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Evaluation of ascitic fluid in dogs

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Abstract
Ascites is referred as accumulation of serous fluid in peritoneal cavity. The difference between serum and ascitic fluid albumin concentration correlates directly with portal pressure. A total of 18 animals were selected for ascitic fluid examination and evaluated for different parameters. Variety of ascitic fluid colour was observed according to different clinical conditions.

Keywords: Peritoneal fluid, ascites, protein

1. Introduction
A small amount of lubricating fluid is found in the peritoneum, which comes from the surrounding tissue and vessels. If the fluid exceeds the normal amount, an individual may have an effusion. If an effusion is present, the collection and retention of fluid inhibits the function of the organ (Mondal *et al*. 2012) [3]. Regardless of localization, classification of serous effusions as transudates or exudates is the first step in the diagnosis of the cause of abnormal fluid collections. The formation of transudates is attributable to systemic factors that result in development of a diluted clear fluid, whereas the formation of exudates is attributable to inflammation or neoplasia resulting in a fluid that may macroscopically resemble plasma (Braun *et al*. 2001) [1].

2. Materials and Methods
2.1 Location and Place of Work
The proposed work was conducted in the Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur, Madhya Pradesh.

2.2 Duration of Work
The study was conducted for a period of six months i.e. from September 2019 to March 2020.

2.3 Selection of cases
A total of 174 dogs brought to veterinary clinical complex (VCC) with the complaint of abdominal distension were screened for ascites and were subjected to detail study. Out of 174 dogs, 18 dogs were selected for therapeutic study along with six apparently healthy dogs as healthy control group.

2.4 Peritoneal fluid analysis
Following Parameters are considered for this study
- Colour
- TLC (cells/mm³)
- Neutrophils (%)
- Lymphocytes (%)
- Total Protein (g/dL)
- Albumin (g/dL)

Cell count was done by the help of haemocytometer counting chamber for TLC.

10 ml of ascitic fluid are collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1500 r/min for 10 min. Nine milliliters of the supernatant are discharged and 40 µL of the remaining ascitic fluid are diluted with 800 µL of Turks's fluid, gently shaken and used to fill the counting chamber. The cells were counted (40 x objective) in four large square by multiplying the number of cells with 50, Number of white blood cells per cubic millimeter were calculated (Riggio and Angeloni, 2009) [9].

2.5 Process of DLC estimation

A smear was prepared from the sediment of the ascitic fluid. Slide was air dried and stained with the Leishman stain. Leishman stain was poured on slide for 2 min, diluted with the help of distilled water for 10 minutes then washed the slide under running tap water and observed slide under the oil immersion microscope. Neutrophils and lymphocytes were counted and smear was also observed for other cells.

2.6 Determination of total protein concentration

Total protein concentration in each sample was measured by the use of a clinical refractometer. Before determining total protein concentrations with a clinical refractometer, the 1.000 density reading was validated with distilled water. Twenty microliters of sample was then pipetted on the prism and the protein concentration was read on the serum protein scale (Braun *et al.* 2001) [1].

For this study a total of 18 ascitic dogs were selected and divided into 3 groups as T_1 to T_3 having 6 dogs in each group. Six coeval apparently healthy dogs served as a healthy control group T_0.

The recorded experimental data were analysed by ANOVA and mean was compared by DMRT as per the standard procedure outlined by Snedecor and Cochran (1994) [12].

3. Results and Discussion

3.1 Colour

The colour of ascitic/ peritoneal fluid was observed in dog suffering from ascites. The peritoneal fluid showed difference in colours viz. transparent, yellowish, turbid and reddish in all the affected cases of ascites. In the cases with yellow or red tinged fluid was indicative of haemoperitoneum.

The colour of ascitic fluid was yellowish, reddish and turbid on day 0 pre-treatment which gradually improved on days 7 and 14 post-treatment (plate 10). In treatment groups the colour improved to turbid and transparent in most of the cases. In group T_2 there was complete change of ascitic fluid color to transparent.

Table 1: Colour of ascitic/ peritoneal fluid in dogs in different treatment groups

Group	Dog	Ascitic/ peritoneal fluid colour		
		Day 0	Day 7	Day 14
T_1	1	Transparent	Turbid	More Turbid
	2	Reddish	Reddish	Turbid
	3	Reddish	Light Reddish	Transparent
	4	Yellowish	Yellowish	Transparent
	5	Transparent	Yellowish	Transparent
	6	Yellowish	Turbid	Turbid
T_2	1	Yellowish	Transparent	Transparent
	2	Transparent	Transparent	Transparent
	3	Yellowish	Transparent	Transparent
	4	Yellowish	Turbid	Transparent
	5	Turbid	Transparent	Transparent
	6	Turbid	Transparent	Transparent
T_3	1	Yellowish	Turbid	Turbid
	2	Turbid	Yellowish	Transparent
	3	Light Reddish	Transparent	Transparent
	4	Turbid	Turbid	Transparent
	5	Yellowish	Turbid	Turbid
	6	Transparent	Transparent	Transparent

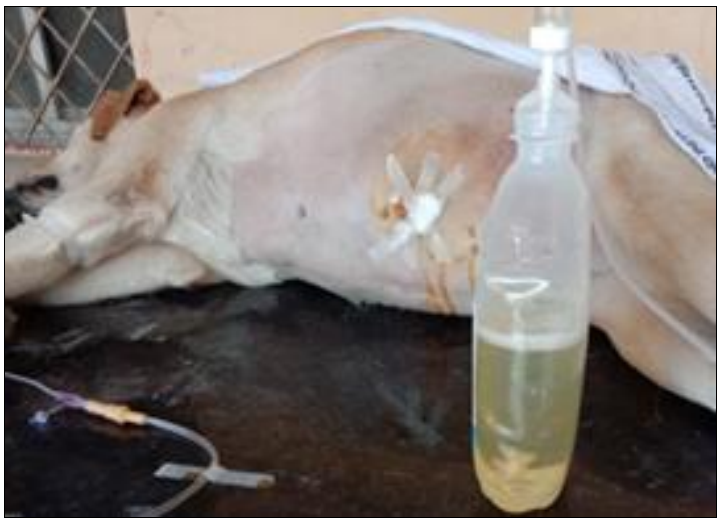

Fig 1: Showing of ascitic/ peritoneal fluid colour in bottle

Clear and colourless peritoneal fluid is transudate whereas, clear and colourless like transudate but slightly turbid is modified transudate and exudates may vary in color from white to amber to pink, but they are usually turbid in nature (Nottidge *et al*., 2003) [4]. Clear yellow in ruptured bladder, acute diffuse peritonitis (yellow, turbid) infarction or necrosis of gut wall (thin red tinged). Milk-colored peritoneal fluid may indicate disease conditions such as carcinoma, lymphoma, tuberculosis or infection. Blood-stained fluid usually due to traumatic tap, peritoneal carcinoma, and ascitic fluids, which remain homogeneous blood-stained fluid throughout the tap and could indicate malignancy, pancreatitis, intestinal infarction and tuberculosis (Runyon *et al.*, 1988) [10]. Bloody fluid may indicate tumor or trauma. Bile-stained fluid may indicate gallbladder problems. High specific gravity and high protein content are indicative of vascular damage and leakage of plasma protein as in peritonitis (Mondal *et al.*, 2012) [3].

3.2 Cellular components

The ascitic fluid was also analysed for the presence of cellular components such as neutrophils, lymphocytes, red blood cells and epithelial cells.

3.3 Ascitic fluid total leucocyte count

The mean ascitic fluid TLC (cells/mm^3) in the dogs of all three treatment groups were recorded on day 0 pre-treatment, and on day 7 and 14 post-treatment. There was high number of total leucocytes count observed in ascitic fluid. The mean values of ascitic fluid TLC is significantly decreased in group T_2 and T_3 from day 0 to day 14.

3.3 Ascitic fluid Neutrophils: The ascitic fluid neutrophil (%) in the dogs of all groups was recorded post treatment. The mean values of ascitic fluid neutrophil (%) in treatment groups T_2 and T_3 significantly decreasing on day 7 and day 14.

3.4 Ascitic fluid lymphocyte

The mean values of ascitic fluid lymphocyte (%) in treatment groups T_2 and T_3 significantly increasing on day 7 and day 14 compared to pre-treatment.

The cell count is probably the single most important ascitic fluid test. Increased total leucocyte count indicates towards the infection. An elevated ascitic fluid total leucocyte count may be seen in malignant diseases and in all inflammatory processes. Significantly higher values of TLC in the ascitic fluid in the present study denote possible bacterial infection which was similar to Phom *et al.* (2019) [7] and contrary to the findings of Regmi and Shah (2017) [8].

Increase in neutrophil (%) pointing towards bacterial and inflammatory condition. Neutrophils are the common cause of cloudy ascites in patients with portal hypertension but this is not specific. Normal neutrophils appear much as they do in peripheral blood. Neutrophils are usually well preserved (non-degenerate) in non-septic inflammatory lesions. As the neutrophils age, the nuclei become hyper segmented and eventually pyknotic. In septic inflammatory lesions, the neutrophils undergo rapid degeneration and eventual rupture. Normal lymphocytes appear much as they do in peripheral blood. Immature lymphoid cells (lymphoblasts) are characterized by their large size and the presence of nucleolus (Mondal, 2012) [3]. The findings of neutrophils and lymphocytes are similar to Kumar and Srikala (2014) [2]; Phom *et al.* (2019) and contrary to the findings of Regmi and Shah (2017) [7].

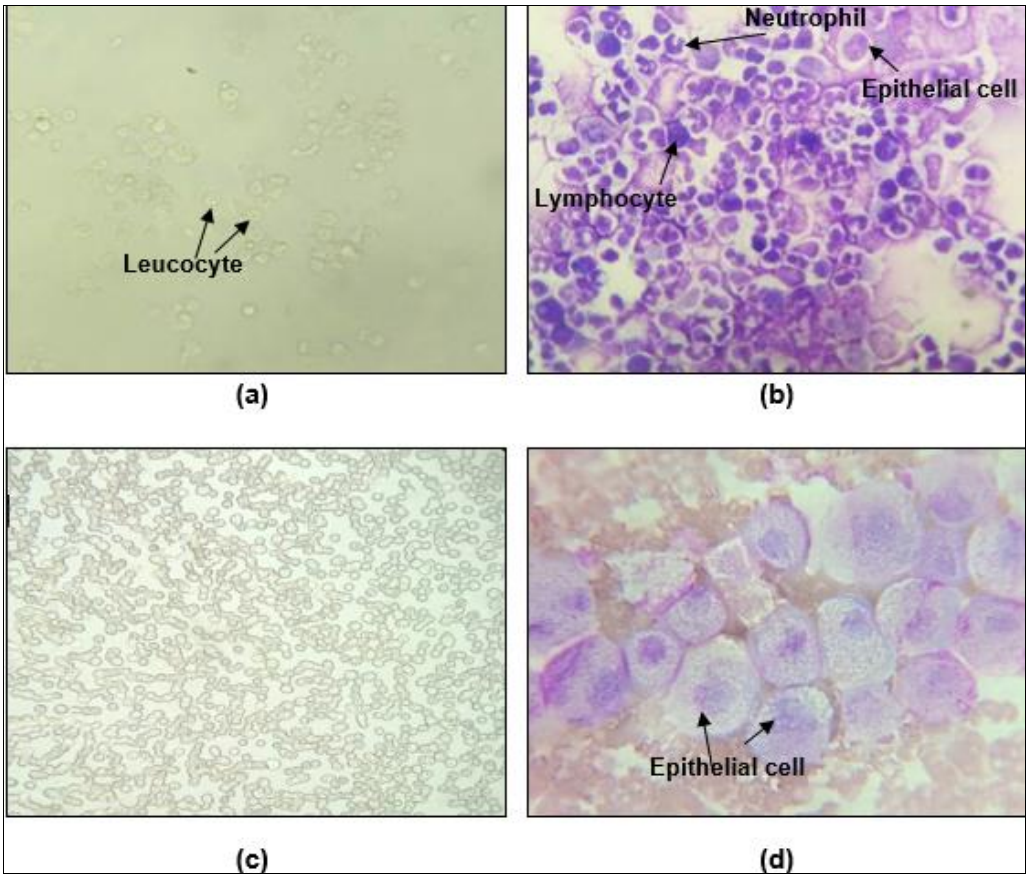

Fig 2: Microscopic examination of ascitic fluid sediment showing: a) Numerous leucocytes in unstained slide (400x), b) Neutrophils, few lymphocytes and epithelial cells in Leishman staining (1000x), c) Numerous RBC (400x), d) Numerous RBC and occasionally bunch of epithelial cells in Leishman staining (400x)

3.5 Ascitic fluid total protein

There was increased total protein recorded in ascitic fluid. The mean ascitic fluid total protein in the dogs of all groups, T_1-T_3 was recorded on day 0 pre-treatment, and on day 7 and 14 post-treatment. The mean values of ascitic fluid total protein in treatment groups T_3 significantly decreasing on day 7 and day 14. Numerical decrease noticed in T_1 and T_2 on day 7 and 14.

3.5 Ascitic fluid albumin

The mean ascitic fluid albumin in the dogs of all groups, T_1-T_3 was recorded on day 0 pre-treatment, and on day 7 and 14 post-treatment. The mean values of ascitic fluid albumin in treatment group T_3 was significantly decreasing on day 7 and day 14.

Table 2: Mean value of ascitic/ peritoneal fluid parameters in dogs

Parameters (Peritoneal fluid)	Groups	Day		
		0	7	14
Total leucocyte count	T_1	875.00±246.89	1248.33±486.93	1136.66±514.37
	T_2	1375.00^{A}±277.41	650.00^{B}±177.01	210.00^{B}±61.96
	T_3	2766.66^{A}±4.77	2000.00^{AB}±570.37	941.66^{B}±296.76
Neutrophils (%)	T_1	76.50±3.46	77.16±2.19	78.16±3.74
	T_2	78.16^{A}±2.82	70.83^{B}±1.62	67.00^{B}±1.15
	T_3	80.50^{A}±1.25	76.83^{AB}±1.66	74.16^{B}±1.90
Lymphocyte (%)	T_1	23.50±3.46	19.50±3.22	21.83±3.74
	T_2	21.83^{B}±2.82	29.16^{A}±1.62	33.00^{A}±1.15
	T_3	19.16^{B}±1.47	23.16^{AB}±1.66	25.83^{A}±1.90
Total protein concentration (g/dL)	T_1	3.26±0.26	2.80±0.23	2.60±0.30
	T_2	2.31±0.54	2.00±0.44	1.68±0.32
	T_3	4.13^{A}±0.51	3.08^{AB}±0.42	2.13^{B}±0.42
Albumin concentration (g/dL)	T_1	0.83±0.12	0.80±0.16	0.86±0.20
	T_2	0.73±0.24	0.45±0.17	0.23±0.11
	T_3	1.35^{A}±0.31	0.58^{AB}±0.22	0.45^{B}±0.27

Mean values with different superscripts (uppercase) differ significantly (p<0.05)

The use of ascitic fluid total protein to classify ascitic fluids as either an exudate or a transudate has contributed to reflect the different processes of fluid formation. Total protein and albumin increase in ascitic fluid indicate the exudative nature of ascitic fluid. Treatment has reduced the amount of total protein and albumin in ascitic fluid, resulted the improvement in ascitic condition. The majority of the variation in ascitic fluid total protein concentration in patients is due to chronic liver disease. TP has for many years been the major criterion used in the differential diagnosis of ascites (Tarn and Lapworth, 2010) [13]. The findings of total protein and albumin of ascitic fluid were elevated which showed both modified transudative as well as exudative ascites in present study similar to the findings of Papasouliotis and Dewhurst (2005) [6]; Saravanan *et al.* (2014) [11]; Ogechi (2019) [5] and Phom *et al.* (2019) [7].

4. Conclusion

On the basis of recovery pattern of ascites and change in ascitic / peritoneal fluid, ascitic dogs of group (T_2) showed maximum recovery followed by dogs of T_3 group and least in dogs of T_1 group

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