



Cytology and Biochemical Analysis of Pleural Effusion in Dogs

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ABSTRACT

Pleural effusion is the accumulation of fluid in the pleural space due to disruption of the hydrostatic and Starling forces which maintain the pressure of pleural cavity. A total of 42 dogs presented to Madras Veterinary College Teaching Hospital Small Animal Medicine Outpatient Unit with a history of cough and dyspnea were selected. Pleural effusion was diagnosed and classified into cardiac, hepatic and tumour and others group based on physical examination, radiography, electrocardiography, ultrasonography, echocardiography, cytological and biochemical evaluation of pleural fluid. The incidence of pleural effusion was 13.5% of the respiratory cases. Cytology of pleural fluid revealed the presence of lymphocytes, neutrophils, macrophages, RBCs, fibrin and mesothelial cells. Cytology of tumour group revealed mesothelioma (3), adenocarcinoma (1) and lymphoma (1). Cytology of other group revealed the presence of numerous RBCs and a few mesothelial cells in hemothorax and mature and degenerated neutrophils along with the presence of numerous clumps of bacteria and macrophages in pyothorax. Estimation of LDH of pleural effusion and ratio of pleural fluid to serum LDH was effective to classify pleural fluid into transudate, exudate and modified transudate.

Keywords: Cytology, Dog, LDH, Pleural effusion, Thoracocentesis

Pleural effusion is the abnormal filling of fluid in the pleural space (Kopcinovic and Culej, 2014). It occurs due to the disruption of the hydrostatic and Starling forces which maintain the pressure of the pleural cavity. There are five pathophysiological mechanisms responsible for accumulation of pleural fluid: (a) increased transpleural pressure (Congestive heart failure), (b) increased capillary permeability, (c) impaired lymphatic drainage (malignancy, neoplasms), (d) transdiaphragmatic movement of fluid from peritoneal space, (e) Pleural effusion of extravascular origin (Sahn, 2012). Common clinical signs include exercise intolerance, lethargy, weight loss, cough, tachypnea, dyspnea, open mouth breathing, orthopnea, pyrexia and cyanosis, but the degree of signs depends on: (a) the rate of fluid accumulation, (b) the nature of the fluid, (c) the volume of fluid, (d)

concurrent respiratory and metabolic conditions and (e) underlying cause of pleural effusion (Murphy and Papsouliotis, 2011). Cytological evaluation of effusions is one of the main diagnostic method for detection of neoplasia although it does not have as much sensitivity to that of tumor markers. The usefulness of tumor markers in the diagnosis of neoplasia is increasing in the recent years in the field of veterinary medicine (Teixeira *et al.*, 2014). Early diagnostic or prognostic information is understood with LDH values as LDH activity is more than 15 times higher in exudates than transudates on an average. Determining the effusion: serum LDH ratio plays a major

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role in differentiating the two types of effusion. Hence, the present study was undertaken with the objective to study the cytology and biochemical analysis of pleural effusion in dogs.

MATERIALS AND METHODS

Cases presented with signs of cough or dyspnea to the small animal outpatient unit of Madras Veterinary College Teaching Hospital were subjected to further examination. A total of 42 cases with pleural effusion identified by imaging techniques such as radiography, electrocardiography, ultrasonography and echocardiography were taken up for this study. Various diagnostic techniques used included electrocardiography, pleural fluid cytology and pleural fluid biochemical analysis to rule out underlying cause of pleural effusion with a concentration towards pleural fluid biochemistry and cytology to help identify the causative agent and the cases were grouped into cardiac group, hepatic group and tumour group and others.

All cases were subjected to radiographic examination to check for the presence of pleural fluid in thoracic cavity and also to check for thoracic or abdominal mass with digital X-ray (Brant and Helms 2012). Electrocardiography (McEwan *et al.*, 2003), abdominal and thoracic ultrasound (Smonetta *et al.* 2017) and echocardiography were performed as per standard procedures (Kienle and Thomas, 2002).

Pleural fluid sample was subjected to spin cytology by centrifuging them at 10,000 rpm for 15 minutes, supernatant discarded and sediment blot dried and smears were made using the sediment. The slide was stained using modified Giemsa stain and viewed under Olympus compound microscope for cytological examination (Rebar and Thompson, 2010). Quantitative estimation of LDH in the pleural fluid was carried out using specific diagnostic kits supplied by Agappe Diagnostics Private Limited, India, following the manufacturer's recommendations for respective estimations to classify the pleural fluid into transudate, exudate and modified transudate based on light's criteria (Light, 2007).

Cytological findings were documented and the LDH values results were statistically analysed, utilizing SPSS-version 14 statistical software package and discussed critically.

RESULTS AND DISCUSSION

Out of forty-two dogs, thoracocentesis was performed on thirty-five dogs. Fourteen (40 per cent) dogs had transudate type of effusion, fifteen (42.8 per cent) dogs had modified transudate type of effusion and six (17.2 per cent) dogs had exudate type of effusion. Out of forty-two dogs, five (11.9 per cent) dogs had effusions due to tumour origin, eight (19.1 per cent) dogs due to hepatic origin, twenty-six (61.9 per cent) dogs due to cardiac origin and three (7.1 per cent) dogs had pyothorax/hemothorax. Out of five dogs that were diagnosed with pleural effusion due to tumour origin, three (60%) dogs had transudate type of effusion, exudate and modified transudate type of effusion were seen in one (20%) case each. Out of eight dogs that were diagnosed with pleural effusion due to hepatic dysfunction, six (75 per cent) cases had transudate type of effusion and two (25 per cent) cases had exudate type of effusion. Out of twenty-six dogs that were diagnosed with pleural effusion due to cardiac origin, thoracocentesis was performed for nineteen cases, of which fourteen cases (73.7 per cent) were modified transudates, three (15.8 per cent) were transudates and two (10.5 per cent) were exudates.

Radiography findings

The common radiographic findings in dogs with pleural effusion were fluid shadow in thorax (71.4 per cent) (Fig. 8), fluid shadow in abdomen (28.6 per cent), cardiomegaly (14.28 per cent), hepatomegaly (14.2 per cent), splenomegaly (7.1 per cent), normal study (7.1 per cent), pulmonary infiltration (4.8 per cent), gas filled intestinal loops (4.8 per cent), intra-abdominal mass (2.4 per cent) and alveolar pattern (2.4 per cent) (Fig. 1).

Presence of mediastinal changes (shadow in thorax) and pulmonary involvement (infiltration, alveolar pattern) were noticed through radiography which is in agreement to the statement made by Quereshi and Gleeson (2006).

In the present study, radiography helped to visualize presence or absence of fluid after drainage but not to identify any lesion. Murphy and Pappasoulitis (2011) documented that taking a radiograph immediately after drainage was not useful to diagnose underlying etiology.

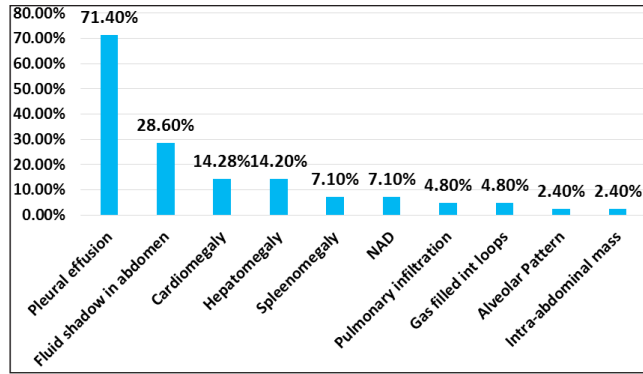


Fig. 1: Radiographic findings in dogs with pleural effusion (n=42)

Electrocardiography findings

Electrocardiography findings in dogs with pleural effusion were reduced ‘r’ wave (30.9 per cent) (Fig. 9), sinus tachycardia (28.6 per cent), normal study (23.8 per cent), ventricular enlargement pattern (14.3 per cent), atrial fibrillation (4.8 per cent), deep ‘s’ wave (2.4 per cent), deep ‘q’ wave (2.4 per cent), ventricular tachycardia (2.4 per cent) and atrial enlargement (2.4 per cent) (Fig. 2).

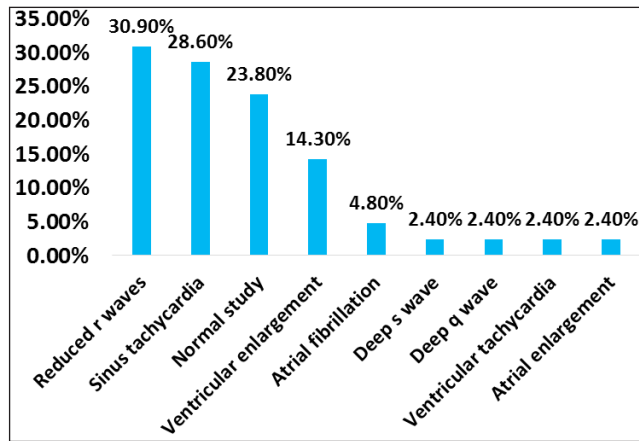


Fig. 2: Electrocardiographic findings in dogs with pleural effusion (n=42)

These findings in cardiac group are in partial agreement to the findings of McEwan *et al.* (2003) who documented that most commonly recorded ECG findings in DCM cases are atrial fibrillation and ventricular tachycardia.

The most common electrocardiographic finding was reduced ‘R’ wave among all the affected groups. The low voltage of the R peak is a consequence of the relative

higher distance between the heart and the electrodes (Manthous, 1993).

Ultrasonography findings

Ultrasonography findings in dogs with pleural effusion were thoracic effusion (100 per cent) (Fig. 10b), hepatic congestion (38.1 per cent), abdominal effusion (30.9 per cent), hepatomegaly (16.7 per cent), hepatic cirrhosis (4.8 per cent) (Fig. 10b), pancreatitis (2.4 per cent) and splenomegaly (2.4 per cent) (Fig. 3).

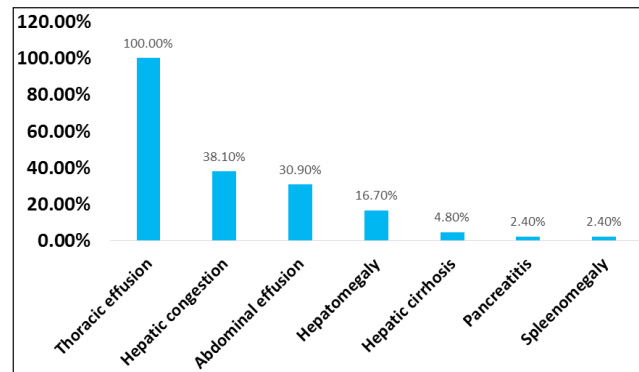


Fig. 3: Ultrasonographic findings in dogs with pleural effusion (n=42)

Thoracic ultrasonography was 100 per cent sensitive for effusions as reported by Quereshi and Gleeson (2006) and this was in full agreement with the present study.

Other findings included abdominal effusions, hepatomegaly, hepatic congestion and hepatic cirrhosis which is in agreement to the findings of Heidelbaugh (2006) who documented that ultrasonography provided valuable information regarding gross appearance of liver and blood flow in portal and hepatic veins in suspected cases of cirrhosis.

Echocardiography findings

Echocardiography findings in dogs with pleural effusion were dilated cardiomyopathy (69.2 per cent) (Fig. 11 a) and mitral valve changes (27 per cent) (Fig. 11 b) and intrathoracic mass (3.8 per cent) (Fig. 4).

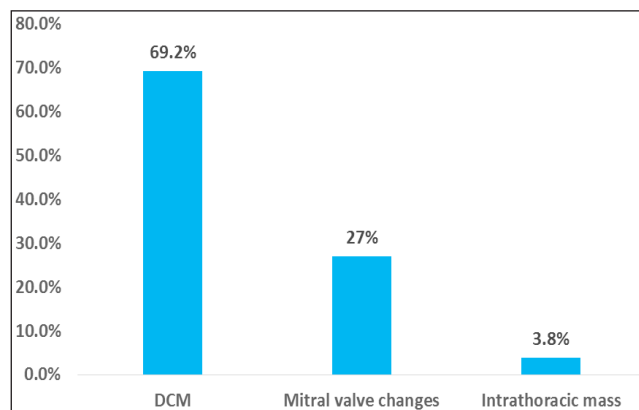
The common echocardiographic finding in the present study is dilated cardiomyopathy (69.2 per cent) which is in complete agreement to the findings of Martin *et al.* (2009) who documented that 74 per cent of the cases that showed signs of pleural effusion were dilated cardiomyopathy.

**Table 1:** Cytological findings of pleural effusion

Sl. No.	Group	Findings	Number
1	Cardiac (n=19)	Lymphocytes	12/19
		Neutrophils	10/19
		Macrophages	7/19
		RBCs	5/19
		Mesothelial cells in cardiac group	1/19
2	Hepatic (n=8)	Lymphocytes	7/8
		Neutrophils	5/8
		Macrophages	2/8
		Fibrins	2/8
		RBCs	1/8
3	Tumor (n=5)	Mesothelioma	Varying sized mesothelial cells with macronuclei and occasional binucleation and cytoplasmic vacuolation. Inflammatory cells and RBCs were also seen. 3/5
		Adenocarcinoma	Medium to large epithelial cells which appeared as clusters of cohesive cells, foamy or vacuolated cytoplasm, fine chromatin and variable prominent nucleoli. 1/5
		Lymphoma	Medium to large lymphocytes with fine chromatin, deep blue granular cytoplasm. Occasional multinuclei, multinucleoli and mitotic figures. 1/5
4	Others (n=3)	Hemothorax	Numerous RBCs and few mesothelial cells 2/3
		Pyothorax	Mature and degenerated neutrophils, numerous bacteria and macrophages 1/3

Table 2: Comparison of pleural fluid biochemical findings between various aetiologies

Group	Pleural fluid				f value
	1(Tumor)	2(Hepatic)	3(Cardiac)	4(Others)	
LDH	152.2±23.95 ^b	108.2±13.8 ^{ab}	88.5±7.9 ^a	105.3±35.3 ^{ab}	3.313*
TP	2.46±0.33 ^a	2.24±0.43 ^a	1.73±0.15 ^a	2.10±0.42 ^a	1.407 ^{NS}

**Fig. 4:** Echocardiographic findings in dogs with pleural effusion (n=26)

In the present study mitral valve changes were recorded in 27 per cent which is in agreement to the findings of Bonagura and Schober (2009) who documented that echocardiography helped in identifying mitral valvular disease.

Cytological findings

Data of the cytology findings from pleural fluid samples are listed in Table 1.

The cytological findings of pleural fluid from cardiac group revealed the presence of lymphocytes (63 per cent), neutrophils (53 per cent) (Fig. 7), macrophages (37 per cent) (Fig. 6), RBCs (26 per cent) and mesothelial cells (5 per cent).

The cytological findings of pleural fluid from hepatic group revealed the presence of lymphocytes (87 per cent), neutrophils (62 per cent), macrophages (25 per cent), fibrins (25 per cent) and RBCs (12.5 per cent).

Out of 42 cases, five cases were diagnosed cytologically as tumour cases. Among the 5 cases, three were diagnosed cytologically as mesothelioma which revealed varying sized mesothelial cells with macronuclei and occasional binucleation and cytoplasmic vacuolation. In two cases, inflammatory cells and RBCs were also present.

Rebar and Raskin (2006) who reported that presence of increased number of mesothelial cell clusters and rafts is a common finding with reactivity. They also reported that in modified transudates, the proportion of inflammatory cells increase and neutrophils rarely account for 30 per cent of cell population. This statement is in full agreement with this study.

Among 5 cases, one was diagnosed cytologically as adenocarcinoma which revealed medium to large epithelial cells which appeared as clusters of cohesive cells, foamy or vacuolated cytoplasm, fine chromatin and variable prominent nucleoli. However, the primary organ of tumour was not identified. This finding is in agreement to the findings of Kushwaha *et al.* (2008) who reported that cytological findings of adenocarcinoma were clusters of large tumour cells having vacuolated cytoplasm with dense nuclei and pale cytoplasm.

Among 5 cases, one case was diagnosed cytologically as lymphoma which revealed medium to large lymphocytes (Fig. 5) with fine chromatin, deep blue granular cytoplasm. Occasional multinuclei, multinucleoli and mitotic figures were seen. This is in full agreement with the findings of Das (2005) who documented that serous effusions involved by an aplastic large cell lymphoma revealed numerous medium to large lymphoid cells with frequent

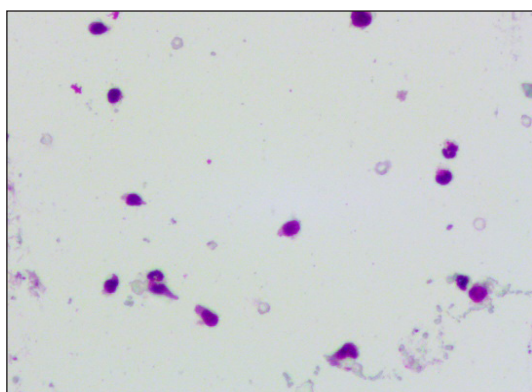


Fig. 5: Cytology of pleural fluid showing lymphocytes, few showing “tennis racket” appearance indicative of lymphoma

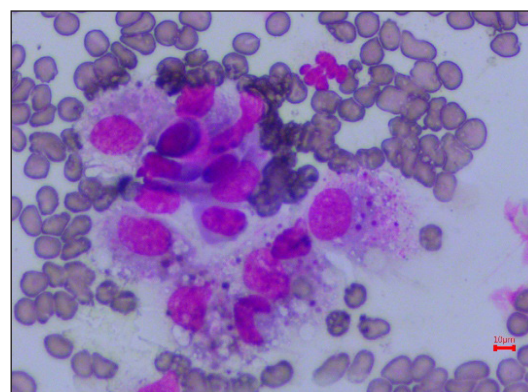


Fig. 6: Cytology of pleural fluid showing macrophages which are indicative of inflammatory response

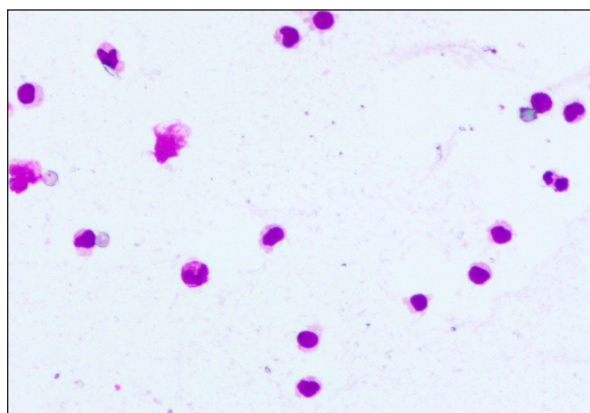


Fig. 7: Cytology of pleural fluid showing neutrophils along with lymphocytes indicative of acute inflammatory response

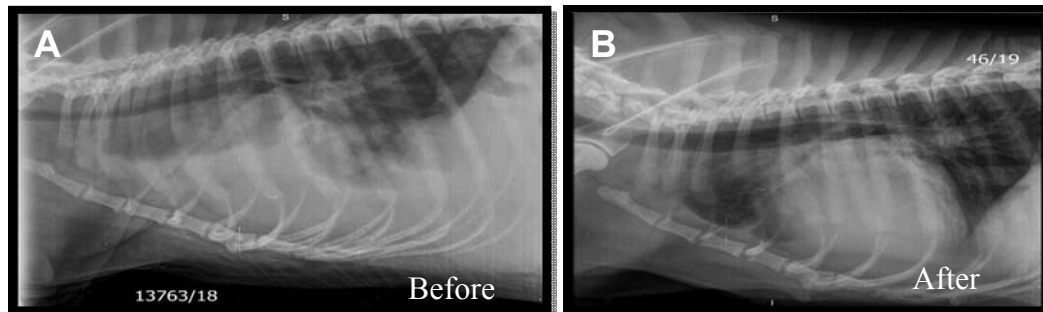


Fig. 8: Radiograph images of thorax showing pleural effusion. (A) Lateral view of thorax showing presence of pleural fluid before thoracocentesis; (B) Lateral view of thorax showing absence of pleural fluid after thoracocentesis

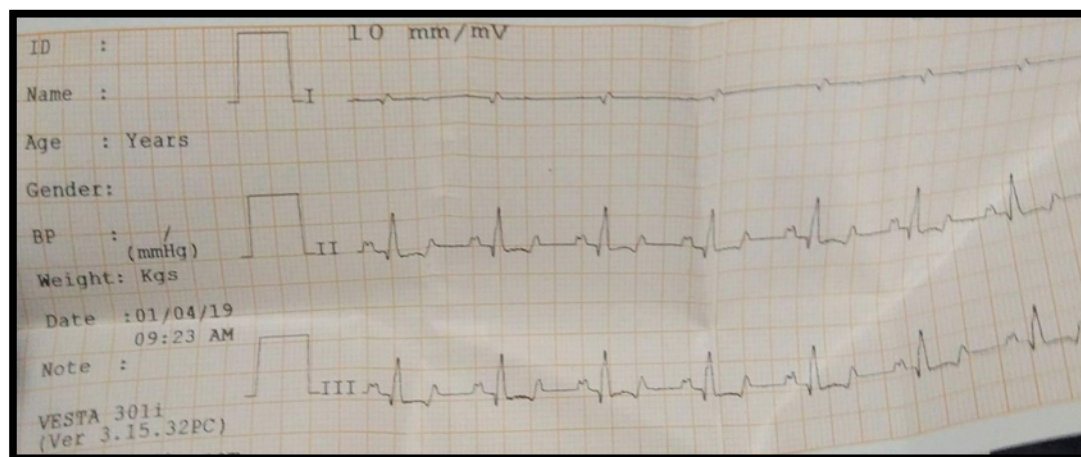


Fig. 9: Electrocardiogram which shows sinus tachycardia and reduced 'R' amplitude indicative of edema or effusion

nuclear irregularities.

Among the cases in others group, two cases were diagnosed as hemothorax. Cytology revealed the presence of numerous RBCs and a few mesothelial cells. Burges (2004), Segura (2004) and Hassan *et al.* (2012) documented that hemothorax cases showed few or no eosinophils along with numerous RBCs which is in full agreement with the present study.

Among the cases in others group, one case was diagnosed as pyothorax. Cytology revealed a greater number of mature and degenerated neutrophils along with the presence of numerous clumps of bacteria and macrophages. Ettinger and Feldman (2010) documented that cytology of pyothorax revealed predominantly degenerate neutrophils, inflammatory cells and large number of intracellular bacteria and agreement with the present study.

In the present study lymphocytes and macrophages were

commonly noticed in pleural fluid which might be due to inflammatory reaction and chronicity of presented cases. Neutrophils were also noticed in most acute cases which could be due to inflammatory response of the cells in the fluid.

Pleural fluid biochemistry findings

The comparison of values of lactate dehydrogenase and total protein of pleural fluid of various aetiologies are listed in Table 2.

The mean values of Lactate Dehydrogenase in pleural fluid (g/dL) in tumour group, hepatic group and cardiac group were 152.2 ± 23.95 , 108.2 ± 13.8 and 88.5 ± 7.9 . A significant difference ($P < 0.05$) in LDH value was observed in hepatic group, tumour group and cardiac group. Tumor group showed elevated LDH values and hepatic group had mildly elevated LDH values when compared to cardiac group.

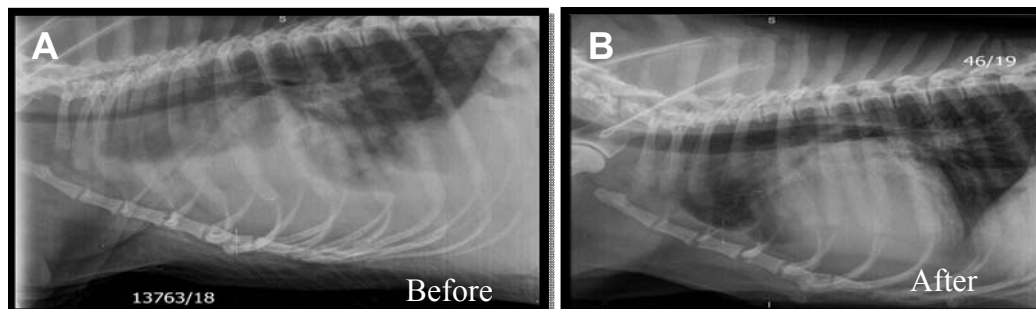


Fig. 10: Ultrasonographic findings of canine pleural effusion, (A) Ultrasound image showing hyperechoic parenchyma of liver indicative of chronic hepatitis/cirrhosis; (B) Ultrasound image showing anechoic area in the thoracic cavity which shows the presence of pleural fluid

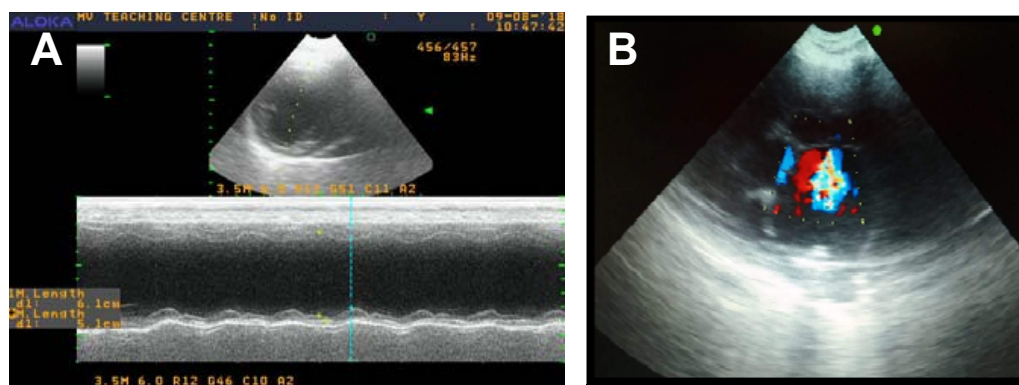


Fig. 11: Echocardiographic findings of canine pleural effusion. (A) Echocardiographic image showing dilated ventricles in a case of dilated cardiomyopathy; (B) Echocardiographic image showing regurgitation of blood in a case of mitral valve disease

Ali *et al.* (2007) stated that the cytoplasmic, cellular enzymes, such as lactate dehydrogenase in extra cellular space are suggestive indicators for disturbance of the cellular integrity induced by pathological conditions. This might be the reason for the elevation of LDH in cases of tumour and hepatic.

The elevated LDH was in cases in which the fluid was an exudate. In cardiac cases, the fluid was predominantly transudate or modified transudate, hence the LDH value was reduced. In hepatic and tumour cases, the fluid was predominantly an exudate and hence the value of LDH was elevated. This is supporting the findings of Smuts *et al.* (2016).

Dev *et al.* (1994) found a significant difference in LDH values, stating that LDH value was minimum in cardiac failure which is in full agreement to this study.

The mean values of Total protein in pleural fluid (g/dL) in tumour group, hepatic group and cardiac group

were 2.46 ± 0.33 , 2.24 ± 0.43 and 1.73 ± 0.15 . There was no significant difference in total protein value between tumour group, hepatic group and cardiac group.

CONCLUSION

Biochemical and cytological analysis of pleural effusion helps to identify the types of effusion and aids in the diagnosis of the root cause of pleural effusion in dogs. Pleural fluid LDH value aids in differentiating exudates and transudates and can be used as a criterion to classify the effusions. Total protein value of the effusion was not useful in the diagnosis of pleural effusion.

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