on one side. Five dogs (41.67%) were showing respiratory distress, 7 (58.33%) dogs had tachycardia, and 2 (16.67%) dogs were showing subcutaneous edema of limbs. Other clinical signs were reduction in body weight, weakness, and dullness.

Haematological study

There was non-significant decrease in Hb in all groups as compared to control and group ‘K’ had significant decrease in mean Hb level (Table 2). There was non-significant increase in TLC mean level in group ‘H’, ‘L’ and ‘O’ than control, and there was non-significant decrease in TLC value in group ‘H+L’. Packed Cell Volume (PCV) of group ‘K’ was significantly lower than other groups and control while PCV of group ‘O’ had higher mean value than other groups and control. Group ‘H’ and ‘L’ had mean PCV level non-significantly lower than control. There was significant increase in neutrophil percentage in all groups as compared to control, but no significant difference was recorded in eosinophil percentage among the groups. Lymphocyte percentage of group ‘K’ dogs was significantly lower than other groups including control. There was significant decrease in platelet count in all groups as compared to control, except group H, where there was non-significant decrease as compared to control. Group H + L and K had significantly lower RBC count as compared to control. The mean MCV value of group H + L was significantly higher and that of Group H, L and O had non-significantly higher as compared to control. However, group K had non-significant decrease in MCV value. Group O had significantly lower mean value of MCHC as compared to control.

Biochemical findings

The mean serum total bilirubin & direct bilirubin of group L was significantly higher than other groups and control. The mean serum AST activity of all the groups was significantly higher than control. The AST values of group H+L was higher than group H, L followed by that of K and O. The ALT mean level of all groups were significantly higher than control except that of group O where there was a non-significant increase than control (Table 3). The total protein and albumin values varied non-significantly in all the groups when compared with control. There was significantly lower globulin level in group O as compared to other groups and control. There was no significant variation in A: G ratio among all groups and control. Dogs in group K had significantly higher blood urea nitrogen, serum urea & serum creatinine than other groups and control.

Post treatment clinical pathology

Post treatment haematological examination on day 15 revealed increase in Hb and PCV in all groups, and there was significant ($p \leq 0.05$) increase in Group K. There was significant decrease in TLC count in Group K as compared to day 0 mean level. There was significant decrease in neutrophil and increase in lymphocyte count in all groups on day 15 as compared to respective day 0 mean level. There was significant increase in monocyte count in group K as compared to respective day 0 mean level. Significant increase in platelet count was recorded in group L, H+L and K as compared to respective day 0 level. There was significant decrease in MCV in group H+L as compared to day 0. There was significant decrease in mean AST and ALT value on day 15 as compared to day 0 in all groups except group O. There was significant decrease in mean ALP value on day 15 as compared to day 0 in group L, H+L and K. There was significant increase in total protein in group O and decrease in blood urea nitrogen (BUN), serum urea and serum creatinine in group K and O on day 15 as compared to day 0.

The total protein, albumin, globulin and A/G ratio in serum and ascetic fluid, SAAG of 4 ascietic dogs are given in the Table 4. SAAG of two cases were more than 1.1 g/dl suggestive of presence of portal hypertension and another two cases had SAAG <1.1 g/dl.

The colour of ascitic fluids was generally clear to pale yellow or straw colour while in three cases, it was found blood tinged. The ascetic fluid was found negative for any malignant cells in cytology and no organisms were found in Z-N stain.

Radiographic examination of thoracic cavity revealed enlargement and rounding of heart, hydro pericardium, mild congestion of lungs and enlargement of heart. Abdominal radiographic findings revealed empty intestine floating in the ascetic fluid, loss of liver density, ground glass appearance of abdomen, free fluid in abdomen, empty intestinal loops floating in free fluid, loss of liver density, few stones in the colon.

Electrocardiography: The notable ECG changes in 9 out of 12 cases revealed absence of P wave, prolonged QT interval, elevation of ST segment, low voltage QRS complex and right axis deviation in one case.

Discussion

The cavitary fluids examinations assist in the diagnosis of diseases in veterinary medicine, but sometimes, this is not much requested. The aetiopathological diagnosis of cavitary effusions varies according to species, age group and gender. These differences greatly assist the clinician to address clinical suspicion associated with the type of fluid-present, history and general examination [17, 3]. Ihedioha *et al.* [3] reported prevalence ranging from 0.6-1% with a mean of 0.78% in Enugu State, Nigeria whereas the present investigation recorded incidence of 0.34%. According to age distribution of ascites, dogs above 3 year (50%) were highly susceptible to ascites, followed by 1 year to 2.9 years (41.67%) age group, and the lowest prevalence was recorded in <1 year age group (8.33%). This implies older dogs have more prevalence than younger one, this might be due to chance of vital organs insufficiency like heart, liver and kidney is more in older age groups. Ihedioha *et al.* [3] reported that most of the ascites cases were largely due to congestive heart failure (50% of cases); other causes were cirrhotic liver disease (14.3%), chronic active hepatitis (21.4%) and kidney disease (14.3%). The present finding of maximum cases of heart-related ascites (25%) confirms the earlier findings.

There was significant decrease in Hb and PCV value in ascetic dogs with renal disorder, suggestive of anaemia, which might be due to the inability of the damaged kidney to produce erythropoietin needed for RBC production [18, 19] Further, there was significant increase in Hb and PCV value in ascetic dogs with renal disorder on day 15 post-treatment. There was significant increase in neutrophil percentage in all groups as compared to control. The higher neutrophil counts has earlier been reported in in ascetic dogs [3]. Neutrophil percentage decreased significantly in all ascetic dogs after 15 days post treatment in the present experiment after treatment.

The mean serum AST activity of all the groups was significantly higher than control and the ALT value of all groups was significantly higher than control except group of other causative types, where there is non-significant increase. Elevation of plasma transaminases such as ALT and AST were indicative of altered hepatocellular membrane permeability, hepatocellular necrosis and inflammation with degree proportion to number of injured hepatocytes [9]. The finding of elevated levels of ALT in dogs is in agreement with the reports of Milne *et al.* [11] on ascitic cattle with cardiac disease. The very high serum AST activity recorded for dogs with CHF is thought to be due to cardiac muscle damage [20, 21], while the elevated serum AST activity recorded for dogs with kidney diseases may be attributed to hypoxia associated with the anaemia reported in these dogs [21].

Significantly higher serum ALP activity was recorded in ascetic dogs with kidney and liver disease. Increased ALP values in ascites related to liver disorder has also been reported by Ihedioha *et al.* [3] and Tantary *et al.*, [22]. It might be due to cholestatic disorders associated with liver damage [21]. ALP is a membrane bound enzyme and luminal surface of biliary epithelial cells and its iso-enzymes are present in kidneys, intestine, placenta and bone but elevation in its level in >1 year old is usually indicative of hepatic origin unless bone disease coexists, since isoenzymes from other organs are having extremely short half-lives. Marked increase in activities of ALP and GGT has been reported in conditions causing cholestasis, cholangiohepatitis, biliary cirrhosis, biliary obstruction and cholecystitis of cholelithiasis [23].

There was decrease in total protein and albumin values in all the ascetic dogs, which might be due to reduced protein synthesizing ability of the liver as most of the hepatocytes had been replaced by fibrous tissue in liver disease. It is presumed that dogs with kidney diseases had hypoproteinemia due to protein loss in urine that occurs in kidney disease [21, 24]. The significantly lower serum albumin recorded for all the ascitic dogs is typical of most ascitic states and in most cases, those were contributory factors to the fluid accumulation process [21]. Significant increase in total protein on day '15' post treatment as compared to day '0' in dogs with renal disorder and ascites of other causes. Tantary *et al.* [22] observed increase in total protein, albumin and globulin on '15' day post-treatment. Liver being the main sites of synthesis and degradation of most of the proteins, any hepatic disorder (chronic hepatitis and cirrhosis) are responsible for decrease in albumin concentration [25]. Total plasma protein might also have decreased due to marked decline in the diet intake, malabsorption and ongoing protein losing enteropathies like gastroenteritis, gastrointestinal ulceration and chronic gastritis. But hypoalbuminemia may also occur without impairment in hepatic albumin synthesis due to either leakage of albumin from hepatic lymph or increase in volume of albumin from hepatic lymph or increase in volume of distribution as in cases of ascites [22] (Tantary *et al.*, 2013).

The decreased glomerular filtration rate commonly associated with kidney disease is believed to be responsible for the significantly elevated serum levels of BUN, creatinine and urea recorded in dogs with kidney damage [3] (Ihedioha *et al*, 2013). SAAG> 1.1 g/dl is suggestive of presence of portal hypertension [7]. Serum ascites albumin gradient (SAAG) of two cases was > 1.1 g/dl indicating portal hypertension. Low voltage QRS complex is a common finding in ECG of ascites dog [23]. Other ECG changes are absence of p wave, prolonged QT interval and non-uniform R-R interval in one case, elevation of ST segment in one case, suggestive of myocardial ischaemia, which may be due to right ventricular enlargement [26].

References

1. O'Brien P, Lumsden J. The cytologic examination of body cavity fluids. Semin Vet Med Surg. 1988;3:140-156.
2. Alleman AR. Abdominal, thoracic and peritoneal effusion, Veterinary Clinic, North Am*:* Small Animal Practice. 2003;33:89-118.
3. Ihedioha JI, Victor OA, King ANE. Prevalence of and clinicopathologic findings associated with ascites in dogs in Enugu State, Nigeria. Comp Clin Pathol. 2013;22:185-193.
4. Moore KP, Wong F, Gines P, Bernardi M, Ochs A, Salerno F *et al*. The management of ascites in cirrhosis: report of the consensus conference of the international ascites club. Hepatology. 2013;38:258-266.
5. Peden WM. Canine Ascites, Iowa State University Veterinarian. 1982;44(1):11-16.
6. Farzaneh BA, Mehdi AM, Reisi-Vanani V. Post-operative ascites of unknown origin after laparoscopic cholecystectomy: Case report. Annals of Medicine and Surgery. 2022;76:103-431.
7. Runyon BA, Montano AA, Akriviadis EA. The serum ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. Ann Intern Med. 1992;117:215-220
8. Runyon BA. Care of patients with ascites. N Engl J Med. 1994;330:337-342.
9. Gines P, Cardenas A, Arroyo V, Rodes J. Management of cirrhosis and ascites. N Engl J Med. 2004;350:1646-1654.
10. Branco-Ferreira M, Pedro E, Barboss MA, Carlos AG. Ascites in hereditary angioedema. Allergy. 1998; 53:543-545.
11. Milne MH, Mellor DJ, Barret DC, Fitzpatrick JL Observations on ascites in nine cattle. Vet Rec. 2001; 148:341-344.
12. Bohn AA. Analysis of canine peritoneal fluid analysis. Vet Clin North Am Small Anim Pract. 2017;47:123-133.
13. Barrs VR, Beatty JA. Feline pyothorax-new insights into an old problem: part 1. Aetiopathogenesis and Diagnostic Investigation Vet J. 2009;179:163-170.
14. Morges MA, Zaks K. Malignant melanoma in pleural effusion in a 14-year-old cat. J Feline Medicine Surg. 2011;13(7):532-535.
15. Skardova I. Occurrence of ascites abdominalis in dogs. J Nutr. 1991;121:S159-S160.
16. Parker MD. An unusual cause of abdominal distension in a dog. Vet Med. 2002;78:189-195.
17. de Oliveira AME, Jaguezeski AM, da Silva CB, Barbosa NV, de Andrade CM. Retrospective study of the aetiopathological diagnosis of pleural or peritoneal effusion examinations of dogs and cats. Comparative Clinical Pathology. 2021;30:811-820.
18. Krantz SB. Erythropoietin. Blood. 1991;77:419-434.
19. Alpers CE. The kidney. In: Kumar V, Abbas AK, Fausto N (eds), Robbins and Cotran pathologic basis of disease, 5th edn. Saunders, Philadelphia. 2004;955-1021.
20. Schoen FJ. The heart; In: Kumar V, Abbas AK, Fausto N (eds) Robbins and Cotran pathologic basis of disease, 7th edn. Saunders, Philadelphia; c2004. p.555-618.
21. Stockham S, Scott MA. Fundamentals of veterinary clinical pathology, 2nd edn. Blackwell, Iowa; c2008.
22. Tantary HA, Soodan JS, Chirag S. Diagnostic and Therapeutic Studies in Ascites in Dogs, Veterinary Research. 2013;6(5-6):111-114.
23. Kumar V, Abbas AK, Fausto N. Robbins and Cotran pathologic basis of disease, 7th edn. Saunders, Philadelphia; c2004.
24. Coles E. Veterinary clinical Pathology 4th ed WB Saunders company Philadelphia. London, Toronto, Mexico, Riodejenario, Sydney, Tokyo & Hong Kong; c1986. p. 136-70.
25. Maity S, Das D, Ambatipudi K. Quantitative alterations in bovine milk proteome from healthy, subclinical and clinical mastitis during *S. aureus* infection, Journal of Proteomics. 2020;223:103815. https://doi.org/10.1016/j.jprot.2020.103815
26. Martin M. Small animal ECGs an introductory guide_2nd edition, Blackwell publishing; c2007.