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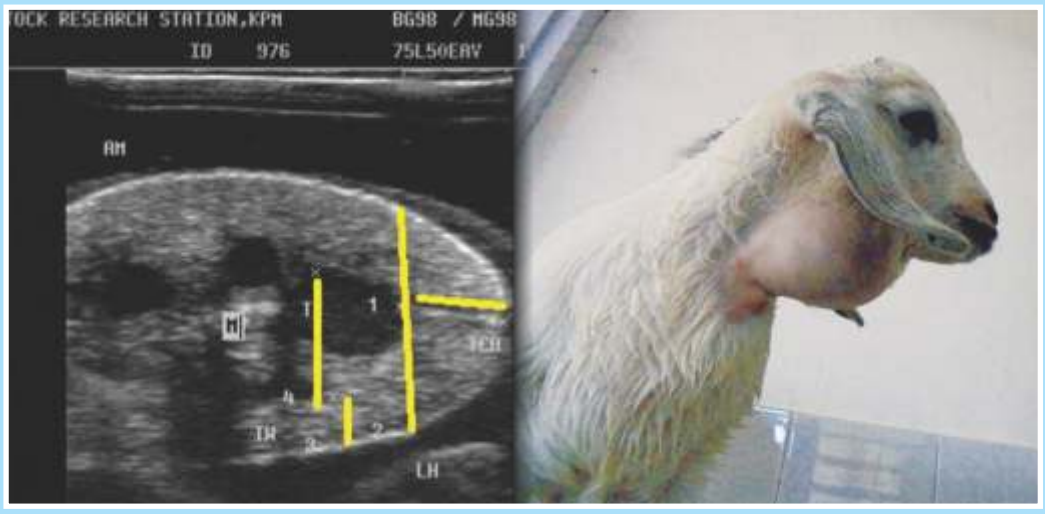

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Research Articles

2D and 3D ultrasonographic study of hepatobiliary disorders in dogs and their etiological pattern

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Abstract

Liver disease in dogs can develop as a result of many different insults. A clinical study was conducted on clinical cases presented to the Small Animal Medicine Referral Clinic of Madras Veterinary College from 2010-2012. Cases presented with signs such as anorexia, lethargy, ascites, icterus, pigmented urine and vomiting were screened for liver disorders. Out of 23,289 dogs with gastrointestinal disorders, 100 dogs were found to have liver diseases of different kinds. Ultrasonography of liver was taken for diagnostic assessments. Liver Disease Group, was further subdivided into three groups; Biliary tract disorders, Parenchymal disorders and Neoplastic disorders. 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. The incidence of liver disease was found to be 0.15 per cent of dogs in the hospital population and formed 0.43 per cent of gastrointestinal caseloads of the hospital. Non-descript dogs and the age group of 4-8 years were commonly affected. Dogs less than 4 years were also found to be affected with a higher incidence, but requires further studies to ascertain breed predisposition of nondescripts and assessment of risk factors such as infectious or toxic agents. Higher prevalence of biliary disorders in males and nondescript dogs also warrants studies on breed predisposition and on risk factors. 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. 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.

Keywords: Liver disorders, Hepatobiliary disorders, Ultrasonography

Liver diseases in pets as well as people are very complex. The liver disease may be frustrating to diagnose and every veterinary practitioner feels of it. Liver disease in dogs can develop as a result of many different insults. In the present scenario with increasing number of pets in cities and urban areas, the owners themselves do inappropriate therapies or the practitioners are forced to go for overzealous medications. Many drugs have been found to adversely affect the functioning of liver leading to signs of hepatopathy. This paper documents the incidence, etiological pattern and ultrasonographic studies of hepatobiliary disorders encountered in the university small animal practice, as these disorders account for considerable time and efforts needed for their veterinary care.

Materials and Methods

The clinical study was conducted with the clinical cases presented to the Small Animal Medicine Referral Clinic of Madras Veterinary College over a period of two and half years (2010-2012). Cases presented with signs such as anorexia, lethargy, ascites, icterus, pigmented urine and vomiting were chosen and screened for liver disorders. Chief complaints, age at onset, management practices, medication history and chronology of events were assessed. The data on breed, age, sex were collected for demographic studies. Findings of Clinical Examination, Clinico-pathological Studies, Coagulation Analysis, Radiography and Ultrasonography were taken for diagnostic assessments. Records of 100 Confirmed Cases were analysed for this study. Grouping was done as follows: 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. 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)

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Results and Discussion

The incidence of gastro intestinal disorders in the present study were observed to be 35 per cent comprising of 23,289 dogs out of a hospital population of 66,450 dogs presented to the Small Animal Medical Out-Patient Unit. Out of these 23,289 dogs with gastrointestinal disorders, 100 dogs were found to have liver diseases of different kinds and accounted for 0.43 per cent incidence of hepatobiliary disorders during the study period. When compared to the hospital population of 66,450 dogs, the incidence of hepatobiliary disorders was found to be 0.15 per cent. The current incidence level was in concurrence with the reports of previous studies (Nambi ,1993; Boomhens *et al*., 2004 and Poldervaart *et al*., 2009).

Many authors had classified liver diseases based on clinical, clinicopathologicial and ultrasound imaging (Reed, 1985). 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 (Brovida and Rothuizen , 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 (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 hepatits 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. In the present study 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) which 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 in three dogs, one dog had hepatocellular carcinoma and another had cholangiocellular carcinomas which were primary liver tumours and remaining four dogs had tumour which were unclassified.

Breed specific incidence of liver disorders are of clinical importance for the practitioners. Doberman pinchers were documented had four times greater than the expected level of incidence of liver diseases as observed from retrospective studies (Strombeck *et al*., 1988). 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 Pomerarian 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. 1).Incidence in German shepherd was 12 per cent and that of Doberman

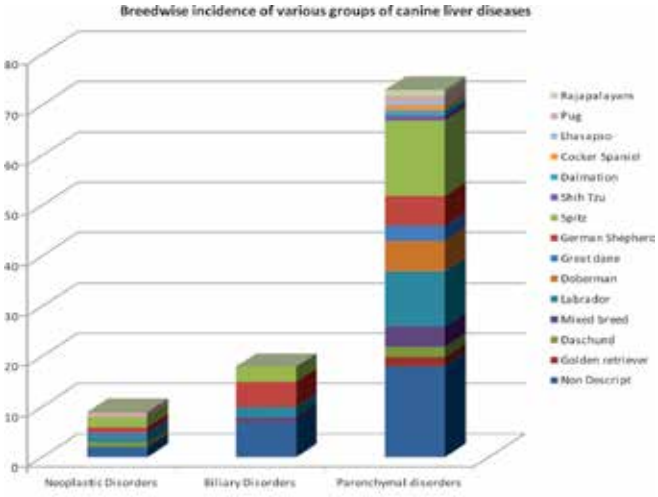

Fig. 1: Breed wise incidence of various groups of canine liver diseases

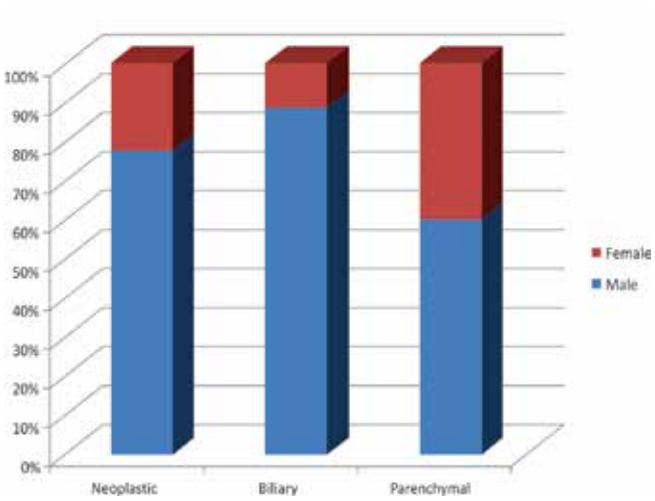

Fig. 2: Age wise incidence of various groups of canine liver diseases

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 Dobermans were comparable to the incidence levels in the previous reports (Shih *et al*., 2007 and Poldervaart *et al.,* 2009)

Age 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 (Strombeck and Gribble, 1978). A wide variation (8 month to 10 years) was reported in the age of dogs diagnosed with cirrhosis (Thornburg *et al*., 1983). Mandigers *et al*. (2004) reported that liver disease was present between 4 and 6 years of age. In the present study (Fig 2) 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 *et al*., 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 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.

In an ultrasonographic study (Plate 18-29) 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 splenomegaly 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) (Table 1).

In an ultrasonographic study of 21 dogs, Shih *et al.* (2007) reported changes in echogenicity which

Table 1: Sonographic findings in canine liver diseases

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

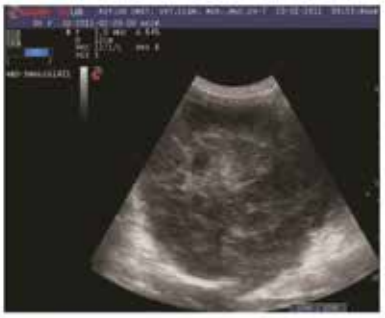

Plate 18. A large markedly heterogenous cavitated mass in the liver (Hepatic tumour)

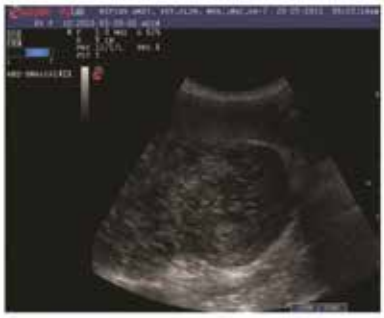

Plate 19. Circumscribed hypoechoic mass with hyperechoic border (Hepatic abscess)

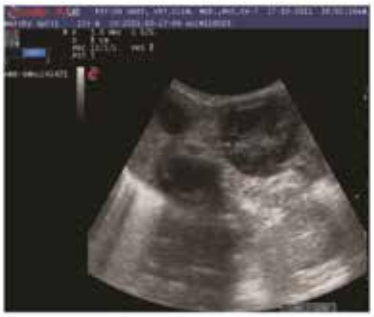

Plate 20. Multifocal hypoechoic mass (Liver neoplasia)

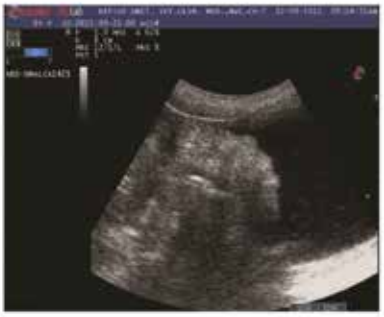

Plate 21. Hyperechoic liver with irregular border and cellular ascites (Cirrhosis)

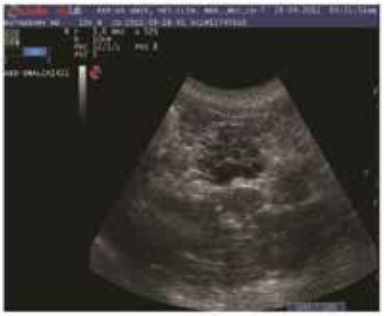

Plate 22. Poorly defined mass contains irregular hypoechoic and anechoic cavitations (Hepatic tumour)

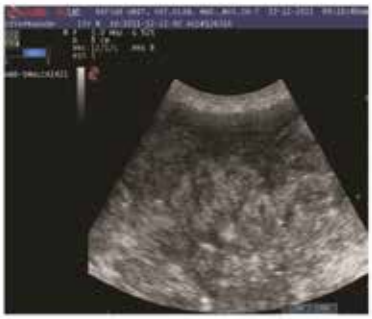

Plate 23. Mixed echogenicity pattern (Malignant tumour)

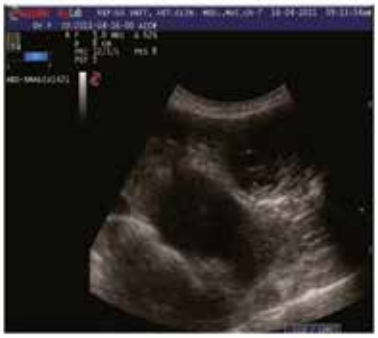

Plate 24. Circumscribed hypoechoic mass with ascites

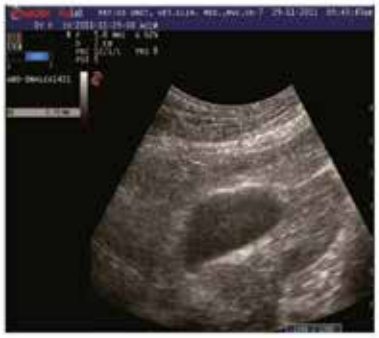

Plate 25. Gallbladder wall thickening

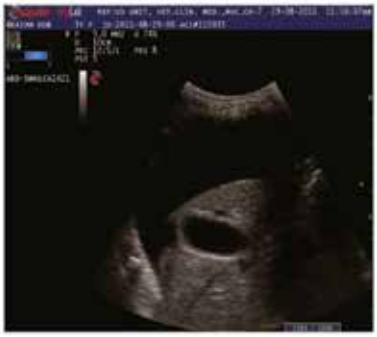

Plate 26. Hyperechoic double walled gallbladder with ascites

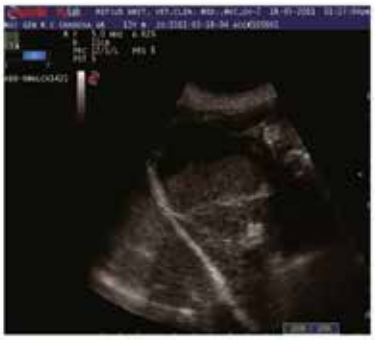

Plate 27. Gallbladder sludge

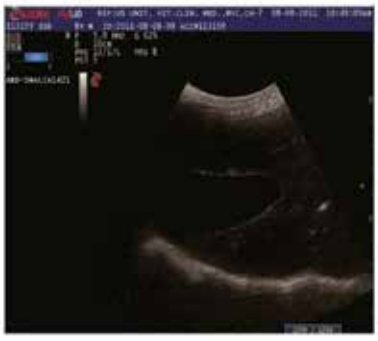

Plate 28. Distended gallbladder

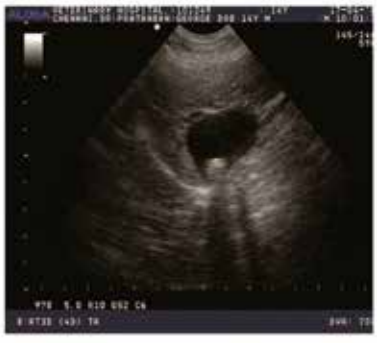

Plate 29. Cholelithiasis (Hyperechoic structures with acoustic shadowing)

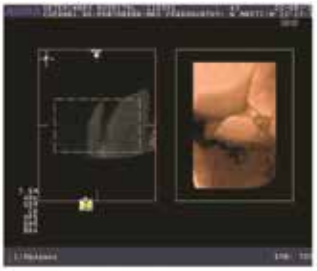

Plate 30. Comparative visualisation of 3D USG and 3D USG image of liver lobes

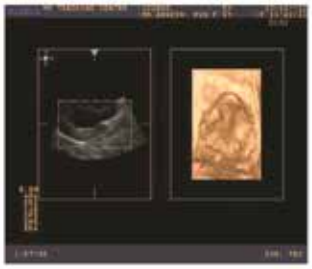

Plate 31. Comparative visualisation of 2D USG and 3D USG image of hepatic lymphoma

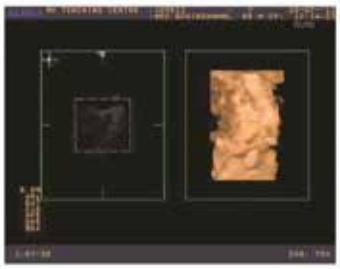

Plate 32. Comparative visualisation of 2D USG and 3D USG image of liver cirrhosis

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 splenomegaly, 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).

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.

Hypoechoic thickening was reported to be associated with gall bladder mucocele, cholangiohepatitis and hypoproteinemia (Barr, 1990; Newell *et al*. 1995; 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 baldder (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.

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 hypoecoic 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 slpenomegaly 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).

In the present study 3 dimensional ultrasound studies were attempted in 6 dogs which were normal and those with ascites, cirrhosis and neoplasia(Plate 30-32). 3D ultrasonography had been shown to provide a more accurate and repeatable method of evaluating anatomic structures and disease entities (Downey *et al.,* 2000). 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 in a better way.

Conclusions

The incidence of liver disease was found to be 0.15 per cent of dogs in the hospital population and formed 0.43 per cent of gastrointestinal caseloads of the hospital. 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 were also found to be affected with a higher incidence and it requires further studies to ascertain breed predisposition of nondescripts and assessment of risk factors such as infectious or toxic agents. Higher prevalence of biliary disorders in males and nondescript dogs also warrants further studies on their breed predisposition and on risk factors. 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. 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.

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Total anthelmintic failure on gastro intestinal parasites of sheep and its impact on blood parameters

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Abstract

A study was conducted to investigate the efficacy of anthelminthics against gastrointestinal nematodes of sheep and changes in blood profiles before and after dosing. The FECRT results revealed development of resistance to albendazole, fenbendazole, closantel and levamisole at 95% upper and lower confidence limit. The efficacy of albendazole, fenbendazole, closantel and levamisole was 68%, 75%, 77% and 84% respectievely. The blood parameters did not reveal any significant increase in their levels post treatment except Levamisole.

Keywords: Faecal culture, FECRT, Haematology, Resistance, Sheep

Gastrointestinal nematode parasitism is well recognized as a major production-limiting disease. Gastrointestinal parasites cause heavy economic losses in meat and wool production (Gordan, 1974). Control of helminth parasites for the past thirty years is mainly done with anthelmintics Currently resistance to anthelmintics by gastrointestinal nematodes of sheep and goat is a widespread problem. Anthelmintic resistance is defined as a decrease in the efficacy of anthelmintics against a population of parasites that were originally susceptible (Sangster and Gill**,** 1999). The extensive use of anthelmintics for control of gastrointestinal nematodes has resulted in development of resistance to one or more of the widely used anthelmintics in many countries including India (Maingi *et al*., 1998**;** Dhanlakshmi *et al.,* 2003; Jeyathilakan *et al.* 2003; Deepa and Devada, 2007; Easwaran *et al*., 2009; Buttar *et al.,* 2012, and Kumar *et al*., 2014).

The task of selecting the drug of choice to control gastrointestinal nematode becomes easier if the resistance status of the parasite is known. Thus regular monitoring of the status of anthelminthic resistance is required as an integral part of worm control programme particularly on organized farm.

Effective monitoring of resistance is vital in order to maintain the efficacy of the currently available anthelmintics and to prevent further selection for resistance. Hence a study was designed to detect the resistance to the most commonly used anthelmintics viz. albendazole, fenbendazole, closantel and levamisole against gastrointestinal nematodes of sheep using *in vivo* faecal egg count reduction test. Blood is an important and reliable medium for assessing the health status of individual animals (Oduye, 1976), hence hematological analysis was done for assessing the degree of damage to host tissue as well as severity of infection (Otesile *et al.,* 1991).

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Materials and Methods

Study area: ILFC farm, Veterinary College, Hassan

Sheep flock selected for the study was managed in a semi-intensive system of grazing by day on pasture and housing at night. Regular anthelminthic drenching was carried out in the farm, 4 times a year with fenbendazole and albendazole. Before the trial it was ensured that sheep were not dosed with anthelmintics for the past 12 weeks.

In this study, fifty adult sheep irrespective of sex were divided randomly into 5 groups of 10 animals each. The animals were individually identified by putting ear tags. Sheep of Group I were dosed with albendazole @ 5 mg/kg B.Wt orally (Bizole, Brilliant Biopharma ltd), Group II animals were dosed with fenbendazole @ 7.5 mg/kg B.Wt orally (Panacur® Vet, Intervet India Pvt ltd.), Group III animals were dosed with closantel @ 15 mg/kg B.Wt orally (Zycloz®, Zydus animal health limited) Group IV animals were dosed with Levamisole @ 7.5 mg/kg B.Wt orally (Nilverm, Virbac animal health India Pvt ltd.), Group V animals remained untreated and served as controls. Faecal samples were collected from the rectum of each sheep before treatment and 14th day post treatment. Quantitative assessment of the faecal egg count in terms of eggs per gram (epg) was done using modified McMaster method (Coles *et*

Table 1: Analysis of faecal egg count reduction test.

Anthelmintic	AM post treatment EPG	Per cent reduction in egg count after 14 day of treatment	Lower 95 % confidence limit	Upper 95 % Confidence limit	Results
Albendazole	1150	68	84	32	Resistance
fenbendazole	890	75	86	56	Resistance
Closantel	800	77	90	48	Resistance
Levamisole	580	84	96	29	Resistance
Control	3540	-	-	-	-

al., 1992). The percent faecal egg count reduction and efficacy for each anthelminthic were calculated as per RESO FECRT analysis programme (Version 2, Revised 17-7-90).

Pre treatment pooled faecal samples from all the animals were cultured and third stage larvae (L3) were identified (Zajac and Conboy, 2012)**.** Similarly, on day 14 post-treatment faecal samples from each group were pooled and cultured separately for identifying the third stage infective larvae.

Blood samples (5 ml) were collected from jugular vein of each sheep in a vial containing EDTA before and Post treatment. Various Haematological parameters like, Total Leucocyte Count (TLC), Haemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Platelet count (PLT) were analyzed using haematoanalyzer. Collected data were statistically analyzed using Paired "T" test.

Results and Discussion

In this study albendazole and fenbendazole reduced the faecal egg count by 68 and 75 % respectively, with less than 95 lower 95% confidence limit. Levamisole was 88% effective in eliminating infection and the lower 95% confidence limit was 95 %. Closantel treated group reduced the egg count by 77 % with less than 95 lower 95% confidence limit. (Table1). Culturing of faecal sample showed the presence of Haemonchus sp, Trichostrongyle sp, Cooperia sp and Oesophgostomum sp before and after treatment. (Table 2a, Plate. 1, 2, 3 and 4). The anthelminthic having efficacy less than 95% with lower 95% confidence limit less than 90 was considered as development of resistance in the target parasite against the drug based on FECRT, according to Coles *et al*., (1992).

The changes in the haematological parameters of different groups before and after treatment have been mentioned in table 3a. Upon conducting Paired "T" test it was found that there was no significant variation in the Albendazole, fenbendazole and closantel treated group. However there was significant rise in total leucocyte, Lymphocyte, Haemoglobin and Packed cell volume in Levamisole treated group (Table 3b).

In this study the gastrointestinal nematodes were found to be resistant to albendazole and fenbendazole. The lower efficacy and resistance to fenbendazole and albendazole might be attributed to their continuous and prolonged use in controlling gastrointestinal nematodes in these farms. This is in opinion with Dhanalakshmi *et al.,* 2003; Eashwaran et al., 2008 and Kumar et al., 2014.

Levamisole had 84 % efficacy with less than 95 lower and 95 % confidence limit. A nearly similar finding was reported by Jeyathilakan *et al.,* (2003); Eashwaran et al., (2009); This result is suggestive of cross-resistance development between the group of anthelmintics because levaminsole is being used in this farm for the first time.

Closantel had 77 % efficacy with less than 95 lower and 95 % confidence limit, which has been already reported by Gupta *et al.,* (2003); Thomaz Soccol *et al*., (2004) and Vohra *et al.,* (2013). The reduced accumulation of drug in parasite body by mechanism such as reduced feeding, failure to dissociate the drug-albumin complex in the gut or increased efflux of

Table 2a: Gastro intestinal nematodal larvae in cultures of faecal samples obtained from sheep before and after treatment.

Before treatment	After treatment
Haemonchus spp.,	*Haemonchus spp.,*
Trichostrongyle spp.,	*Trichostrongyle spp.,*
Cooperia spp.,	*Cooperia spp.,*
Oesophgostomum spp.,	*Oesophgostomum spp.,*

Table 3a. Mean and Standard deviation of Haematological parameters before and after treatment with drug.

Drug	Hb (g %)		PCV		T E C (× 10^6 cells /µl)		WBC (× 10^3 cells /µl)		LY (× 10^3 cells /µl)		MO (× 10^3 cells /µl)		GR (× 10^3 cells /µl)		LY %		MO %		GR %		PLT × 10^3	
	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post
A	7.6± 0.73	6.27± 0.39	24.67± 1.67	22.01± 1.17	7.907± 0.64	7.193± 0.51	10.15± 0.68	12.57± 1.5	8.9± 0.52	11.16± 1.24	0.58± 0.11	0.67± 0.13	0.69± 0.14	0.77± 0.18	88.25± 1.79	89.26± 1.13	5.36± 0.8	5.03± 0.55	6.39± 1.09	0.57± 0.65	186.6± 17.85	272.7± 33.72
F	6.62± 0.36	6.7± 0.19	21.68± 1.08	20.73± 0.58	7.35± 0.47	7.668± 0.36	12.78± 1.18	12.24± 0.97	11.07± 0.98	10.92± 0.86	0.79± 0.11	0.68± 0.09	0.91± 0.17	0.62± 0.09	86.97± 1.25	89.15± 1.01	5.62± 0.72	5.52± 0.62	6.9± 0.74	5.32± 0.43	181.0± 24.51	230.1± 18.47
C	4.91± 0.5	5.6± 0.52	16.31± 1.56	19.21± 1.73	5.61± 0.52	6.789± 0.53	11.5± 1.73	14.82± 0.98	10.41± 1.55	11.29± 1.1	0.53± 0.1	1.39± 0.55	0.56± 0.1`2	2.28± 0.95	91.18± 1.08	78.29± 6.67	4.28± 0.52	7.83± 2.11	4.54± 0.58	13.88± 4.59	136.8± 27.62	220.9± 24.86
L	5.85± 0.49	6.75± 0.41	18.97± 1.36	22.95± 1.14	6.348± 0.55	7.565± 0.44	11.28± 1.52	13.87± 1.53	9.1± 1.58	11.46± 1.66	1.02± 0.34	1.23± 0.43	1.16± 0.31	1.18± 0.32	78.02± 7.77	81.35± 7.15	10.19± 4.05	9.64± 4.16	11.79± 3.88	9.01± 3.01	134.9± 30.28	158.7± 39.33

A-Albendazole, F- Fenbendazole, C-Closantel, L-Levamisole

Table 3b. Change in Haematological parameters after treatment with drug (sample size in each group is 10 animals)

Drugs	WBC ×10^3 cells /µl)	LY ×10^3 cells /µl	MO ×10^3 cells /µl	GR ×10^3 cells /µl	LY%	MO%	GR%	T E C (× 10^6 cells /µl)	Hb (g %)	HCT/ pcv	MCV	MCH	MCHC	PLT ×10^3
Albendazole	1.04	1.26	-0.12	-0.12	1.01	-0.33	-0.69	-0.714	-0.23	-2.66	-0.63	-0.65	-1.03	12.1
Fenbendazole	0.16	0.15	-0.11	-0.09	2.18	-0.61	-1.58	0.311	0.08	-0.95	0.03	-0.14	-0.73	13.1
Closantel	0.16	0.15	-0.01	-0.09	2.18	-0.61	-1.58	0.311	0.08	-0.95	0.03	-0.14	-0.73	14.1
Levamisole	2.59*	2.36*	0.21	0.02	5.33	-0.55	-2.78	1.217	0.6*	3.98*	0.21	0.23	-1.31	13.8

The Values with * are statistically significant at 0.05%

There was significant increase in the values of WBC, LY, Hb (g%), and HCT/pcv before and after treatment with Levamisole.

Table 2b, Plates showing Third stage larvae of *Haemonchus spp, Trichostrongyle spp, Cooperia spp. and Oesophgostomum spp.*

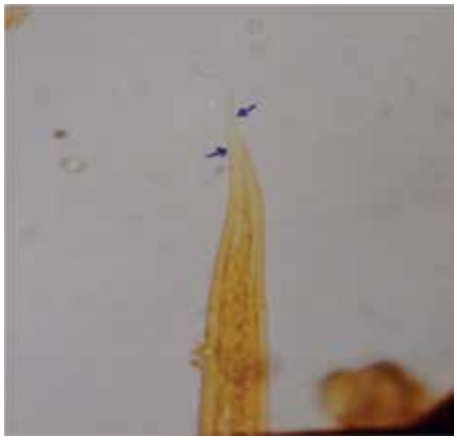

Plate 1. Third stage larvae of *Haemonchus* species with a kink in the tail sheath 40x.

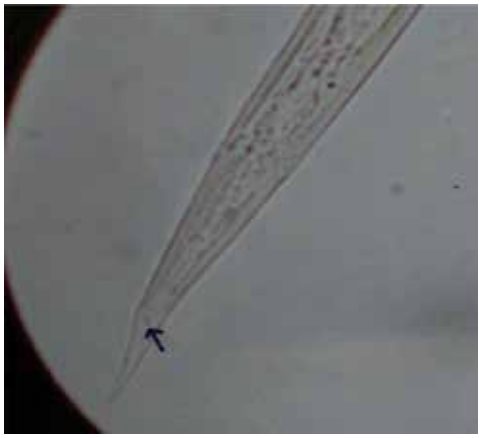

Plate 2. *Trichostrongylus* species with tail sheath is having one or two tuberosities or indistinctly rounded tail 40 x.

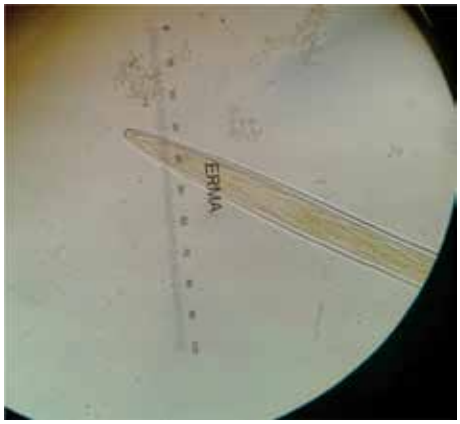

Plate 3. Head of *Cooperia* third stage larva showing two refractile bodies at anterior end of oesophagus 40x

Plate 4. Third stage larvae of *Oesophagostomum* species with wrinkled sheath 40x.

closantel from resistant worms attributed to anthelmintic resistance (Rothwell and Sangster, 1997).

In this study Haemonchus sp and *Trichostrongyle sp* were found predominant before and after treatment. These are in agreement with the findings of Maingi, 1991; Dhanalakshmi *et al.,* 2003; Kumar *et al.,* 2014, Höglund *et al.,* 2009; Atle*, et al.,* 2012 and Meenakshisundarm (2014). High biotic potential of GI nematodes especially *H.contortus* contributes to rapid selection for resistance as large number of generations of worms are produced within a short time (Deepa and Devada, 2007). Since *H. contortus* was the predominant GI nematode species observed in this study, this factor also might have contributed to the selection for resistance.

Hematological parameters revealed no improvement in their levels before and after treatment with albendazole, fenbendazole and closantel. This can be attributed to the fact that the parasites were resistant to the drug and were not eliminated after treatment. However even though there was resistant to levamisole there was a significant rise in haemoglobin, packed cell volume and lymphocyte. Which can be attributed to the Haemopoiesis enhancing and immunomodulatory effect of levamisole (Wauwe and Janssen, 1991).

In conclusion, the detection of anthelmintic resistance in GI nematodes of sheep necessitates implementation of urgent measures to slow down the development of resistance. Periodical screening of flocks for resistance by FECRT is also recommended.

The results of the present study have indicated the need for strategic drenches in combination with grazing management for slowing down the spread of anthelmintic resistant worm populations in sheep and goats. The presence of multiple resistance to chemicals leads to the urgent need for developing an alternative gastrointestinal parasite control program, based on epidemiological dates and periodical post treatment faecal examination to monitor performance of individual anthelmintcs.

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Cardiac evaluation in anaemic dogs with clinical Babesiosis caused by *Babesia gibsoni*

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Abstract

Fifty anaemic dogs (haemoglobin 3.5 to 5.0 g/dl) with confirmed clinical babesiosis were evaluated for cardiac functioning employing cardiac auscultation , pulse examination , electrocardiography and estimation of cardiac troponin-I at the time of referral. Anaemic dogs with babesiosis, caused by *Babesia gibsoni*, showed increased heart rate and respiratory sinus arrhythmia .On electrocardiographic examination, changes such as sinus tachycardia (large breed dogs ≥ 140 per minutes or small breed dogs ≥ 160 bpm) , sinus arrhythmia , enlarged 'R' and broad 'QRS' ,ST depression (0.2 to 0.4 mV) , ST elevation (0.15 to 3.0 mV); enlarged 'T'(≥ 25% of 'R') and complex changes were detected in 15 (30%),4 (8%),3 (6%),8 (16%),7 (14%),6 (12%) and 7(14%) cases . Cardiac troponin I levels varied from 0.5 to 14.6 ng/ml with an average of 6.65 ± 0.42 ng/ml and median of 6.0 ng/ml suggesting varying degree of cardiac insult in these anaemic dogs having *Babesia gibsoni* infection. Dogs having higher level of cTn-I (> 7.0) succumbed invariably.

Keywords: Arrhythmias; *Babesia gibsoni*; canine babesiosis; cardiac injury; cardiac troponin-I ; electrocardiography.

Canine babesiosis is a tick-borne potentially fatal blood protozoan disease caused by several species of Babesia.Of these *B. canis* and *B.gibsoni* are well documented in India. Naturally occurring cases of babesiosis are manifested by a wide variety of inconsistent and vague clinical signs ranging from anorexia, anaemia, neurological deficits, lymphadenopathy, splenomegaly to .hepatomegaly . Both intra and extra vascular haemolysis in babesiosis results in regenerative anaemia, anaemic hypoxia, anaerobic metabolism, metabolic acidosis and excessive systemic inflammatory response that may lead to cardiac lesions (Reyers *et al*.,1998). Haemodynamic changes secondary to anaemia may lead to cardiac remodeling owing to anaemic hypoxia and activation of systemic inflammatory syndrome that may worsen heart failure (Horwich *et al*.,2002). Dvir (2001) reported that electrocardiographic changes were present in 40 % cases of canine babesiosis caused by *B. canis rossi*. In 2002 Lobetti *et al*.(2002) observed high cardiac troponin-I values in non-survivor dogs suffering from babesiosis caused by *B.canis rossi* infection and suggested that high cTn-I values were associated with poor outcome . In India, it appears that there is no definite evidence to suggest cardiac invovement in severely anaemic cases of canine babesiosis caused by *B.gibsoni*. Therefore the present study was undertaken to evaluate cardiac insult, if any, in severely anaemic dogs with babesiosis, caused by *B.gibsoni*.

Materials and Methods

Fifty anaemic client owned dogs with clinical babesiosis, confirmed on periphral blood smear cytology, formed the material for the present study. The dogs were subjected to detailed clinical examination and haemogram (Celltac MEK64- Nihon Kohden). Dogs having haemoglobin ≤ 5.0 g/dl were subjected to electrocardioghraphy in right lateral rcumbancy employing hex axial lead system (Magic R - Maestros) in a calm and comfortable room and quantitative estimation of Troponin I (chemiluminescent enzyme immunoassay method (CLEIA) PATHFAST, Mitsubishi Kagaku Iatron Inc, Tokyo, Japan) .

Results and Discussion

During the period of 3 years , 50 severely anaemic dogs (haemoglobin 3.5 to 5.0 g/dl) of different breeds (German Shepherd, 12; Nondescripts, 8; Labrador, 8; Pug, 5: Boxer, 4; Greatdane, 4; Pomeranian, 4; Dobermann, 3; Dachshund,1 and Cocker Spaniel,1;), age (4 months to 10 years) and sex (male, 30, female, 20) with confirmed babesiosis (caused by *B. gibsoni*) were investigated for cardiac abnormalities. These cases were referred at the hospital after treatment failure for 6.0 to 15 days. The disease was clinically characterized by anorexia, vomitting, varying temperature (102.6 to 104 ^{0} F), weight loss, severely pale mucus membranes, respiratory distress, arrhythmia and tick infestation as observed in other

cases of babesiosis at home and abroad. Blood smear cytology confirmed *B. gibsoni* infection and haemogram showed severe anaemia (haemoglobin 3.5 to 5.0 g%.) in these dogs. Electrocardiograms revealed sinus tachycardia(large breed dogs ≥ 140 per minutes or small breed dogs ≥ 160 bpm), sinus arrhythmia , enlarged 'R' and broad 'QRS' ,ST depression (0.2 to 0.4 mV) , ST elevation (0.15 to 3.0 mV) ; enlarged 'T' (≥25% 0f 'R') and complex changes in 15 (30%),4 (8%),3 (6%),8 (16%),7 (14%),6 (12%) and 7 (14%)cases respectively. Higher prevalence of sinus tachycardia and sinus arrhythmias seems to be due to haemodynamic changes in severely anaemic dogs with babesiosis resulting into hypoxia and consequent metabolic changes stimulating chemo receptors and sympathetic activity (Metivier *et al*.,2000). Increased in 'R' wave amplitude and wide QRS in three dogs suggested left heart enlargement owing to overload related to compensatory haemodynamic changes and corroborate the findings previously reported in dogs with anaemia (Scheel and Williams ,1985; Champion *et al*.,2013). Horwich *et al*. (2002) reported that chronic hypoxia owing to severe anaemia causes structural remodelling in cardiac myocytes with activation of inflammatory process worsening heart failure. ST segment changes, both ST elevation (0.15 to 0.3 mV) and ST depression (0.2 to 0.4 mV), observed in 15 dogs seems related to ventricular repolarization and were suggestive of impaired myocardial perfusion and oxygenation. Enlarged T wave (≥ 25% of 'R') was also related to impaired ventricular repolarization and myocardial hypoxia (Tilley,1992).

Though cardiac arrhythmias or electrocardiographic abnormalities were evident in anaemic dogs with babesiosis, it is difficult to predict myocardial insult with certainty as diagnostic sensitivity of electrocardiography or echocardiography to diagnose minor or reversible myocardial injury is poor. An early prediction of myocardial insult seems to have prognostic perspective. These days' cardiac troponins (I and T) are being claimed to exhibit myocardial tissue specificity and high sensitivity. The level of cTn-I remains elevated for a much longer period of time (6-10 days), thus providing for a longer window of detection of cardiac injury (Oyama and Sisson, 2004). Therefore serum cTnI ,a more sensitive indicator of myocardial cell injury, was estimated in 50 severely anaemic dogs suffering from babesiosis caused by *B. gibsoni* .The study revealed that cardiac troponin I levels varied from 0.5 to 14.6 ng/ml with an average of 6.65 ±0.42 ng/ml and median of 6.0 ng/ml in these dogs. Of these two dogs had comparatively low cTn-I (0.5 or o.6 ng/ml) and rest had levels ≥ 3.5 ng/ml . Ralli *et al.* (2005) observed increased levels of cTn-I in 50.9% humans with anaemia and indicated that anaemia is a factor for increased mortality by heart failure .The variability in the level of cTn-I in the severely anaemic dogs with *B.gibsoni* may be due to variability and duration of cardiac insult . cTn-I concentration is correlated with survival i.e. higher cTn-I concentration reduces the chance of survival (Oyama,2006). Reversible cardiac insult has recently been reported in cases of canine ehrlichiosis in India (Varshney *et al.,*2015). Acute or chronic cardiac injury induces release of cTN-I into circulation. In normal dog without cardiac insult, cTn-I levels are very very low varying from 0.03 to 0.07 ng/ml with a median of 0.02 ng/ml (Sleeper *et al*.,2001). The observation that myocardial dysfunction found during sepsis is reversible also supports the idea of troponin release associated with reversible injury (Parker *et al,* 1984). While cTn-I elevations indicate myocardial injury, they do not give any information as to the mechanism of injury. Troponin level can rise with very small amounts of myocardial cell damage. (Burgener *et al*.,2006; Hamm *et al*.,1997) . Cardiac damage in severely anaemic dogs with babesiosis , caused by *B. gibsoni*, may be related to the severe systemic inflammatory response and hypoxia.

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Teat ultrasonographic assessment of milk flow disorders in hand milked dairy cows

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Abstract

While many studies had been done on teat morphology of machine milked animals, no major studies are available for hand milked cows. Interpretation of ultrasonographic measurement is very crucial for better understanding on the recovery of teat tissue before and after milking. Towards this ultrasonographic assessment of teat morphology was undertaken on 32 hand milked cows. Milk Flow Disorders were diagnosed in 23 (71.86 %) cows. Ultrasonography of the teat was found to have better diagnostic yield in the detection of Milk Flow Disorder (64.28 %) when compared to clinical detection (35.75 %) as well as in location of the lesion and in identifying the type of lesion.

Keywords: Ultrasonography, Hand milked cows, Milk Flow Disorder,

Ultrasonography is increasingly used in Large Animal Medical Practice for the diagnosis of anatomical, pathological and physiological conditions of all parts of the mammary gland (teat and parenchyma). The application of ultrasound in the evaluation of milk flow disorders is emerging and is a biggest challenge in developing countries like India, where in hand milking is the most common among farmers. There are lots of studies carried out on mastitis but only a few studies were carried out on milk flow disorders. Many of the ultrasound studies are done in machine milked cows, but not in hand milked cows. This study reports the ultrasonographic assessment of milk flow disorders in hand milked dairy cows.

Materials and Methods

The present research work was carried out in Madras Veterinary College, University Research Farms and private dairy farms in and around Chennai. After a screening of 450 cows for milk flow disorders and with mastitis, 32 animals were selected for ultrasonographic study. Based on milk yield they were divided into low, medium and high yielders. Physical examination as well as Ultrasonographic examination of udder and teat was performed to identify the Milk flow disorder, location of the lesion and type of lesion by water bath technique using Esaote Mylab-20 Ultrasound Station with 5.0 MHz convex and 7.5 to 12.5 MHz linear transducers, respectively (Fig.1). Using 7.5 to 12.5 MHz linear probe ultrasonographic morphometry (teat canal length-TCL, teat wall thickness-TWT, and teat end width-TEW and teat cistern width-TCW) were evaluated on normal teats and milk flow disorder affected teats, before and after hand milking. Milk samples were collected from all the quarter for mastitis testing.

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994). The statistical software package – SPSS 20.0 for windows was utilized and the results were analyzed based on one way ANOVA, Duncan's technique, t-test and Chi-square test.

Results and Discussion

Out of 32 animals assessed with Teat Ultrasonography, 23 cows were found to have Milk flow disorders (MFD) and the remaining 9 were without Milk flow disorders. Out of 128 teats examined in the present study, 28 teats were found to have milk flow disorders. Milk flow disorder was found to affect all quarters, almost equally (left fore = 6, left hind = 8, right fore = 8, right hind = 6).

The mean values of ultrasonographic variables of healthy teats (left fore and left hind teat as well as right fore and right hind teats) before and after milking in low, medium and high yielders are presented in Table Ia,b (Fig.2a & 2b). The values for affected teats, are presented in Table IIa,b (Fig. 3a, 3b).

In present study the types of lesions identified in quarters with Milk Flow Disorders included intraluminal growth, teat cistern growth, milk clots, teat fistula, tip injury and covered teat injury each. Teat

cistern mucosal growth type of milk flow disorder was encountered at higher percentage 35.71(10/28) followed by teat tip injury, covered teat injury 17.85 (5/28). Franz *et al.,* (2009) reported of similar findings. Milk flow disorders are the main indications for ultrasonographic examination of the teat. Teat injuries, improper milking technique and partial or total teat stenosis are common reasons for the disturbance of milk flow in dairy cows (Selvaraj *et al*., 2016).

In this study the teat canal length and teat wall thickness were found to be increasing in the entire teat after hand milking (Table Ia, b). Similar findings were reported for machine milked cows by various authors (Zecconi *et al.,* 1992; Neijenhuis *et al*., 2001; Gleeson *et al.,* 2005 and Stojnović and Alagić 2012). The increase in the measurements indicates that the time taken for recovery of the teat to normalcy was more, thereby exposing the teat for possible infections. Various authors have discussed about the teat recovery time. Recovery time of teats is interpreted as the time that it takes for the teats to decrease the penetrability of the teat canal to endotoxin or teat canal diameter after milking (Hamann and Burvenich, 1994; Schultze and Bright, 1983; McDonald, 1975). Teat-canal penetrability is an important parameter for management aspects as with increased teat penetrability the possibilities for infections are more. Hence animals need to be allowed to stand for 1-2 hours after milking so as to avoid contact of the teat with the bedding materials and/ or floor.

The teat penetrability is dependent on the biochemical composition of the teat tissue, the opening of the sphincter, and the changes in teat tissue. The teat permeability cannot be studied with ultrasound. However, it is assumed that teat tissue changes could reveal teat-canal penetrability. To establish the relationship between teat-canal penetrability and changes in teat thickness, it requires advanced ultrasographic techniques and assessment and need to be studied further.

The teat cistern width had varying pattern (Table Ia, b) in different types of yielders. However the changes were not statistically significant. Neijenhuis et al. (2001) observed that, the teat end width had different patterns after milking in machine milked cows. Similar findings were observed in this study with hand milked cows. The reason for such changes might be due to the fact that measurement was being carried out at the most pliable part of the teat, which is difficult to be held during measurement. This study was carried out at different places in Chennai where the farmers practiced hand milking. The technique of milking (stripping or by full hand method) by different milkers could also influence the teat canal width. This could also be attributed to improper milking and or complete or incomplete milking.

The teat end width in various yielders was found to be decreased after milking in the entire teat. This was divergent from the findings of Neijenhuis *et al.,* (2001). Current study was conducted with hand milked animals whereas the other studies referred were conducted on machine milked animals. Again this present study comprised of mixed breed population at different places where as the referred studies were carried out on Holstein cows from a particular farm. Differences in breed characteristics in the teat morphometry and genetic selection might also be a reason for such differences and further studies in this regard in our Indian animals and breeds needs to be undertaken.

The teat wall thickness and teat cistern width change through the milking process in opposite directions (as cistern decreases in dimensions the teat-wall thickness increases), as the milk is withdrawn from the teat cistern during milking (Neijenhuis *et al.*, 2001). Similar observations were noticed in this study except for the left fore teat in both low and high yielders, right fore teat in high yielders and right hind teat in medium yielders where in they did not show any changes. These few insignificant differences could be overseen as these measurements were carried out at the most pliable part of the teat. Fluctuation was seen with the ratio between teat-wall thickness and teat-cistern width. While no explanation could be given for this, few authors (Schultze and Bright, 1983; McDonald, 1975) used endotoxin penetrability or radiography, and observed a similar pattern.

In the current study the teat canal length and teat wall thickness had comparable increase in the entire teat after milking in both MFD affected and unaffected teats (Table IIa, b). The pattern was different in various teats with regard to teat canal length when compared between MFD affected and unaffected group. In MFD unaffected group the after milking values of left fore teats and left hind teats were higher than the MFD affected group where as it was found to be reverse in the right fore and right hind teats. The teat wall thickness was found to be more in fore teats compared to hind teats

Table: ia. Mean±se values of fore teat ultrasonographic variables before and after milking

		Low yielders				Medium yielders				High yielders				
		BM (mm)	AM (mm)	D (mm)	D%	BM (mm)	AM (mm)	D (mm)	D%	BM (mm)	AM (mm)	D (mm)	D%	F-value
TCL	LF	10.18 ±0.45	10.35 ±0.40	0.17 ±0.21^{a}	1.66	10.4 ±0.70	11.77 ±0.96	1.32 ±0.74^{a}	12.7	13.36 ±0.49	14.70 ±0.98	1.34 ±0.76^{a}	10.0	1.623^{NS}
	RF	10.99 ±0.89	11.16 ±0.98	0.17 ±0.15^{a}	38.7	12.83 ±1.64	13.34 ±1.70	0.51 ±0.27^{a}	3.9	13.50 ±0.91	14.13 ±0.79	0.62 ±1.064^{a}	4.6	0.154^{NS}
TWT	LF	7.51 ±0.31	7.62 ±0.25	0.11 ±0.17^{a}	1.5	7.73 ±0.57	9.18 ±0.52	1.44 ±0.16^{b}	18.6	7.25 ±0.45	7.56 ±0.39	0.31 ±0.67^{ab}	4.3	3.1401^{NS}
	RF	7.92 ±0.61	8.82 ±0.57	0.89 ±0.56^{a}	11.2	6.94 ±0.81	8.95 ±0.84	2.01 ±0.59^{a}	28.9	7.75 ±0.90	8.00 ±0.71	0.25 ±0.96^{a}	3.2	1.2461^{NS}
TCW	LF	9.63 ±1.34	10.08 ±1.23	0.45 ±0.67^{a}	4.67	10.38 ±1.09	9.73 ±1.21	-0.65 ±0.80^{a}	-6.26	6.86 ±1.17	8.60 ±0.79	1.74 ±1.16^{a}	25.3	0.538^{NS}
	RF	9.04 ±0.99	8.79 ±1.64	-0.25 ±0.90^{a}	-2.76	10.00 ±1.54	8.57 ±1.56	-1.43 ±0.84^{a}	-14.3	8.62 ±0.65	9.25 ±1.58	-0.63 ±1.6^{a}	-7.3	0.164^{NS}
TEW	LF	21.75 ±0.74	20.04 ±1.21	-1.71 ±1.08^{a}	-7.9	20.74 ±0.75	20.56 ±0.81	-0.17 ± .35^{a}	-0.82	21.51 ±0.72	20.62 ±0.57	-0.89 ± 0.21^{a}	-4.1	0.873^{NS}
	RF	21.96 ±0.73	21.36 ±0.69	-0.603 ±0.12^{a}	-2.7	21.73 ±0.54	21.64 ±0.47	-0.08 ± 0.34^{a}	-0.37	23.1 ± 1.17	21.87 ±0.81	-1.25 ± 0.59^{a}	-5.4	2.049^{NS}
TWT/	LF	0.95 ±0.14	1.10 ±0.15	0.15 ±0.03^{a}	15.7	0.86 ±0.11	1.27 ±0.13	0.41 ±0.09^{a}	47.7	0.91 ± 0.12	1.17 ± 0.22	0.27 ± 0.19^{a}	29.7	1.352^{NS}
TCW	RF	1.05 ±0.23	1.32 ±0.23	0.27 ±0.11^{a}	25.7	0.68 ±0.14	1.28 ±0.27	0.6 ± 0.20^{a}	88.2	0.88 ± 0.20	1.12 ± 0.22	0.23 ± 0.29^{a}	26.1	0.815^{NS}

** (P<0.01) – Statistically highly significant, * (P<0.05) – statistically significant, NS (P>0.05) - Statistically non significant, Mean bearing same superscript are not differed significantly; [Low yielders (LF and RF n= 11, and 10 respectively); Medium yielders (LF, and RF n= 7 and 6 respectively); High yielders (LF, and RF n= 8 and 8 respectively)]; BM-Before milking, AM-After milking, D-Difference)

Table: ib. Mean±se values of rear teat ultrasonographic variables before and after milking

		Low yielders				Medium yielders				High yielders				
		Before Milking (mm)	After Milking (mm)	Difference (mm)	Difference %	Before Milking (mm)	After Milking (mm)	Difference (mm)	Difference %	Before Milking (mm)	After Milking (mm)	Difference (mm)	Difference %	F- value
TCL	LH	11.06 ±0.85	11.56 ±0.86	0.49 ±0.09^{a}	4.43	11.0 ±0.64	11.70 ±0.67	0.67 ±0.27^{a}	6.09	13.00 ±0.45	15.19 ±0.82	2.18 ±0.55^{b}	16.8	7.63**
	RH	10.71 ±0.61	11.24 ±0.64	0.52 ±0.34^{a}	4.9	11.38 ±0.61	11.85 ±0.78	0.48 ±0.34^{a}	4.2	12.81 ±0.89	12.97 ±0.51	0.70 ±0.36^{a}	5.46	0.221 ^{NS}
TWT	LH	8.50 ±0.62	9.20 ±0.72	0.69 ±0.39^{a}	8.1	7.23 ±0.62	10.59 ±1.41	3.36 ±1.53^{b}	46.5	8.45 ±1.48	9.14 ±1.51	0.69 ±0.56^{a}	8.2	3.026 ^{NS}
	RH	8.39 ±0.55	9.10 ±0.42	0.70 ±0.36^{a}	8.3	7.06 ±0.58	8.82 ±0.75	1.92 ±0.23 ^{b}	27.2	7.34 ±0.59	8.41 ±0.39	1.08 ±0.300^{ab}	14.7	3.42*
TCW	LH	10.59 ±1.69	8.27 ±1.14	-2.32 ±0.63^{a}	-21.9	12.45 ±1.41	7.52 ±0.81	-4.93 ±0.45^{a}	-39.5	11.92 ±1.63	6.21 ±0.99	-5.71 ±0.799^{a}	-47.9	2.27 ^{NS}
	RH	7.95 ±0.99	7.30 ±0.77	-0.65 ±0.82^{a}	-8.17	9.45 ±1.17	9.87 ±1.89	-0.42 ±0.17^{a}	-4.44	9.37 ±1.51	6.63 ±0.96	-2.74 ±0.57^{a}	-29.2	0.088 ^{NS}
TEW	LH	21.35 ±0.70	20.90 ±0.74	-0.45 ±0.09^{a}	-2.1	21.50 ±1.45	20.5 ± 0.96	-1.44 ± 1.1^{a}	-6.69	21.12 ±0.80	21.00 ±0.71	-0.13 ± 0.28^{a}	-1.46	1.367 ^{NS}
	RH	21.47 ±0.55	20.19 ±1.85	-0.18 ±0.13^{ab}	-0.84	20.37 ±1.02	19.59 ±0.97	-0.82 ± 0.21^{a}	-4.02	20.57 ±0.80	20.48 ±0.82	-0.087 ±0.33^{a}	-0.42	2.809 ^{NS}
TWT/ TCW	LH	1.07 ±0.14	1.28 ±0.17	0.21 ±0.07^{a}	19.6	0.79 ±0.13	1.51 ±0.22	0.72 ± .18^{b}	91.1	1.19 ± 0.26	1.69 ± 0.31	0.49 ± .15^{ab}	41.2	4.103*
	RH	1.10 ±0.15	1.39 ±0.16	0.27 ±0.14^{a}	24.5	0.71 ±0.11	1.10 ±0.19	0.43 ± 0.09^{a}	60.6	0.90 ± 0.10	1.49 ± 0.26	0.59 ± 0.22^{a}	65.5	1.1048 ^{NS}

* (P<0.01) – Statistically highly significant, * (P<0.05) – statistically significant, NS (P>0.05) - Statistically non significant, Mean bearing same superscript are not differed significantly; [Low yielders (LH and RH n= 10 and 11 respectively); Medium yielders (LH and RH n= 7 and 7 respectively); High yielders (LH and RH n= 8 and 7 respectively)]

Fig. 1. Ultrasonography of teat: Water bath technique and Direct gel contact technique (7.5 to 12.5 MHZ linear probe)

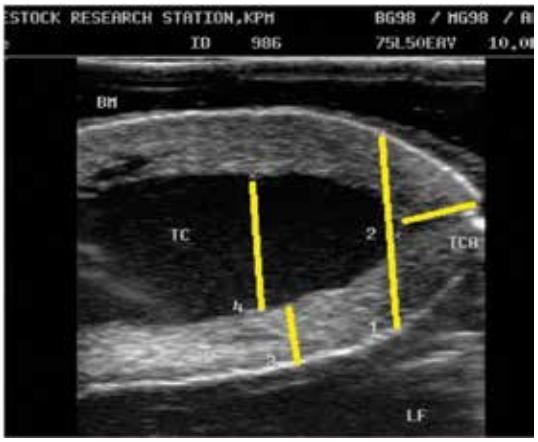

Fig.2a. Ultrasonographic measurement of normal teat before milking 1. TEW-24.2 mm, 2.TCL-8.59 mm, 3. TWT-7.13 mm, 4. TCW- 16.4 mm

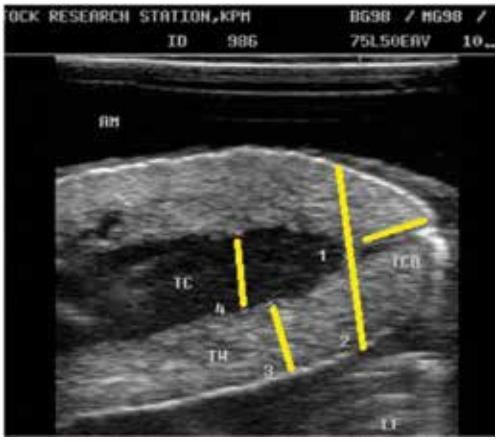

Fig. 2b. Ultrasonographic measurement of normal teat after milking 1.TCL-9.74 mm, 2.TEW-23.4 mm, 3.TWT-9.12 mm, 4.TCW-9.12 mm.

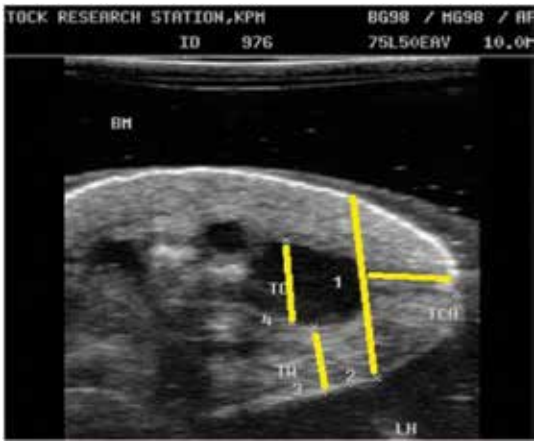

Fig.3a. Ultrasonographic measurement of Milk flow disorder affected teat before milking 1. TCL-9.76 mm 2. TEW-22 mm, 3. TWT-8.06 mm, 4. TCW- 10 mm

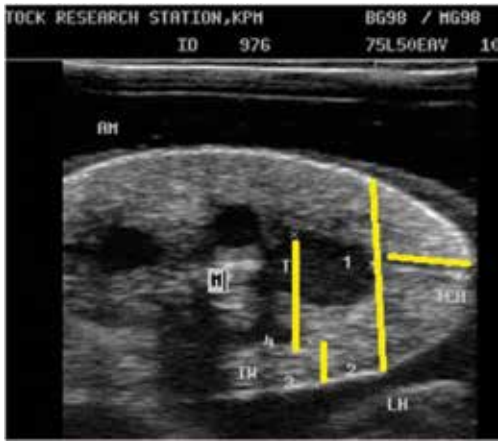

Fig.3b. Ultrasonographic measurement of Milk flow disorder affected teat after milking 1.TCL-10.1 mm, 2.TEW-22.2 mm, 3.TWT-4.93 mm, 4.TCW-12.3 mm.

Table: IIa. Mean±se values of fore teat ultrasonographic variables of cows during before and after milking in mfd affected and unaffected

		MFD affected					MFD unaffected				
		Before Milking	After Milking	Difference (mm)	Difference %		Before Milking	After Milking	Difference (mm)	Difference %	T
TCL	LF (n=6)	12.95 ±0.84	13.03 ± 0.62	0.09 ± 0.58	0.7	LF (n=26)	10.41 ± 0.53	12.11 ± 0.56	1.7 ± 0.69	16.3	1.204^{NS}
	RF (n=8)	9.72 ±0.42	10.36 ±0.38	0.67 ±0.15	6.9	RF(n=24)	12.44 ±0.63	12.81 ±0.65	0.38 ±0.35	3.05	0.47^{NS}
TWT	LF (n=6)	7.73 ± 0.82	8.96 ± 1.108	1.23 ± 1.02	15.9	LF (n=26)	7.37 ± 0.25	8.30 ± 0.33	0.93 ± 0.36	12.6	0.34^{NS}
	RF (n=8)	7.48 ±0.86	8.95 ±0.83	1.1 ±0.32	14.7	RF (n=24)	7.74 ±0.42	8.69 ±0.39	0.95 ±0.43	12.3	0.19^{NS}
TCW	LF (n=6)	10.96 ± 1.62	10.89 ± 1.58	-0.06 ± 0.24	-5.5	LF (n=26)	10.24 ± 0.83	7.90 ± 0.67	-2.33± 0.89	-22.8	1.21^{NS}
	RF (n=8)	12.33 ±1.43	10.35 ±0.88	-2.49 ±1.06	-20.2	RF (n=24)	10.84 ±0.97	8.10 ±0.93	-2.74 ±0.98	-25.3	0.14^{NS}
TEW	LF (n=6)	22.35 ± 0.95	21.86 ± 1.00	-0.48 ± 0.18	-2.1	LF (n=26)	21.62 ± 0.43	19.82 ± 0.91	-1.8 ± 0.9	-8.3	0.69^{NS}
	RF(n=8)	22.26 ±0.91	22.18 ±0.86	-0.29 ±0.09	-1.3	RF (n=24)	22.37 ±0.50	21.71 ±0.39	-0.67 ±0.23	-2.9	0.92^{NS}
TWT/ TCW	LF (n=6)	0.83 ± 0.19	0.94 ±0.19	0.11 ± 0.1	13.3	LF (n=26)	0.84 ± 0.07	1.42 ± 0.28	0.58 ± 0.29	69.0	0.75^{NS}
	RF (n=8)	0.68 ±0.12	0.93 ±0.13	0.26 ±0.08	38.2	RF (n=24)	0.92 ±0.12	1.58 ±0.27	0.67 ±0.3	72.8	0.78^{NS}

NS (P>0.05) - Statistically non significant

Table: IIb. Mean±se values of rear teat ultrasonographic variables of cows during before and after milking in mfd affected and unaffected

		MFD affected					MFD unaffected				
		Before Milking	After Milking	Difference (mm)	Difference %		Before Milking	After Milking	Difference (mm)	Difference %	T
TCL	LH (n=8)	12.46 ±1.4	12.83 ± 1.28	0.37 ± 0.26	2.96	LH (n=25)	11.50 ± 0.44	12.57 ± 0.56	1.07 ± 0.25	0.093	1.41^{NS}
	RH (n=6)	10.42 ±0.46	10.81 ±0.54	0.39 ±0.2	3.7	RH (n=25)	11.86 ±0.42	11.90 ±0.54	0.04 ±0.39	0.33	0.58^{NS}
TWT	LH (n=8)	7.36 ± 0.73	8.24 ± 0.88	0.87 ± 1.11	11.8	LH (n=25)	8.10 ± 0.57	9.32 ± 0.67	1.22 ± 0.51	15.1	0.31^{NS}
	RH (n=6)	8.73 ±0.65	9.76 ±0.64	1.03 ±0.36	11.7	RH (n=25)	7.26 ±0.37	8.48 ±0.28	1.22 ±0.23	16.8	0.4^{NS}
TCW	LH (n=8)	10.48 ± 1.72	10.16 ± 1.92	-0.33 ± 1.89	-3.1	LH (n=25)	9.34 ± 0.70	7.14 ± 0.56	-2.2 ± 0.51	-23.6	1.37^{NS}
	RH (n=6)	9.99 ±1.18	8.65 ±1.23	-1.34±0.36	-13.4	RH (n=25)	9.81 ±0.77	7.78 ±0.76	-2.03 ±0.48	-20.6	0.74^{NS}
TEW	LH (n=8)	21.94 ± 0.56	20.93 ± 0.62	-1.01 ± 0.34	-4.6	LH (n=25)	21.12 ± 0.53	20.44 ± 0.45	-0.68± 0.33	-3.21	0.51^{NS}
	RH (n=6)	21.27 ±0.69	20.96 ±0.65	-0.31±0.14	-1.5	RH (n=25)	20.61 ±0.50	19.46 ±0.74	-0.42 ±0.19	-2.0	0.28^{NS}
TWT/ TCW	LH (n=8)	0.93 ± 0.25	1.35 ± 0.55	0.42 ± 0.53	45.2	LH (n=25)	1.03 ± 0.11	1.47 ± 0.13	0.45 ± 0.09	43.6	0.1^{NS}
	RH (n=6)	0.95 ±0.14	1.25 ±0.16	0.3 ±0.08	31.6	RH (n=25)	0.85 ±0.076	1.33 ±0.13	0.48 ±0.12	56.5	0.81^{NS}

NS (P>0.05) - Statistically non significant

in MFD affected teats than MFD unaffected teats after milking. It was found after meticulous and extensive screening of literatures that there were no published reports available in this aspect. Hence, assumptions are made that the difference could be due to the differences in hand milking techniques adopted by the milkers (stripping or by full hand method).

The teat cistern width in all the teats were found to be decreased in both MFD affected and unaffected cows after milking. This study also revealed that after milking values in MFD unaffected teats were found to decrease than the values for corresponding teats in MFD affected group. Decreases in the teat cistern width after milking were reported by Neijenhuis *et al.,* (2001). Studies on milk flow disorders revealed that milk is being retained in the teat cistern signifying that the teat cistern width may not decrease when compared to MFD unaffected teats as observed by Querengässer *et al.,* (2002). In this study the ultrasound parameters were also suggestive of milk retention in MFD affected teat. The teat end width was found to be decreased after milking in the entire teat in both MFD affected and unaffected teat. Neijenhuis *et al.,* (2001) reported of increase in teal wall thickness after machine milking in healthy teats. Teat end width (TEW) decrease was more in MFD affected teats than unaffected teats except for left hind teat. The differences recorded might be due to the fact that measurements are being carried out at the most pliable part of the teat.

From the current study, Ultrasonography of the teat was found to have better diagnostic yield in the detection of MFD (64.28% (18/28)) when compared to clinical detection (35.75 % (10/28)). Teat ultrasonography has the advantage for localization of the lesions and in identifying the type of lesions. Ultrasonography of the teat can be included as one of the parameter in selection of good quality dairy cattle as well as probable high yielder cows. No published reports are available on ultrasonography of teat with regard to milk flow disorders especially in hand milked cows. Hence the current ultrasonographic teat parameters gains significance; however further study in a larger sample sizes for different Indian breeds, for utilization of ultrasound for high yielding dairy cattle selection in India.

Conclusions

The values of ultrasonographic variables for teat parameters were found to increase after milking compared to those before milking; but they were statistically non significant. The increase in the measurements indicates that the time taken for recovery of the teat to normalcy was more, thereby exposing the teat for possible infections. Further study on various Indian breeds will help in establishing a robust mastitis and milk flow disorder control programmes. Ultrasonography of the teat needs to be included in diagnosis and management of MFDs, as there is a higher detection rate with ultrasound with clinical examination alone.

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Cross antigenicity of gut derived antigen of *Oesophagostomum columbianum* with other helminthes by western blotting

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Abstract

Cross antigenicity studies to identify non cross reactive antigen in Gut Derived Antigen (GDAg) of *Oesophagostomum columbianum* were conducted with hetrologous hyperimmune sera (HIS) raised against different helminth as well as experimental sera of sheep and goat against different helminth found as concurrent infection in sheep and goat by western blotting. Immunoaffinity purified gut derived antigenof *O columbianum* with HIS against *Haemonchus contortus* reacted to 130 and 68kDa polypeptides whereas experimental sera of sheep infected with *H. contortus* reacted to 130kDa polypeptides. HIS against *Paramphistom epiclitum* reacted to130, 100, 68 and 50kDa polypeptides of GDAg whereas experimental sera raised in goat against *P. epiclitum* reacted to 130 and 68kDa. HIS against *Fasciola gigantica* reacted to 170,130,56, and 48 kDa polypeptides of GDAg whereas experimental sera of sheep infected with *F.gigantica* reacted to 130,100,68 and 50 kDa polypeptides of GDAg. Non cross antigenic polypeptides were 11,17,30.32,35,38,150. The study indicated GDAg of *O. columbiamun* as a good source of non cross antigenic polypeptides as evidenced by western blotting which could be exploited for immunodiagnosis of *O. columbianum* infection.

Keywords: Cross antigenicity, *Oesophagostomum columbianum*, gut derived antigen

Oesophagostomum columbianum, is one of the major gastrointestinal parasite of small ruminants commonly called nodule worm inhabiting large intestine of sheep and goat. It causes nodular enteritis or pimply gut leading to considerable morbidity and huge economic losses to meat processing industry due to condemnation of sheep intestine throughout the Asian subcontinent Mohanta *et al.,(* 2007). The pathogenic effect is mainly caused by the migratory prepatent stages of the parasite. Most serious problem seen in *Oesophagostomum* infections arise from larvae penetrating the mucosa of the intestine. Recent trends in the parasite control emphasize the need for integrated management programmes to reduce or overcome the problems of drug resistance, chemical residues and high costs of treatment. For effective management of pimply gut infection an early diagnosis during prepatency is required as the prepatent period is longer and treatment is delayed due to lack of any suitable diagnostic test available in hand at present. Since differentiation of *O. columbianum* infection by presence of eggs from other G.I nematodes is difficult by faecal sample examination, a suitable immunodiagnostic test may be of great value for the clinicians. It can only be achieved with a purified antigen avoiding cross antigenicity because it is prevalent as mixed infection with other helminths (Philipp & Rumjaneck 1984; Cuquerella *et al*.1994; Molina *et al.* 1999). Gut of nematode has been a very good source of polypeptides which are present in excretory- secretary antigen also. Therefore, present study was undertaken to identify some polypeptides in the gut derived antigen to be exploited further for immunodiagnosis.

Materials and Methods

Collection of parasites: Adult *O. columbianum* were collected from the intestines of infected sheep and goat, procured from the local abattoir at Bareilly, Uttar Pradesh. The parasites were washed several times in Phosphate Buffer Saline (PBS) having pH 7.2 making these free from debris and then kept in PBS.

Identification of *O. columbianum*: *O. columbianum* was identified as per characters given by Yamaguti, (1961) and Levine (1980)

Preparation of Gut Derived Antigen (GDAg): The antigen was prepared as per technique described by Munn *et al.* (1997) for *Haemonchus contortus.* Adult *O.columbianum* were collected from intestines of sheep and goat. Collected worms were washed in PBS and kept at 80 ºC. Frozen worms were homogenized in solution containing 1% Tween-20 and thesit. After complete homogenization, the homogenate was centrifuged at 14000 rpm for 45 minutes. Supernatant was mixed with polyethylene glycol 20,000 solution in a required amount. After overnight incubation the

extract was recentrifuged at 20,000 rpm for 30 minutes and the supernatant collected was designated as gut derived antigen.

Protein estimation: Protein concentration in the GDAg was estimated as per Lowry's method (Lowry,1951).

Characterization of Antigen by SDS-PAGE: SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed to observe the polypeptide profile in the GDAg of *O.columbianum*. Molecular weights of the polypeptides were determined with help of standard protein marker (11-170 KDa), as per the method described by Laemmli (1970).

Raising of hyperimmune sera: Two New Zealand white rabbits were used for hyperimmunization. Hyperimmune sera was raised against GDAg of *O. columbianum* in two rabbits as per the procedure of Hudsan and Hay (1989).One rabbit was kept as a control. Before immunization the experiment, blood samples were collected from all the rabbits intra cardially to obtain preimmunized normal rabbit sera and stored at -20°C. For raising hyperimmune sera, stable water in oil emulsion of equal volume of antigen Freuend's complete and Freuend's incomplete adjuvants (FCA & FIA) were used as inoculums. Dose and route of antigen, adjuvant used and the schedule of hyperimmunization Jas *et al.* (2010)

-Western Blotting: Western blotting was performed using i-blot(Invitrogen). The SDS- PAGE gel was removed carefully from the glass plates and kept in triple distilled deionized water for 10 minutes. The i-blot instrument was assembled and operated as per the manufracturer's instructions and the proteins from the gel were transferred to the NC membrane. This membrane was kept in blocking buffer for 2 h at 37^{o}c and washed with PBS-T three times at five min interval each and was subjected to hyperimmune serum. This was incubated at 37^{o}c for 2 h and then washed again three times with washing solution. Secondary antibody (Goat anti- Rabbit IgG HRP conjugate 1:10000) was added and incubated at room temperature for 1h. Final washing was done as previously. DAB substrate was added to develop bands of reacting polypeptides.

Immunoaffinity purification of Gut derived antigen: GDAg was purified using amino link matrix. The column containing matrix was equilibrated at room temperature and regenerated by washing with coupling buffer. The column was further loaded with purified immunoglobulin (5mg/ml). 20mg purified immunoglobulin was loaded in the column and 200µl of amino link reductant solution was also added. It was incubated overnight at 4^{0}C with end to end shaking. Next day the column was washed to remove excess uncoupled antibody. After complete washing the antigen (5mg/ml) was loaded to the column and incubated at room temperature with end to end shaking overnight at 4^{0}C. Antigen was removed through washing buffer and finally the bound polypeptides were eluted through elution buffer (pH 2.5) and fractions were collected. After recording the OD values of fractions, the fractions of interest were pooled and concentrated. The OD values of pooled and concentrated fraction were estimated.

SDS-PAGE of purified fractions: SDS-PAGE of immunoaffinity purified fractions of GDAg was performed in the similar manner as described before.

Cross Antigenicity studies: Western blot was performed utilizing eluted purified fractions of GDAg with hetrologous HIS as well as experimental sera (sheep and goat) of other GI helminths *viz H. contortus, Paramphistomum epiclitum* and *Fasciola gigantica* Polypeptides showing reactivity to heterologous sera were identified for further identification of non crossreactive polypeptide in *O. columbianum* antigen.

Results and Discussion

Collection of parasites and identification of parasite: From 20 intestines 544 worms were collected. Microscopic examination of the worms showed cuticle forming mouth collar in form of truncated cone. Cervical grooves extending around ventral surface of the body and cuticle anterior to this groove inflated to form cephalic vesicle. Lateral alae originated immediately behind cervical groove, extending almost whole length of the body and cervical papillae pierced the anterior extremities of the lateral alae. The buccal capsule was shallow, external corona radiata consisting of 20-24 elements, internal radiata having two small elements to each that of external leaf crowns. Oesophagus was club shaped with oesophago- intestinal valve. Male bursa was well developed with equal size spicules which do not extend from bursal lobe. Vulva of female was slightly prominent, opening little anterior to anus. Vagina was very short, transverse, leading into kidney shaped ovijectors or pars ejectrix. The morphological features confirmed to *O.columbianum*.

Antigen preparation and characterization by SDS-PAGE: GDAg of the adult worms prepared by standard protocol had the protein concentration 4.22 mg/ml. Protein profile of GDAg of adult *O. columbianum* determined by12% SDS –PAGE which revealed prominent protein bands of 11, 20, 29, 30 ,40, 68,100 ,130 KDa.

Identification of immunodominant polypeptides GDAg by Western-blotting: Protein bands fractionated by SDS-PAGE were subjected to western blotting using hyperimmune sera raised against GDAg. Polypeptide bands of 11, 17, 26, 30, 35, 53, 68, 100, 110,130, and one above 130 kDa showed strong reactivity. These polypeptides were designated as immunodominant polypeptides.

SDS-PAGE of immunoaffinity purified GDAg: Protein profile of purified fractions of GDAg antigen was determined by 12% SDS-PAGE. Clear Intense bands of 17, 30, 32, 35, 68, 72, 100& 130 KDa in purified GDAg and several faint bands were observed (Figure 1).

Western blot with immunoaffinity purified GDAg of *O.columbianum* with homologous hyperimmune sera: Immuno-blot of purified gut derived antigen of *O.columbianum* and anti-GD Ag antibody (HIS) reacted with polypeptides of 11, 17, 30, 32, 35, 50, 100, 130 and 150 kDa (Figure 2)

Cross–antigenicity studies by western blotting: Western blotting was performed with Immunoaffinity purified GDAg and heterologous sera(hyperimmune and experimental) infected with *H. contortus, P. epiclitum* and *F.gigantica.* In western blotting purified GDAg showed reactivity of 130 and 68 KDa polypeptide with HIS against *H.contortus* reacted to130 KDa polypeptide only (Figure 3). Western blotting with HIS against *P. epiclitum* reacted to polypeptides 130, 100,68, and 50 KDa (Figure 4) whereas experimental sera of goat infected with *P. epiclitum* reacted only to 130 and 68 KDa polypeptides (Figure 5). HIS against *F. gigantica* reacted to 170. 130, 50 and 48 KDa of purified gut derived antigen of *O. columbianum* ((Figure 6)) whereas experimental sera of sheep infected with *F.gigantica* reacted to 130, 100, 68 and 50 KDa (Figure 7).

Keeping in view antigenicity of gut derived antigen in *H.contortus,* present study was undertaken to identify immunodiagnostic polypeptides in *O. columbianum* utilizing cross antigenicity studies by western blotting. Sharing of polypeptides among GI nematodes has already been described by Siefker and Rickard (1998) who recorded sharing of carbohydrate epitopes in intestinal protein of bovine gastrointestinal nematodes. In western blotting these workers recognized sharing of high molecular weight protein bands ranging between 111-298 KDa of *H.placei* of cattle with several nematodes such as *O.ostertegi*, *Cooperia punctata, H.contortus,and O.radiatum.* These polypeptides were conserved in these species. In *O. radiatum* the polypeptides were semi conserved. They also confirmed their finding with immunohistochemical studies verifying the intestinal location of the epitopes of the antigens. During present study cross antigenicity of *O.columbianum* with *F.gigantica* and *P. epiclitum* has been done through western blotting for the first time.Cross antigenicity of somatic antigen of *H. contortus* with *O.columbianum* has been reported by Prasad (2007) and also with *F.gigantica* but not with *P.epiclitum.* During the present study it was found that cross antigenicity of purified fraction of *O.columbianum* with *H.contortus*, *P.epiclitum* and *F.gigantica* were mainly among high molecular weight polypeptides. Further work is needed to identify most promising polypeptide which may be exploited singly or in combination to develop a field oriented immunodiagnostic test. Further the polypeptides in combination may be exploited for immuno prophylaxis. It was found that 130, 72 and 68 KDa polypeptides which are very prominent polypeptides in SDS-PAGE of immunoaffinity purified GD Ag are highly cross reactive to *H. contortus, P. epiclitum* and *F.gigantica* which are commonly found in sheep and goat. Only experimental sheep sera infected with *F. gigantica* did not react to 68 KDa polypeptide of *O. columbianum.* It was therefore inferred that polypeptides with 100and 72 KDa among high molecular weight polypeptides and 17-35 KDa among low molecular weight polypeptide may be exploited for immunodiagnosis. Low molecular weight polypeptides which are less prominent in SDS-PAGE more useful for immunodiagnosis.

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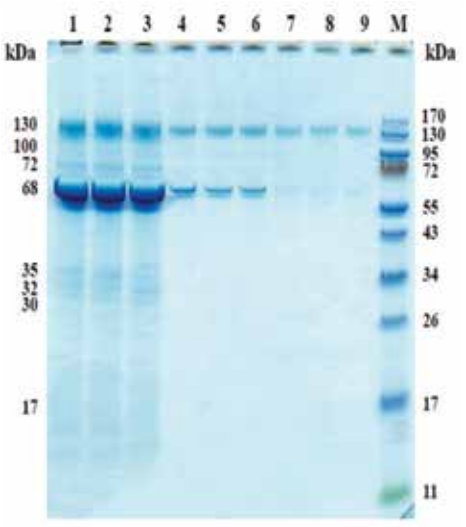

Fig. 1: SDS- PAGE of immunonaffinity purified gut derived antigen of *O. columbianum.*

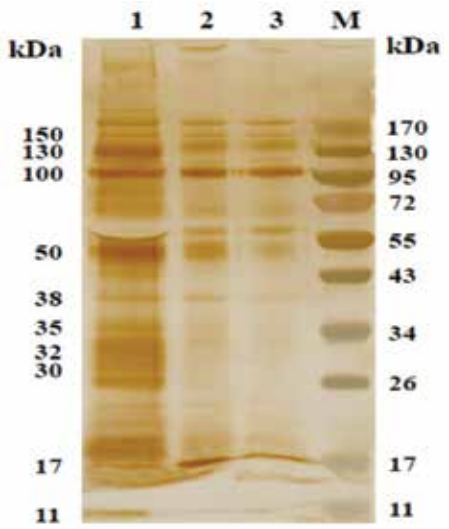

Fig. 2: Western blotting of immunonaffinity purified GDAg of *O. columbianum* with homologous HIS (anti-GDAg antibody)

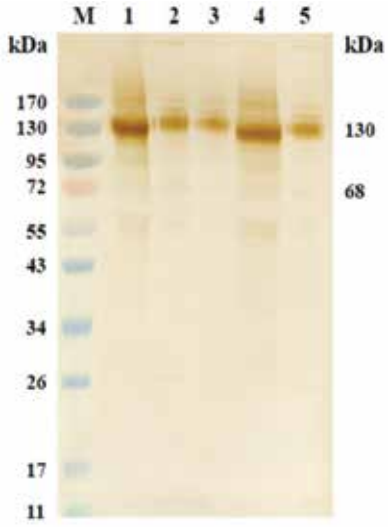

Fig. 3: Cross-antigenicity of immunoaffinity purified GDAg of *O. columbianum* with HIS against *H. contortus* in western blotting

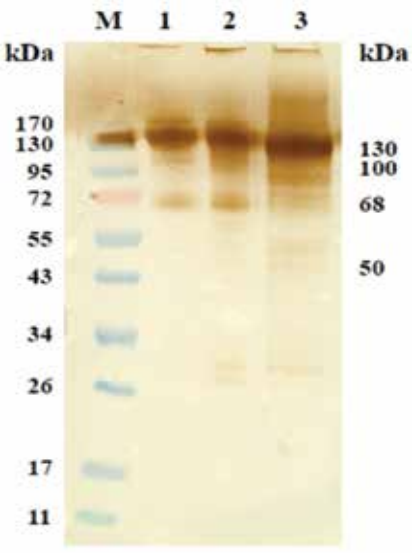

Fig. 4: Cross-antigenicity of immunoaffinity purified GDAg of *O.columbianum* with anti- *P. epiclitum* antibody (HIS) in western blotting

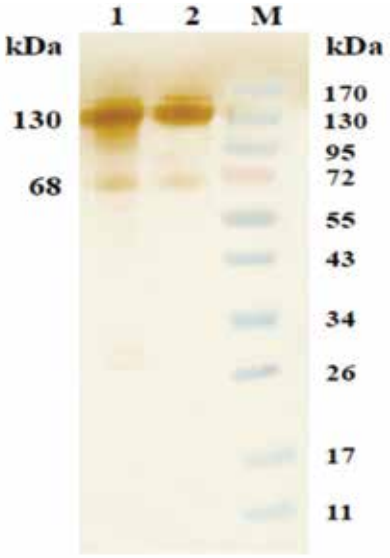

Fig. 5: Cross-antigenicity with immunoaffinity purified GDAg of *O.columbianum* with experimental serum of goat infected with *P. epiclitum* in western blotting

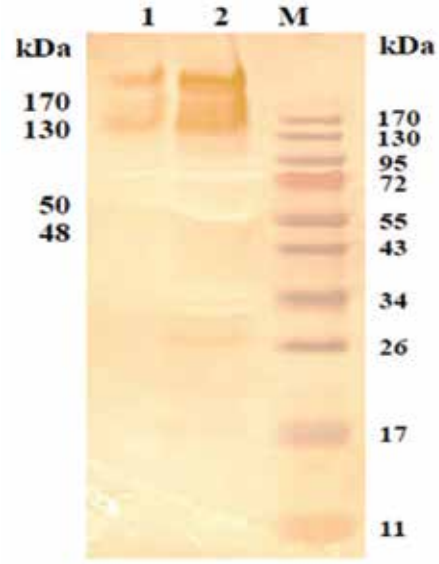

Fig. 6: Cross-antigenicity of immunoaffinity purified GDAg of *O.columbianum* with anti- *Fasciola* antibody (HIS) in western blotting

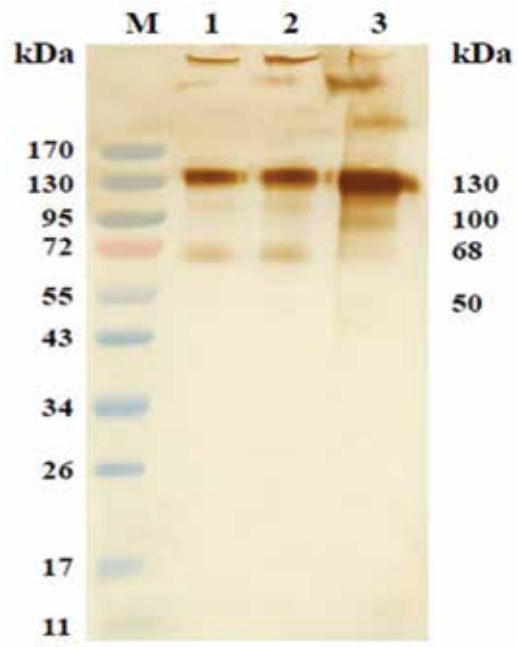

Fig. 7: Cross-antigenicity with immunoaffinity purified GDAg of *O.columbianum* with experimental serum of sheep infected with *Fasciola gigantica* in western blotting

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Clinicopathologial changes in hepatobiliary disorders in dogs

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Abstract

Liver disease is a frequent presentation to small animal practitioner posing a diagnostic challenge. To meet this challenge, the study was undertaken to identify the various clinicopathological changes associated with hepatobiliary disorders in dogs presented to the Small Animal Medicine Referral Clinic of Madras Veterinary College over a period of two and half years (2010-2012). Cases presented with signs such as anorexia, lethargy, ascites, icterus, pigmented urine and vomiting were chosen and screened for liver disorders. Out of these 23,289 dogs with gastrointestinal disorders, 100 dogs were found to have liver diseases of different kinds. Liver Disease Group was further subdivided into three groups Parenchymal disorders, Biliary tract disorders and Neoplastic disorders. Findings of Clinical Examination, Clinico-pathological Studies, Coagulation Analysis, were taken for diagnostic assessments. Histopathology of liver tissue was undertaken using Haematoxylin and Eosin stain. 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 while dogs with neoplastic liver disorders showed clinical signs such as weakness, anaemic signs like tachycardia /tachypnea. Significant anemia was evident in all three kinds of liver disorders viz. parenchymal, biliary and neoplastic disorders indicating chronic inflammatory process and inefficient iron utilization. The chronic inflammatory process was evidenced by significant leukocytosis, neutrophilia hypoalbuminemia in all the three groups. Gamma glutamyl transferase was found to be significantly elevated in biliary disorders. Hepatic histopathology is gold standard test for the confirmatory diagnosis and classification of various liver disorders.

Keywords: Liver, Hepatobiliary disorders, Coagulation analysis, Haematobiochemistry

Liver disease is a frequent presentation to small animal practitioner posing a diagnostic challenge. 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. Assessing the small animal patients with suspected primary hepatobiliary disease is rarely a simple process because no single diagnostic test currently available has perfect sensitivity and specificity (Hess and Bunch, 2000).The challenge for the veterinarian is to choose the most appropriate diagnostic test to arrive at the most accurate diagnosis (Webb *et al*, 2002). Towards this goal, the study was undertaken to identify the various clinicopathological changes associated with hepatobiliary disorders in dogs.

Materials and Methods

The clinical study was conducted with the clinical cases presented to the Small Animal Medicine Referral Clinic of Madras Veterinary College over a period of two and half years (2010-2012). Cases presented with signs such as anorexia, lethargy, ascites, icterus, pigmented urine and vomiting were chosen and screened for liver disorders. Chief complaints, age at onset, management practices, medication history and chronology of events were assessed. Findings of Clinical Examination, Clinico-pathological Studies, Coagulation Analysis, were taken for diagnostic assessments. Haematological parameters were analysed using auto haematology analyzer BC-2800 Vet, Mindray. The serum was separated and analysed with automatic blood biochemistry analyzer, A15 random access analyzer, Biosystems, Barcelona. Plasma was used for the estimation of PT and aPTT using Mispa\\Clog which is an opto-mechanical coagulation analyser which applies the turbodensitometric measuring principle (Agappe Diagnostics Ltd). The tissue Specimen fixed as per routine procedure and the section were stained with Haematoxylin and Eosin stain and examined under light microscope. Records of 100 Confirmed Cases were analysed for this study. Grouping was done as follows: Group I: Apparently Health Dogs acting as Control group, Group II: Liver Disease Group, Group

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Table 1. Medical history of canine liver diseases

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

II was further subdivided into three groups as Group II A: Parenchymal disorders, Group II B: Biliary tract disorders and Group II C:Neoplastic disorders.

Results and Discussion

In the present study the incidence of gastro intestinal disorders were observed to be 35 per cent (23,289 dogs) out of 66,450 dogs presented to Small Animal Medicine Referral Clinic of Madras Veterinary College.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

Parenchymal and neoplastic disorders in this study had nonspecific complaints like anorexia, weight loss and vomiting while the biliary disorders had some specific complaints like high coloured urine (100 per cent) and icterus (77.78 per cent) besides nonspecific signs(Table 1). Rutgers and Haywood (1988); Rutgers *et al.*(1993); Shih *et al.,* (2007); Alvarez and Whittemore*et al.*(2009) and Pooja *et al.* (2010) observed that lethargy, inappetance, vomiting,

Table 2. Clinical presentation of canine liver diseases

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

Table 3. Mean ± SE values of erythrogram in canine liver diseases

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

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 historyshall sensitize the clinician and he/she has to maintain a high suspicion for biliary disorders if such medical history is presented.

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).* 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 than the other two groups (IIA and IIC). Lecoindre and Arpaillange (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. Hence these signs could be taken as clinical indicators for the presence of biliary disorders.

Anorexia and weight loss(75 %); polydipsia/ polyuria (50 %) whilst others had anaemia and hypovolemic shock, secondary to tumour rupture; palpable massin the cranial abdomen (30 %) and abdominal bloating while jaundice (18 %) were recorded in cases with liver tumors(Thamm, 2001). In the present study the predominant signs observed included anaemia and weakness (66.6 % each), anorexia (55.56 %), palpable distension of liver and poor haircoat (44.44 % each), weight loss and ascites (33.33 % each). These observed signs were in accordance with the

Table 4. Mean ± SE values of leucogram in canine liver diseases

Sl. No.	Group	Total WBC count (cells / mm^3)	Neutrophils (cells / mm^3)	Lymphocytes (cells / mm^3)	Monocytes (cells / mm^3)	Eosinophils (cells / mm^3)
1	Control (n=6)	8683.33± 470.76 [a]	6,181.50± 347.80 [a]	1897.33± 132.68	442.17± 12.51	162.33± 56.16[a]
2	Parenchymal Disorders (n=73)	18,220.55± 895.36 [b]	14,610.89± 779.03 [b]	2733.01± 171.77	528.11± 52.91	325.16± 27.70 [ab]
3	Biliary Disorders (n=18)	21,094.44± 1,660.84 [b]	17,218.67± 1,480.80 [b]	2813.83± 222.31	510.50± 62.26	356.44± 47.02 [ab]
4	Neoplastic Disorders (n=9)	15,088.89± 2,079.29 [b]	11,974.22± 1,681.97 [b]	2204.22± 339.84	486.67± 101.11	394.00± 89.21 [b]
5	F Value	4.883	5.050	1.172	0.106	1.385

Table 5. Mean ± SE values of coagulation parameters in canine liver diseases

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

findings of Thamm (2001). Interestingly weakness and anaemia was observed as predominant signs (66.67 per cent), only in dogs with neoplastic disorders than the other two gropus (IIA and IIB)

Significant reductions were observed in Haemoglobin,PCVand RBC count in all the three groups while there were no significant changes in erythrocytic indices (Table 3).Dogs with neoplastic disorders 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 other groups (IIA and IIB). 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. Lecoindre and Arpaillange (2010); Pastor and Bachs (2010) and Brovida and Rothuizen (2010) also observed that anaemia in hepatic disease was associated with chronic inflammatory reactions and related defective iron utilization. Significantly elevated leucocyte count and neutrophil count observed in this study (Table 4) indicated the ongoing inflammatory process in the parenchymal and biliary disorders of liver while in neoplastic disorders could be the result of inflammation and necrosis associated with large tumours as observed by Pastor and Bachs (2010). This inflammatory process could have caused the anemia as observed in previous studies.

Elevations in the values (Table 6a&b) of liver enzymes such as ALT, AST, ALP, GGT was observed in parenchymal and neoplastic 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 ; 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).

The biochemical changes observed in biliary disorders (Table 6a&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). Besides these changes, nonsignificant elevations were also observed in liver enzymes such as ALT, ASTas well as metabolites like BUN, Creatinine and Cholesterol. In these dogs the liver enzyme elevations revealed a

Table 6 a. Mean ± SE values of serum biochemical parameters in canine liver diseases

Sl. No.	Group	ALT (IU/L)	AST (IU/L)	SAP (IU/L)	GGT (IU/L)	BUN (mg / dl)	Creatinine (mg / dl)
1	Control (n=6)	64.57±7.18	45.15±5.01	87.78±14.73	3.73±0.2[a]	20.00±3.86	0.89±0.10
2	Parenchymal Disorders (n=73)	180.77±27.67	121.78±22.91	365.58±47.34	4.28±0.2[a]	29.05±2.83	2.37±0.52
3	Biliary Disorders (n=18)	78.53±6.34	41.06±3.05	237.03± 39.43	15.07±2.01 [b]	25.14±3.17	1.36±0.58
4	Neoplastic Disorders (n=9)	134.40±85.17	31.52±7.36	125.67±29.21	4.98±0.40 [a]	20.13±2.49	0.79±0.15
5	F Value	1.537	1.914	2.546	36.825	0.802	0.850

Table 6 b. Mean ± SE values of serum biochemical parameters in canine liver diseases

Sl.No.	Group	Total Protein (g / dl)	Albumin (g / dl)	Globulin (g / dl)	Total Bilirubin (mg / dl)	Direct Bilirubin (mg / dl)	Glucose (mg / dl)	Cholesterol (mg / dl)
1	Control (n=6)	8.13± 0.21 [a]	3.65± 0.15 [a]	4.48± 0.15	0.44± 0.026 [a]	0.12± 0.01 [a]	97.50± 2.29	105.65± 4.48 [a]
2	Parenchymal Disorders (n=73)	6.57± 0.20 [b]	2.02± 0.10 [b]	4.55± 1.70	1.20± 0.11 [ab]	1.0949± 0.24 [a]	88.93± 2.60	127.90± 5.50 [ab]
3	Biliary Disorders (n=18)	6.42± 0.32 [b]	2.52± 0.228 [b]	3.90± 0.22	1.55± 0.25 [b]	0.5056± 0.73 [b]	87.06± 3.93	169.57± 21.12 [b]
4	Neoplastic Disorders (n=9)	6.59± 0.22 [b]	2.42± 0.15 [b]	4.17± 0.18	0.66± 0.17 [a]	0.4100± 0.24 [a]	95.22± 2.99	151.01± 23.48 [ab]
5	F Value	2.003	8.378	1.011	3.099	2.954	0.669	3.300

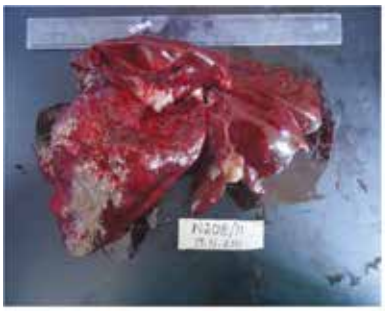

Plate 34. Hepatic cirrhosis

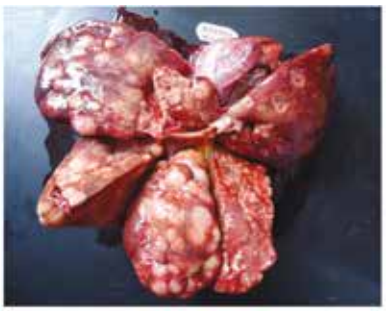

Plate 35. Multiple irregularly formed tumors, often showing central umbilication

Plate 36. Macronodular liver

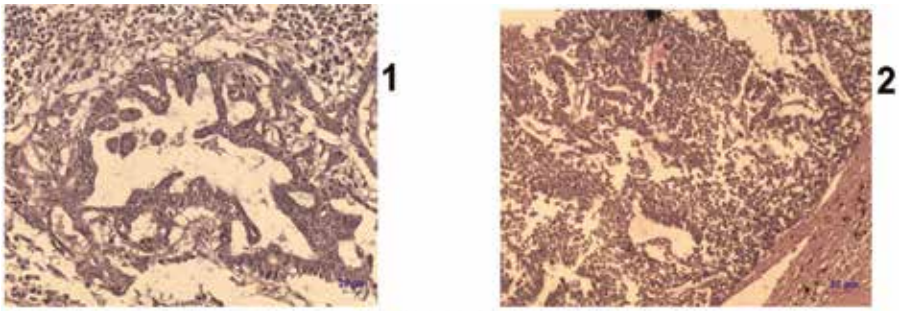

Plate 37. Neoplastic cells were polyhydral in shape(1) and they were arranged in ductular and glandular pattern(2)

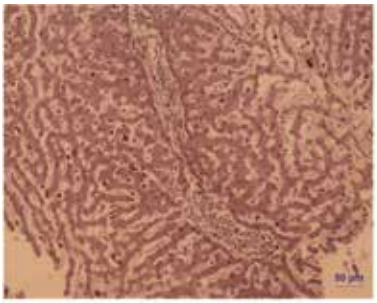

Plate 38. Billiary hyperplasia - Bileduct epithelium showed hyperplastic changes

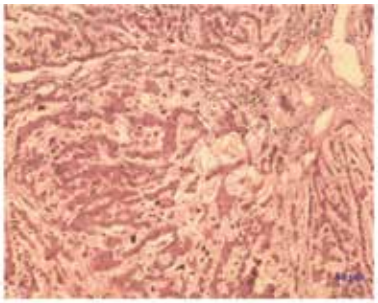

Plate 39. Cirrhosis - Cirrhotic nodules contained dilated sinusoids, thinning of hepatic cords and enclosed by fibrous tissues

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*., 1996).

In the present study elevation of GGT was significant in dogs with biliary disorders, than the other two groups IIA and IIC. 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*at al.,* 1996).

One notable observation in this study was that the dogs with biliary disorders had significantly decreased platelet count with non-significant changes in PT and aPTT (Table 5). Recent studies have documented that there is quantitative and qualitative platelet defects accompanying hepatobiliary disease (Brovida and Rothuizen, 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 thrombopoitin 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 which requires further studies on the entire coagulation cascade to understand the precise mechanism involved.

In the present study there were significant reductions in total protein and albumin (Table 6 b) in all the three groups. The hypoproteinemia could be due to disruption in hepatic protein metabolism. Similar findings were reported in previous studies also (Sevelius, 1995; Center*et al*.,1996*;* Shih*et al*., 2007; Poldervaart*et al* 2009; Brovida and Rothuizen, 2010).

In the present study the cytology in 11 dogs revealed neutrophilic in filtration, while one sample revealed numerous lymphocytic cells which revealed a diffuse hepatic tumor mass on ultrasonographic examination. In the liver cytologic specimens 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 (Mortiz, 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 eight dogs that were sent for post-mortem. The histopathological changes were focal necrosis of hepatocytes with neutrophilic infiltration, hydropic degeration, 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 tests histopathology has to be taken in order to establish a confirmative early diagnosis.

Conclusions

The medical history and 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 while dogs with neoplastic liver disorders showed clinical signs such as weakness, anaemic signs like tachycardia /tachypnea. Significant anemia was evident in all three kinds of liver disorders viz. parenchymal, biliary and neoplastic disorders indicating chronic

inflammatory process and inefficient iron utilization. The chronic inflammatory process was evidenced by significant leukocytosis, neutrophilia hypoalbuminemia in all the three groups. Gamma glutamyl transferase was found to be significantly elevated in biliary disorders. Hepatic histopathology is gold standard test for the confirmatory diagnosis and classification of various liver disorders.

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Diagnostic and therapeutic aspects of subclinical ketosis in crossbred cows of periurban areas of Hyderabad

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Abstract

Out of 280 post parturient crossbred cows between 0 to 60 days post calving presented with the history of reduced feed intake and decreased milk yield, 106 (37.86 %) were found suffering from different subclinical production disorders. On detailed clinical examination and urine analysis, 38 cows (35.85%) were found positive for subclinical ketosis. Blood samples from study group were analyzed for haemoglobin, haematocrit and certain other parameters. Serum samples were analyzed for calcium, glucose, magnesium, phosphorous, AST, ALP, total protein and albumin and compared with that of control cows. Cows with subclinical ketosis were randomly divided in to two different groups. Group I (n=19) animals were administered Ketonil Gel[a] orally @ 1 tube/day for 2 days, followed by half of the tube for next 2 days and Liq. E-Booster[b] @ 200 ml orally BID for 2 days followed by 100ml BID for next 2 days. Group II (n=19) animals received Inj. Wocktrose-25[c] @ 540 ml i.v OD for 3 days, Inj. Ketocort[d] @ 10mg total dose i.m for 3 days and Inj. Tribivet[e] @ 5 ml i.m for 3 days. There was a significant improvement in haemoglobin and serum glucose, calcium, total protein and albumin and significant decrease in serum AST levels after the treatment. The comparative means of healthy, group I and II cows revealed that the treatment given to group II was more effective.

Keywords: Subclinical Ketosis, Crossbred cows, Haematological changes, Biochemical changes, Therapy.

One of the most important disorders in veterinary science and particularly dairy cattle in higher producing herds is production or metabolic diseases. The production diseases of the dairy cow are a manifestation of the cow's inability to cope with the increasing metabolic demands due to high production, and they continue to be a cause of economic loss to the dairy industry (Mulligan and Doherty, 2008). The transition period for dairy cows is generally defined as the time period from 3 weeks prior to parturition through 3 weeks after parturition (Smith, 2005) and is a pivotal time in the production cycle of the dairy animals, in which they are at high risk for the occurrence of most of production diseases. Subclinical form of a disease means simply a condition marked by increased or decreased levels of certain metabolites inside the body without exhibiting the clinical signs or in other words aclinical to the farmer except drastic reduction in milk yield. In general, subclinical disease incidence is far more common than clinical diseases, most often goes unnoticed (Duffield, 2001). Among the subclinical metabolic disorders, detection of subclinical ketosis at an early stage is an important step in order to prevent substantial economic losses. It may be associated with significant clinical disease risks, impaired production and reduced reproductive performance. However, very little vital information is published devoting the impact of this problem and there is dire need to study in detail about possible implications of subclinical ketosis in terms of early detection, conversion into clinical form, in order to minimize the production losses. Therefore the present investigation is undertaken with the objectives to study the incidence, clinical and haemato biochemical findings, therapy and therapeutic efficacy of certain drugs and to evolve a suitable comprehensive therapeutic regimen in cows with subclinical ketosis.

Materials and Methods

280 post parturient crossbred cows between 0 to 60 days post calving presented at Campus Hospital and Dairy experimental station of College of Veterinary Science, Rajendranagar and peri urban dairy farms located in and around Hyderabad, during the study period of 14 months, with the history of decreased appetite and drop in milk yield were selected and screened for subclinical ketosis by examination of urine and blood for biochemical profiles. Urine sample from each cow was collected on day 0 (Day of collection) and on day

5 (post treatment) by stroking her perineum below the vulva, which made her to pass urine. Approximately 5 ml urine was collected from each animal in a sterile glass vial and analyzed for urinary ketones; Urine pH and Glucose by using 'URS-10' strips supplied by M/S Sri Sai Ganesh Agencies, Secunderabad with the help of 'URI-PLUS' urine analyzer supplied by M/S Rapid Diagnostics Pvt. Ltd., New Delhi.

Cows which were positive for urine ketones were selected for the present study and were randomly divided into two groups and given oral therapy for first group and parenteral therapy was provided for another group. To study their efficacy group I (n=19) animals were administered Ketonil Gel[a] orally @ 1 tube/day for 2 days, followed by half of the tube for next 2 days and Liq. E-Booster[b] @ 200 ml orally BID for 2 days followed by 100ml BID for next 2 days. Group II (n=19) animals received Inj. Wocktrose-25[c] @ 540 ml i.v OD for 3 days, Inj. Ketocort[d] @ 10mg total dose i.m for 3 days and Inj. Tribivet[e] @ 5 ml i.m for 3 days.

Apparently healthy animals within two months of calving formed the control group. Blood samples were collected from jugular vein on day '0' (day of collection) and day 5 (post treatment) into vacutainers containing heparin as anti coagulant for haematological estimations and also into vaccutainers without anticoagulant for biochemical estimations. Serum vacutainers were kept undisturbed till serum separation and the serum was transferred into another test tube with the help of a sterile Pasteur pipette. The sera were subjected to centrifugation at 5000 rpm for 5 minutes to obtain clear serum. Then the serum was transferred to eppendrof tubes, labeled accordingly and maintained at 4°C. Haematological estimations viz., Haemoglobin (Hb in g %), Packed Cell Volume (PCV in %), Total Erythrocyte Count (TEC in ×106/µL), Total Leucocyte Count (TLC in ×103/µL), Differential Leucocyte Count (DLC in %), Mean Corpuscular Haemoglobin (MCH in pg) and Mean Corpuscular Volume (MCV in fL) were estimated on the same day of collection with the help of Humacount in the Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad and biochemical parameters were carried out using 'Star 21 Plus' semi automatic Analyzer supplied by M/S Rapid Diagnostics Pvt. Ltd., New Delhi on the same day of collection with the help of biochemical kits supplied by M/S Sri Sai Ganesh Agencies, Secunderabad. The data was subjected to statistical analysis by one way ANOVA using Statistical Package for Social Sciences (SPSS) version 10. Differences between means were tested using Duncan's multiple comparison test and significance was set at 5 percent ($p<0.05$) and also at 1 percent ($p<0.01$). The values were represented as mean ± Standard Error.

Results and Discussion

Out of 280 crossbred cows within 60 days of postpartum with the history of reduced feed intake and decreased milk yield, 106 (37.86 %) were found suffering from different subclinical production disorders. On detailed clinical examination and urine analysis, 38 cows were tested positive for subclinical ketosis which accounted the incidence of 35.85%. Tabrizi *et al. (*2007) also recorded 36% incidence rates, which is in correlation with the present findings suggesting the alarming nature of the disease even in subclinical form and untreated cases might gradually progress into clinical form thus leading to great economic loss in terms of milk yield and milk fat. While higher incidence of 58% was reported by Samie *et al* (2010). However, Jahromi and Nahid (2011) and Padmaja (2009) recorded lower incidence rates as 7.2 and 14.44%, respectively. Though wide ranges are reported by many authors the occurrence of ketosis may probably depend upon extent of care taken by the farmer in early post partum period.

Urine samples of all the cows were screened for glucose and ketone bodies before and after treatment. Group I and II animals had + (Moderate) to ++ (High) for glucose and + (Moderate) to ++ (High) for ketone bodies before treatment and negative after therapy. Anjilappa (2001) and Steen (2001) estimated ketone bodies in urine of milch cattle suffering with indigestion in early lactation to know the etiology of reduced appetite.

a. A proprietary product of M/s. Neospark Ltd. This contain Propylene glycol, Propionic acid, Niacin, Choline, Cobalt, Magnesium, Vitamin A, Vitamin D3, Vitamin E and Vitamin B12 with energy base/10 ml.

b. A proprietary product of M/s. Intas Pharmaceuticals, Ahmedabad. It is a Gluconeogenic precursor fortified with Nicotinamide and Cyanocobalamine.

c. A proprietary product of M/s. Vitoquinol Ltd. Each 100 ml contain 25g Dextrose.

d. A proprietary product of M/s. Intas Pharmaceuticals, Ahmedabad. Each ml contains 4 mg Dexamethasone.

e. A proprietary product of M/s. Intas Pharmaceuticals, Ahmedabad. Each ml contains Vitamin B1 50 mg, Vitamin B6 50 mg and Vitamin B12 500 mcg.

Bremmer, (2004) and Tanwar *et al.,* (2005) suggested that the testing of urine ketones by using Ketostix read within 5 to10 seconds which had the sensitivity and specificity of 79% and 96%, respectively reported to be good diagnostic test and useful in monitoring strategies for therapy and prevention of subclinical ketosis. ketones were more concentrated in urine than in the body, blood stream and milk, therefore a slight color change in urine was acceptable in early lactation of high producing cows (Bass, 2001). Rothera's test and Ketostrips are suitable qualitative tests to detect ketone levels in urine and milk and Sharma (2010) found that urine was about 8 times more sensitive than milk in detecting ketones.

Haematological investigation depicted in Table-1 revealed moderately decreased levels of Hb, TLC, lymphocytes, MCH and MCV in all the affected groups compared to apparently healthy group indicating animals might suffer from anaemia following postpartum due to diminished immunologic status. The present findings are in accordance with Goff and Horst (1997).There was not much difference noticed among haematological parameter before and after treatment, however significant improvement was noticed in Hb ($P < 0.05$) and TLC ($P < 0.01$) in both the groups following therapy. There was a significant ($P < 0.01$) decrease in TLC in subclinical ketosis can be attributed to high level of ketone bodies in the blood was associated with a significant decrease in the total leukocyte count, number of neutrophils and eosinophils (Kuzma *et al* 1997). Increase in granulocytes was significant in group 1 ($P < 0.05$) and increase in monocytes was significant in ($P < 0.05$) group II.

The mean serum glucose levels (Table-2) in group I and II, before and after treatment, were 34.50±1.01 and 41.90±1.08; 32.80±0.82 and 60.16±1.54 mg/dL, respectively. The decreased serum glucose levels before therapy in subclinical ketosis was in accordance with, Sakha *et al.* (2004), Padmaja (2009) and Bahera *et al.* (2011). The values were significantly increased in group I ($P<0.05$) and II ($P<0.01$) following therapy. Glucose deficiency might be the primary theory for hypoglycaemia in ketosis, because 60 to 85% of the available glucose is used in the mammary gland for milk synthesis. There is also a strong interaction between excess fatty acid availability or mobilization and glucose deficiency (Littledike *et al* 1981). In order to compensate the low blood glucose level, glycogenolysis and gluconeogenesis in liver were enhanced. The glucose output to blood by liver was lowered which led to decreased insulin secretion. This might be reason for low glucose level in ketosis positive cows (Bahera *et al.,* 2011). Bergman (1973), in his thorough review of many interrelations between hypoglycemia and ketosis, supported the glucose deficiency theory, whereas Kronfeld (1971) did not agree completely with a carbohydrate shortage theory but states that the lactational demand seems conducive to excess fat. The mean serum calcium levels in group I and II, before and after treatment were 77.90±0.08, 9.66±0.13; 8.07±0.03 and 10.74±0.14 mg/dL, respectively. The findings of decreased serum calcium

Table 1: Mean Haematological Findings Before and After Treatment in Group – I & II Cows Affected With Subclinical Ketosis Compared to Apparently Healthy Cows.

S. No.	Parameter	Apparently healthy cows (n=10)	Group – I (n= 19)		Group - II (n=19)	
			Before Treatment	After Treatment	Before Treatment	After Treatment
1.	Hb (mg %)	9.97±1.58	7.32±0.64	9.78±0.57*	6.95±0.38	9.62±0.34*
2.	PCV (%)	45.96±1.74	41.52±1.46	43.56±1.17	40.31±0.89	45.37±1.43*
3.	TEC ($\times 10^6/\mu L$)	8.23±1.83	7.15±1.61	8.34±1.29	6.85±1.23	8.24±1.40*
4.	TLC ($\times 10^3/\mu L$)	9.97±1.15	7.74±1.53	10.16±1.20**	6.93±1.10	9.67±1.80**
5.	Lymphocytes (%)	19.23±0.19	17.32±0.25	18.17±0.98	18.53±0.20	18.92±0.45
6.	Monocytes (%)	0.98±0.26	0.79±0.26	0.81±0.31	0.93±0.94	1.46±0.67*
7.	Granulocytes (%)	79.92±0.75	64.2±0.47	70.87±0.44*	68.36±0.12	76.52±0.18**
8.	MCH (pg)	15.14±0.52	11.25±0.40	14.14±0.81*	12.63±0.62	15.45±0.69*
9.	MCV (fL)	52.2±0.13	48.6±0.45	56.3±0.94*	43.9±0.39	53.7±0.22*

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

in subclinical ketosis animals were in accordance with Padmaja (2009) and Bahera *et al.* (2011). The reason could be hypocalcaemia which prevented secretion of insulin and tissue uptake of glucose which could enhance lipid mobilization, thus increasing the risk of ketosis (Littledike *et al.,* 1998). Increased serum calcium levels were significant in group I ($P<0.05$) and II ($P<0.01$) after treatment. There was no significant difference in serum magnesium concentration before and after treatment in both the groups and was in accordance with Padmaja (2009). There was no significance difference in serum inorganic phosphorous levels before and after treatment values in both the groups. However, Grunberg (2008) and Padmaja (2009) reported decreased levels, where as Bahera *et al.* (2011) recorded significant increase in mean serum phosphorus concentration in subclinical ketotic post parturient crossbred cows. Serum AST levels were decreased in both groups after the therapy. Decreased levels were significant in group I ($P<0.05$) and II ($P<0.01$). The present findings are in accordance with Padmaja (2009) and Bahera *et al.* (2011). Increased serum AST levels in subclinical ketosis could be due to hypocalcaemia resulting in ischemic necrosis of muscle, thus resulted in release of contents of the enzyme into the blood as also reported by Rao (2010). There was no significant difference between before and after treatment values of serum ALP in both the groups. The mean serum total protein levels were low prior therapy in both groups and increased after the therapy and increased levels were significant in group I ($P<0.05$) and II ($P<0.01$). A similar finding of decreased serum total protein was reported by Bahera *et al.* (2011) and Padmaja (2009). However, Mandali *et al.* (2002) Oliveira *et al. (*2003) found no difference in total serum proteins between postpartum and healthy animals. The decreased level of plasma proteins during early lactation might be attributed to its utilization for milk synthesis (Dahate *et al*., 2004). However, increased serum total protein after parturition was recorded by Randhawa and Chand (2011). In the present study, decreased serum albumin levels were recorded in both the groups when compared to apparently healthy animals, however came to normalcy following therapy. The above findings were in agreement with Padmaja (2009), Rao (2008) and Mahmut *et al.* (2009). Increased levels was significant in both group I ($P<0.05$) and II ($P<0.01$) groups after therapy. Reduced serum albumin levels could be due to the lactation stress. Hypoalbuminaemia occurs when there was excessive loss of albumin or if hepatic production was insufficient to meet demand, as a result of insufficient production or increased consumption in ruminants (Russell and Roussel, 2007).

After therapy, a significant improvement was observed in both the groups on account of milk yield, appetite and absence of ketones in urine. However, the treatment of group II was more effective which could be due to additional beneficial effects of dexamethasone, which might have induced gluconeogenesis resulting in hyperglycaemia (Buchoo and Bhattacharyya 2007) and also suggested that the treatment of ketosis with dextrose, electrolyte and corticosteroid combinations was successful. Tanwar *et al.* (2005), Sarasola and

Table 2: Mean Biochemical Findings Before and After Treatment in Group – I & II Cows Affected With Subclinical Ketosis Compared to Apparently Healthy Cows.

S. No.	Parameter	Apparently healthy cows (n=10)	Group – I (n= 19)		Group - II (n=19)	
			Before Treatment	After Treatment	Before Treatment	After Treatment
1.	Serum Glucose (mg/dL)	58.10±2.96	34.50±1.01	41.90±1.08*	32.80±0.82	60.16±1.54**
2.	Serum Calcium (mg/dL)	11.17±0.27	7.90±0.08	9.66±0.13*	8.07±0.03	10.74±0.14**
3.	Serum Magnesium (mg/dL)	2.69±0.08	2.35±0.05	2.27±0.01	2.63±0.04	2.54±0.02
4.	Serum Phosphorous (mg/dL)	6.27±0.22	5.79±0.08	5.95±0.04	5.84±0.09	6.18±0.12
5.	Serum AST (U/L)	98.37±3.43	142.78±0.70	123.50±0.20*	158.62±0.42	103.35±0.17**
6.	Serum ALP (U/L)	68.44± 4.26	89.58±0.62	82.88±0.81	97.84±0.63	91.63±0.19
7.	Serum Total Protein (g/dL)	7.11±0.16	5.73±0.13	6.52±0.09*	5.25±0.12	7.42±0.12**
8.	Serum Albumin (g/dL)	3.46±0.07	2.65±0.04	3.15±0.04*	2.38±0.03	3.74±0.05**

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Schmidt (2009) and Rao (2010) also suggested the treatment with corticosteroids in ketotic cases. Employing prebiotics in cases of ketosis might counter the negative effect of stress and may increase milk production.

It can be concluded that, for early detection of subclinical ketosis, urine examination and early monitoring of serum glucose levels within 7 days postpartum are useful to prevent progress of subclinical ketosis into clinical form of ketosis. In the present study, treatment given to group II i.e., Inj. Wocktrose-25 @ 540 ml i.v OD for 3 days, Inj. Ketocort @ 10mg total dose i.m for 3 days and Inj. Tribivet @ 5 ml i.m for 3 days was effective when compared to oral therapy given to group I. There was a significant improvement in haemoglobin and serum glucose, calcium, total protein and albumin and significant decrease in serum AST levels after the treatment. The comparative means of healthy, group I and II cows revealed that the treatment given to group II was more effective.

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Evaluation of oxidative stress and haemato-biochemical alterations in canine pyoderma and its therapeutic management.

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Abstract

Canine pyoderma, an infectious disease of dog having zoonotic importance needs a thorough research for its complete recovery.During this study period the prevalence of canine pyoderma was found to be 19.94%in Bhubaneswar which was found to be related to age, sex and breed.20 dogs suffering from pyoderma showing various clinical signs like pus in skin, generalised alopecia,epidermal collarettes, erythema, papules and crusts on the skin as presented at the Teaching Veterinary Clinical Complex of C.V.Sc & A.H, OUAT were selected and their skin swabs were sent to bacteriology lab which revealed the presence of *Staphylococcus aureus* in all samples with highest sensitivity to azithromycin, amoxycilin+clavulanate and cephalexin. There was a significantly lower mean value of Hb, PCV, TEC, lymphocyte count, eosinophil count, albumin-globulin ratio whereas significantly higher ($p<0.05$)mean values of TLC, neutrophil, ALT and AST were detected in pyoderma affected dogs. Erythrocytic oxidative stressmarkers showed asignificantly higher mean value of LPO and lower mean value of SOD and catalase.The selected dogs were treated with azithromycin @ 10mg/kg bwtonce daily orally for three consecutive days in a week for four weeks along with mupirocin ointment applied topically till scar appeared on lesions. Significant improvement in clinical signs, haemato-biochemical parameters and oxidative stress indices were reported by end of treatment period. There was absence of recurrence on day 60 in the affected dogs with LPO, SOD,catalase values at a non-significantly different value on day 30 of treatment however some others showed initial sign of recurrence.

Keywords: CaninePyoderma, Oxidative stress, Azithromyin

Canine Pyoderma , is one of the important skin diseases of the dogs caused by a group of closely related coagulase-positive staphylococcal species (Sasaki *et al*., 2007). This disease is characterized by combinations of erythema, papules, pustules, crusts, scaling and epidermal collarettes (Hillier *et al*.,2006). Dogs are more prone to pyoderma due to the unique characteristics of their skin consisting of a thin stratum corneum, lack of lipid plug in the hair follicles and high skin pH which possess a risk for bacterial invasion, subsequent growth and over colonization (Takashi *et al*., 2007).

An ideal antibacterial agent selected in the treatment of canine pyoderma should have good skin penetration capacity and high antimicrobial activity against the specific bacteria involved. Recurrence of pyoderma in most of the affected dogs is due to improper selection of the antibiotic along with improper dosage and duration of treatment without performing any bacteriological examination or antibiogram prior to the initiation of treatment. The selection of highly sensitive antibiotic for treatment of bacterial pyoderma is being done through culture and antimicrobial sensitivity test (Bloom, 2013).

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Materials and Methods

The present study was carried out in the Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar. Twenty healthy dogs without any signs of pyoderma were selected and grouped as Group 1 as healthy control. Twenty dogs with recurrent pyoderma were selected and taken as Group 2. Blood sample (5 ml) from each dog was collected on day 0, day 15 and day 30 of treatment in heparinised vials for examination of CBC and preparation of RBC hemolysate for estimation of erythrocytic oxidative indices.Erythrocytic oxidative enzymes like SOD, Catalase and LPO were estimated from the 10% RBC haemolysate prepared from heparinised blood by colorimetric method (Placer *et al*., 1966 and Cohen *et al*, 1970). The haematological parameters like Hb, DC, TLC, TEC and PCV were studied as perthe methods suggested by Jain, (1986). Biochemical assay of plasma was done to assay AST, ALT, TSP, albumin and globulin

on auto analyzer using commercially available kits. The statistical analysis of data was done as per the standard methods (Snedecor and Cochran, 1994).

The Group 2 dogs were treated with Azithromycin@ 10 mg/kg.b.wt once daily for three consecutive days in a week (Zur *et al*., 2014) for four weeks as per the culture and sensitivity test of skin swabs and topically mupirocin was applied till scar appeared, whereas the Group 1 animals were given no treatment.

Results and Discussion

Thevarious clinical signs like prurits,erythema, papules, pustules, crusts, epidermal collarettes, pus, alopecia were detected on day 0in the selected dogs affected with pyoderma kept under Group 2 as mentioned in table I and figure I, II & III. On day 15 of the experiment most of the signs like pus, epidermal collarettes, papules, pustules were found to be diminishinghowever alopecia and pruritus at a very lower intense were detected. All the above signs were minimized and apparently absent on day 30 of the experiment. The animals in Group 2 were kept under observation till 60 days. Four of the animals showed recurrence of the disease which may be attributed to the erythrocytic oxidative stress indices which were at a higher level than others in that group on the 30th day of experiment. Except those other dogs did not show any signs of recurrence by day 60

Staphylococcus aureus species was isolated in all the skin swabs from culture and sensitivity test. The antibiogram of antibiotic sensitivity test is given in table no II. The bacteria was found highest sensitive to azithromycin (72.34%) followed by amoxyclavulanate (68.28%),Mupirocin(68.10) and cephalexin (66.41%).

Significantly higher mean LPO (1.53 ± 0.06nmol /mg Hb) values and significantly lower mean Catalase (0.54 ± 0.03units/mg Hb) and SOD (0.69 ± 0.02units/mg Hb) values were recorded in the Group 2 (table III) than the healthy control group on day 0. This indicates existence of oxidative stress at a significantly higher level in pyoderma affected dogs. There was significant decrease in the LPO value on day 30 (0.84 ± 0.03), which is in agreement with Jewell *et al*.(2000), Behera *et al*.(2011), Packer *et al*.(1979), Saskia *et al.*(1993), Rock *et al*. (1996). On day30 of the experiment the significant reduction in the erythrocytic mean LPO values may be due to killing the causative organisms and reducing the altered function in different vital organs through antibiotic therapy.Significant increase in SOD (0.84 ± 0.02) and Catalase (0.99 ± 0.01) values were observed on both day 15 and day 30 which may be due to stimulation of body reticulo-endothelial sytem enhancing antioxidative enzyme level and reducing oxidant level in absence of bacteria related stress.

On day 0, significantly decreased level of mean Hb (10.35 ± 0.17g/dl), PCV (32.62 ± 0.3%) and TEC (5.75± 0.07 x 10^6/mm^3) were recorded in Group 2 (table IV) than Group 1. This is in accordance with the findings of Pal *et al*.(1991), Prathiba *et al*.(2000) and Nair and Nauriyal (2007). The decrease in the values of Hb, PCV and TEC might be due to anaemia caused by the loss of skin protein as reported by Seigmund *et al*.(1986), Bhosale *et al*.(2000) and Deb *et al*.(2000). There was significant increase in Hb (12.64 ±0.25g/dl), PCV (41.38 ±0.21%), and TEC (6.16 ± 0.10x 10^6/mm^3) concentration in the Group 2 from day 0 to day 30. This revealed that treatment with highly sensitive antibiotic in canine pyoderma for 4 weeks enhances

Table I: Showing recovery of various clinical signs

Clinical signs	Day 0	Day 15	Day 30
erythema	++++	+	-
alopecia	++++	+++	+
pruritus	++++	++	-
papules	+++	+	-
crusts	+++	++	-
pustules	++	+	-
epidermal collarettes	+++	+	-
Pus in skin	+++	+	-

Fig-IDAY 0 Fig-II DAY 15 Fig-IIIDAY 30

the haemoglobin, PCV and TEC in a steady state manner. The mean TLC was detected at a statistically significant higher level (20025 ± 125.12 per mm^3) on 0 day in Group 2 than Group 1. The values were in accordance with the reports of Nair and Nauriyal (2007), Gera *et al*. (2009) and Beigh *et al*. (2013). Stress due to dermatitis and bacterial toxins had been suggested as possible reason for marked leucocytosis (Aujla *et al*., 1997). The increased TLC was associated with neutrophilia (87.80 ± 0.51%), and concomitant lymphopenia (10.20 ± 0.61%) onday 0. Neutrophilia may be attributed to the cell injury releasing substances such as leukotrien and leucocytosis promoting factors from the blood into the injured areas. This results in release of more neutrophils into the blood stream. The significant decrease in TLC (11360 ± 109.75 per mm^3) and mean neutrophil percentage (66.60 ± 0.31%)on 30^{th} day could be attributed to decreased tissue damage following therapy (Sharma and Gupta, 2005). Similarly, mean lymphocyte percentage (22.20 ± 0.39 %) showed significant increase after treatment on day 30.There is significant elevation of total protein (9.03 ± 0.19g/dl) noticed in affected dogs as compared to the healthy ones on 0day. An increase in the mean total protein value in the present study could be due to increased inflammatory response associated with the infection. Statistically significant decrease in the mean values of A/G ratio (0.87± 0.02) was noticed in Group 2 which is in agreement with Shyma and Vijaykumar, 2011. There was decrease in mean total protein (7.12 ± 0.05g/dl) and increase in A/G ratio (1.08 ± 0.03) in Group 2 on day 30.On day 0 significantly higher mean ALT (77.90 ± 0.76 U/l) and AST (65.37 ± 0.87 U/l) values were recorded in Group 2, which is in agreement with Biswas *et al*.(2002). The mean ALT and AST values in group 2 decreased on day 15 and further on day 30 and differs nonsignificantly from day 0. This indicates treatment with antibiotics efficiently restore the altered function of the liver towards normal during the stressful conditions of bacterial infection.

Conclusions

The treatment of canine pyoderma with highly sensitive antibiotics both parentrally and topically till significant reduction of oxidative stressindices gives complete recovery with no recurrence. Haemato-biochemcal parameters provides proper guidance about the response of antibiotic against thebacteria, recovery pattern as well as for deciding period of antibiotic therapy.

Table II: Antibiotic sensitivity pattern of S. aureus cultural isolate obtained from pyoderma affected dogs

Sl.No	Antibiotic disc	Sensitive	Resistant
1	Azithromycin (15µg/disc)	72.34%	27.66%
2	Amoxyclavulanate (20+10µg/disc)	68.28%	31.72%
3	Mupirocin	68.10	31.90%
4	Cephalexin (30µg/disc)	66.41%	33.59%
5	Vancomycin (15µg/disc)	34.34%	65.66%
6	Ceftriaxone with sulbactum (30µg/disc)	41.38%	58.62%
7	Amikacin (30µg/disc)	22.41%	77.59%

Table III: Erythrocytic oxidative stress enzymes in different groups of different observation period:

Parameters	Groups (n=20)	Mean± SE		
		0 DAY	15th DAY	30th DAY
MDA (nmol /mg Hb)	G1	0.71 ± 0.02A	0.71 ± 0.03A	0.72 ± 0.02A
	G2	1.53 ± 0.06cB	1.08 ± 0.05bB	0.84 ± 0.03aB
SOD(units/mg Hb)	G1	1.17 ± 0.06B	1.15 ± 0.06B	1.16 ± 0.07B
	G2	0.69 ± 0.02aA	0.77 ± 0.02abA	0.84 ± 0.02bA
CATALASE(units/mg Hb)	G1	1.31 ± 0.17B	1.31 ± 0.16B	1.28 ± 0.16B
	G2	0.54 ± 0.03aA	0.63 ± 0.01A	0.99 ± 0.01bA

(Group 1: healthy control group with no treatment , Group 2: Animals treated with azithromycin.Values (mean±SE) having no common superscripts (small letters in row and capital letters in a column) differ significantly at $p<0.05$).

Table IV: showing the Haemato-biochemical changes with response to the antibiotic treatment in different groups of animals:

Parameters	Groups n=20	Mean± SE		
		0 DAY	15th DAY	30th DAY
Hb (g/dl)	G1	13.44 ±0.36B	13.20 ±0.35	13.56 ±0.26
	G2	10.35 ± 0.17aA	11.27 ±0.31b	12.64 ±0.25b
PCV(%)	G1	43.72 ±0.32B	43.56 ±0.38B	43.93 ±0.33B
	G2	32.62 ± 0.3aA	37.01 ±0.23bA	41.38 ±0.21bA
TEC (10^6/mm^3)	G1	6.2 ±0.05B	6.16±0.05	6.17±0.05
	G2	5.75± 0.07aA	6.05±0.08b	6.16±0.10b
TLC (/mm^3)	G1	11170 ± 183.21A	11320 ± 198.21A	11270 ± 199.47A
	G2	20025 ± 125.12aB	12740 ± 149.22bB	11360 ± 109.75bC
NEUTROPHIL %	G1	66.50 ± 0.62A	66.30 ± 0.68A	66.30 ± 0.40A
	G2	87.80 ± 0.51cB	70.50 ± 0.40bB	66.60 ± 0.31aB
LYMPHOCYTE %	G1	25.20 ± 0.55B	25.80 ± 0.66B	25.80 ± 0.66B
	G2	10.20 ± 0.61aA	22.20 ± 0.39bA	22.20 ± 0.39bA
EOSINOPHIL %	G1	4.70 ± 0.30B	4.60 ± 0.27B	4.90 ± 0.28A
	G2	1.70 ± 0.15aA	3.80 ± 0.20bA	4.20 ± 0.20bA
TOTAL POTEIN (g/dl)	G1	6.18 ± 0.13A	6.11± 0.14A	6.14 ±0.11A
	G2	9.03 ± 0.19bB	8.26 ± 0.04aB	7.12 ± 0.05aB
A/G Ratio	G1	1.35 ± 0.05B	1.33 ± 0.05B	1.34 ± 0.05B
	G2	0.87 ± 0.02aA	1.09 ± 0.01bA	1.08 ± 0.03bA
ALT (U/l)	G1	66.05 ± 0.90A	65.63 ± 0.96A	65.93 ± 0.89A
	G2	77.90 ± 0.76bB	71.62 ± 1.10aB	60.89 ± 1.35aB
AST (U/l)	G1	34.74 ± 0.88A	34.49 ± 0.83 A	34.47 ± 0.92 A
	G2	65.37 ± 0.87cB	50.03 ± 0.41bB	36.61 ± 0.63aB

(Group 1: healthy control group with no treatment , Group 2: Animals treated with azithromycin.Values (mean±SE) having no common superscripts (small letters in row and capital letters in a column) differ significantly at $p<0.05$).

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Tick borne intracellular diseases in dogs and its clinicopathophysiological changes

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Abstract

The study was carried out to explore clinicopathophysiological changes due to various tick borne intracellular diseases of dogs in Uttar Pradesh, India. Dogs presented (3650 nos) during the study period in the Referral Veterinary Polyclinic at Indian Veterinary Research Institute (IVRI) were screened for tick borne intracellular diseases (TBICDs). Microscopic examination of giemsa blood smear was conducted in 650 suspected cases. Blood and serum samples were analyzed for haemato-biochemical changes. Clinical signs and symptom of suspected dogs were recorded to explore clinical pattern due to different TBICDs. Ultrasonographic study was carried out as per the standard procedures to know the hepatic involvement. Study revealed 101nos (2.77%) dog positive for (TBICDs). Varied clinical signs and symptom were noted with overall mean clinical score of 8.39±0.18. USG examination of dogs with TBICD revealed hypo echogenicity of liver, gall bladder distension, splenomegaly, hepato-splenomegaly and ascites. Decreased values of Hb, PCV, TEC and platelet count with increased clotting time recorded. Hypoproteinemia, hypoalbuminemia, hyperglobulinemia and hyperbilirubinemia with increased ALT, ALP and GGT were the predominant feature. Study revealed ehrlichiosis as the most common tick borne intracellular disease in dog followed by mixed TBICDs, babesiosis, anaplasmosis and hepatozoonosis in Uttar Pradesh, India with wide range of clinicopathophysiological changes as compared with healthy dogs.

Keywords: Tick borne intracellular diseases, Clinical signs, Clinicopathology, Dog

Tick borne intracellular diseases (TBICDs) in dog represent an emerging problem in veterinary medicine in the recent years. Ticks are the notorious vectors of various pathogenic protozoa, rickettsiae, bacteria, and viruses that cause serious and life threatening illnesses in humans and animals worldwide (Jongejan et al., 2007). Dog population in India was estimated to be 25 million and within the 'pet' category 5 million dogs were suffering from concurrent tick infection (Megat Abd Rani et al., 2010). In addition to causing serious diseases in tropical and semi-tropical regions (Irwin and Jefferies, 2004), they are now increasingly recognized as a major cause of disease even in temperate climates and urban environments (Shaw et al., 2001). Blood sucking, damaging hides and skins, toxin secretion predisposing to myiasis and dermatophitosis is the predominant feature due to tick infestation (Mtshali et al., 2004). In addition to creating sites for secondary invasion by pathogenic organisms (Kaufman et al., 2006), reduction in body weight gains affects canine management. The major canine tick-borne infections are protozoan origin (caused by *Babesia* spp, *Theileria* spp., *Hepatozoon* spp), rickettsial and bacterial diseases (*Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., *Bartonella* spp., *Coxiella* spp., and *Borrelia* spp.) and viral infections (tick-borne encephalitis). Co-infections of *Babesia* and *Anaplasma,* along with *Ehrlichia, Bartonella, Hepatozoon*, *Leishmania* and *Rickettsia* species have also been reported in dogs (O'Dwyer et al., 2001) and may complicate the clinical signs and pathogenesis of infection (Shaw et al., 2001). Clinical findings can range from incidental hematological findings to severe life-threatening illness. So, the present study was carried out to know the detail clinical signs and symptoms along with hemato-biochemical changes involved in various TBICDs viz. babesiosis, ehrlichiosis, anaplasmosis, hepatozoonosis and mixed infection.

Materials and methods

Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, (UP) during 2010-2012.

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650 ailing dogs with the history of tick infestation, erratic fever, chronic or prolonged illness and unresponsive to routine treatment were subjected to blood and buffy coat smear examination and those found positive with intracellular blood borne parasite were included in the present study. Six apparently healthy dogs of different age, sex and breeds, brought for either health checkup or for vaccination were used for comparison.

Each dog was subjected to detail clinical examination as per standard procedure (Jones et al., 1994) Presence of symptoms/ signs/ involvement of different body systems and systemic states were recorded. A clinical score of each ailing dog was worked out based on 17-points scale (Jones et al., 1994).

To know the hepatic involvement in TBIDs, ultrasonographic study was carried out as per the standard procedures (Nyland and Mattoon, 2002) with Scanner 200 vet (Pie Medical, Netherland) or Sonosite model 600M and a 5.0 MHz AAS transducer.

Blood samples were collected from sephanous/ cephalic vein in clean dry sterilized vial with ethylene diamine tetracetate (EDTA) for hematological analysis. For serum separation, 5ml blood without anticoagulant was collected and centrifuged at 3000 rpm for 5 minute and were stored in deep freeze at (–) 20 ^{0}C for further biochemical and enzymatic estimations.

Smear from blood and buffy coat were examined with standard procedure for confirmation of tick borne intracellular organism viz. *Babesia, Ehrlichia*, *Anaplasma* and *Hepatozoon* organism or mixed infection. At least 200 leukocytes in each blood smear and up to 100 oil immersion fields in each Buffy coat were screened for presence of pathogen.

Hematological parameters viz. hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocytic count (DLC), platelets count, clotting time, red blood cell indices were analyzed as per the standard techniques (Jain, 1896)

Total protein and albumin (Biuret method), creatinine (Alkaline picrate method) and total bilirubin (modified Jendrasik and Grof method) were estimated with the help of commercial kit (Span diagnostic kit,Span Diagnostic Limited, Surat, India).

Serum enzyme profile viz. serum alanine amino transferase (ALT), asparate aminotransaminase (AST/SGOT), alkaline phosphatase (ALP), gamma glutamyl transferase (γ-GT) were measured by standard diagnostic kits (Span diagnostic kit,Span Diagnostic Limited, Surat, India).

All the data were analyzed by using ANOVA test by Statistical Package SPSS 15 (SPSS, Science, Chicago, USA).The results were reported as means ± SE. A value of $P<0.05$ was considered as significant.

Results and Discussion

During the study period, a total 3650 dog presented in the Referral Veterinary Policlinic, IVRI, Izatnagar, Bareilly, India were attended. Off which 650 dogs were suspected for TBICDs and later 101 dogs only were recorded confirmed for TBICD due to parasite origin. This indicated 15.54% confirmed (101/650) cases. The percent prevalence of different TBICDs were 60%, 10%, 5%, 4% and 22% for ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infection respectively. Under mixed infections, 54% (12/22) dogs with ehrlichiosis and babesiosis, 31.8% (7/22) with ehrlichiosis and anaplasmosis and 13.6% (3/22) with ehrlichiosis, babesiosis and hepatozonosis were recorded.

Clinicopathophysiological investigations in dogs with TBICDs were carried out on 101 confirmed cases only. Clinical manifestation of TBICDs in dogs under the study is shown in the Table 1.Presence of ticks (95.05%) in most of the dogs with a lowest incidence of jaundice development (2.97%) were recorded. An overall mean clinical score, based on 17 point scale was 8.39± 0.18 with an individual score varying from 5 to 13. The mean clinical score in mixed infection was highest among other infected groups depicting 9.95±0.30 with an individual score varying from 6 to 13

USG examination revealed that 49.50% cases were hypo echogenicity of liver, out of which 38% had gall bladder distension, 23.76% hepato-splenomegaly, 15.84% ascites and 12.87% splenomegaly in positive cases (Table 2) as compared to healthy group. In this study, it was observed that hypo echogenicity of liver was more prominent in ehrlichiosis followed by mixed infection, hepatozoonosis, anaplasmosis and babesiosis. Gall bladder distension was found of higher proportion in hepatozoonosis than babesiosis followed by anaplasmosis, mixed infection and ehrlichiosis. Increased incidence of splenomegaly was found in anaplasmosis followed by babesiosis, hepatozoonosis,

mixed infection and ehrlichiosis. On the other hand, hepato-splenomegaly was more common in hepatozoonosis followed by babesiosis ehrlichiosis, anaplasmosis and mixed infection. Ascites was found more common in babesiosis infected dogs followed by mixed infection and ehrlichiosis.

The mean ±SE values of hematological parameters of dogs suffering from different tick borne intracellular diseases are shown in Table 3 & 4. There was significant (P<0.05) decrease in the Hb values due to babesiosis, hepatozoonosis and mixed infection when compared with healthy group. But no significant differences were seen within the infected animals. PCV values did not show significant reduction in all infected groups when compared to healthy group (34.8 ± 0.7). Similarly, there was no significant difference recorded amongst the infected groups. Similar picture was revealed with TEC values and platelet count. Lowest platelet count was observed in the dogs with anaplasmosis (0.68±0.09) which differed significantly (P<0.05) from the *Babesia* infected dog (1.16±0.13) though both the group significantly differed from the healthy one (2.50±0.20). The clotting time revealed significantly (P<0.05) higher in all the infected groups when compared to healthy group (3.48±0.08/ min). There was no significant variance of TLC, MCV, MCHC and MCH values of both infected and healthy groups. With regards to DLC, neutrophil percent of mixed infection, ehrlichiosis and babesiosis differed significantly (P<0.05) from hepatozoonosis group which revealed highest neutrophil values. Significantly (P<0.05) highest monocyte values was observed in ehrlichiosis infected dogs and mixed infected dogs when compared to healthy group.

There was significant difference (P<0.05) in the total protein value of ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infected dogs when compared with the biochemical profile of serum samples taken from healthy dogs under study (Table 5). The albumin levels were significantly (P<0.05) decreased in infected groups as compared to healthy group whereas serum globulin levels were significantly (P<0.05) increased. The value of A/G ratio of mixed infected group was significantly (P<0.05) lower

Table 1: Pattern of clinical observation recorded in various intracellular TBDs in dogs

Clinical parameters	Ehrlichiosis (n=60)	Babesiosis (n=10)	Anaplasmosis (n=5)	Hepatozoonosis (n=4)	Mixed infection (n=22)	Total no of cases (n=101)	Percentage (%)
Tick	56	9	5	4	22	96	95.05
LN enlargement	50	8	2	3	22	85	84.16
Staggering gait	47	4	1	4	19	75	74.26
↑Temperature	44	8	4	1	15	72	71.29
Pale MM	29	7	5	3	22	66	65.35
Anorexia	34	8	2	3	17	64	63.37
Petechial hemorrhage	26	8	3	2	12	51	50.50
Respiratory	25	7	1	1	13	47	46.53
Diarrhoea	25	5	2	1	14	47	46.53
Vomiting	22	5	-	3	15	45	44.54
Inappetance	26	2	3	2	5	38	37.63
Nervous sign	18	1	-	1	13	33	32.67
muscular skeletal	17	2	-	4	10	33	32.67
Epistaxis	23	2	5	-	2	32	31.68
Ocular sign	13	5	-	2	9	29	28.71
Malena	13	3	1	1	7	25	24.75
abdominal distension	12	3	-	-	1	16	15.84
Jaundice	1	2	-	-	-	3	2.97

Table 2 : Ultrasonographic changes of liver and spleen in various intracellular TBDs in dogs

Organ	Ehrlichiosis (n=60)	Babesiosis (n=10)	Anaplasmosis (n=5)	Hepatozoonosis (n=4)	Mixed infection (n=22)	Total no of cases (n=101)	Percentage (%)
1. Liver							
1a. Liver hypo echogenicity	31	3	2	2	12	50*	49.50
1b. Gall bladder distention	10/31	2/3	2/ 2	1/ 2	4/ 12	19/50*	38
2.Hepato-splenomegaly	12	2	2	1	5	22	21.78
3. Ascites	12	3	0	0	1/ 22	16	15.84
4. Splenomegaly	5	2	1	1	4	13	12.87

* Gall bladder distention noted in 19 dogs out of 50 dogs showing hypoechogenisity of liver

followed by anaplasmosis, ehrlichiosis, babesiosis and hepatozoonosis infected group as compared to healthy dogs. There was no significant difference of serum BUN levels and creatinine values in healthy and infected groups except babesiosis infected dogs (1.50±0.07 mg/ dl) which differed significantly ($P<0.05$). Significantly ($P<0.05$) higher bilirubin level in ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infected group were recorded as compared to healthy group

Mean serum ALT, AST, ALP and GGT activity in dogs infected with ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infection are shown in Table 6. The ALT activity was significantly ($P<0.05$) higher in dogs with ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infection than healthy dogs. Among TBICDs, mean ALT activity was significantly ($P<0.05$) higher in mixed infected group than ehrlichiosis, babesiosis anaplasmosis infected group. But there was no significant difference of mean ALT activity between mixed infected and hepatozoonosis infected group, though ALT level of mixed infected group was higher. There was no significant difference in case of AST activity of infected and healthy group. Mean activity of ALP (U/L) in dogs of ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infection group differed significantly ($P<0.05$) in comparison to healthy. Similarly, the activity of GGT in infected group was significantly ($P<0.05$) increased from healthy group.

Incidences of tick borne intracellular diseases in dogs have been earlier reported from India (Lakshmanan et al., 2007; Megat Abd Rani et al., 2011; Wise and Tarlinton, 2012). Similarly concomitant infection of *E.canis* with *H.canis* (Sarma et al., 2012) and *E. canis* with Babesia spp (Kumar et al., 2006) were earlier documented. Tick-borne infections like ehrlichiosis, hepatozoonosis, anaplasmosis, rickettsiosis, lyme disease, babesiasis etc. are frequently seen not as independent, but as co-infections because of same vectors incriminated for transmission (O'Dwyer et al., 2001).

Clinical signs and symptoms help clinician to predict type of disease and helps for confirmatory diagnosis. In case of ehrlichiosis, the main clinical signs of present study were in accordance with previous reports (Rungsipipat et al., 2009). The wide variation in clinical picture may be due to many factors like age, breed, immune competence of dogs, clinical phase of the diseases, variation in virulence between different strains etc (Greene, 2006). Lymphadenomegaly was observed mostly in acute condition due to accumulation of large number of white blood cells to the lymph nodes present around the infection to fight it. Pale mucous membrane and hemorrhages may be attributed to a combination of mild thrombocytopenia and vacuities (Harrus et al., 1998)

Variety of clinical signs in naturally occurring cases of canine babesiosis was observed in present study similar to other reports (Chaudhury, 2006). The pathogenesis of neurological symptom in babesiosis is related to parasitized erythrocytes that become sequestrated in the central nervous system microvasculature and to the release of inflammatory mediators and tissue hypoxia, which can lead to neurological signs. Ascites in babesiosis might be due

Table 3 : Hematological profile of dogs with various intracellular TBDs

Groups	Hb (g/dl)	PCV (%)	TEC ($x10^6$µl)	TLC ($x10^3$µl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets ($x10^5$)	Clotting time(min)
Healthy (n=6)	11.95± 0.26^{b}	34.8± 0.7^{b}	5.1± 0.06^{b}	10.38± .46	68.16± 1.52	23.40± 0.65	34.42± 1.29	2.50± .20^{c}	3.48± 0.08^{a}
Ehrlichiosis (n=60)	9.30± 0.38^{ab}	29.38± 0.52^{a}	3.50± 0.11^{a}	10.24± 0.87	87.38± 2.42	26.73± 0.79	31.27± 0.93	0.91± 0.03^{ab}	6.06± 0.26^{b}
Babesiosis (n=10)	8.59± 0.66^{a}	28.90± 1.11^{a}	3.55± 0.31^{a}	13.52± 3.78	89.62± 11.61	24.68± 0.81	29.41± 1.69	1.16± 0.13^{b}	7.36± 0.48^{b}
Anaplasmosis (n=5)	9.82± 0.58^{ab}	29± 1.61^{a}	3.36± 0.20^{a}	9.12± 1.53	86.42± 1.63	29.26± 0.88	33.84± 0.46	0.68± 0.09^{a}	7.32± 0.40^{b}
Hepatozoonosis (n=4)	7.80± 0.45^{a}	26.0± 1.08^{a}	3.03± 0.32^{a}	7.70± 0.88	87.88± 6.55	26.25± 1.78	30.09± 1.80	0.85± 0.05^{ab}	6.65± 0.44^{b}
Mixed infection (n=22)	7.71± 0.59^{a}	29.0± 0.94^{a}	3.29± 0.24^{a}	10.92± 1.41	93.57± 4.36	23.70± 1.15	26.09± 1.45	0.90± 0.10^{ab}	6.93± 0.47^{b}

Values are Mean ±SE; Values in the same column with the different superscripts are significantly different at (P<0.05).

to lower plasma colloid osmotic pressure which leads to the formation of edema including ascites; pleural effusion and pitting edema (Ha-Jung et al., 2006). The large variation in clinical signs seems to be due to a number of factors including differences in the pathogenicity between strains of TBICDs, breeds of the dog, nutritional and immune status of the dog.

The major clinical signs in anaplasmosis infected dogs were observed in present study similar to other reports (Mazepa et al., 2010). Epistaxis and pale mucosa observed in *Anaplasma* affected dogs in present study might be due to reduce platelet count.

Dogs with hepatozoonosis may display waxing and waning signs of disease, which may eventually result in a chronic wasting condition. Gait abnormalities and musculoskeletal pain may be due to myositis and periosteal bone lesions. Alterations of clinical signs in dogs infected with *H. canis* vary depending on age of the host, degree of infection, and association with concurrent infection (Sarma et al., 2012).

Ultrasonographic findings observed in various TBICDs in the present study are in full agreement with earlier reporter (Srivastava and Srivastava, 2011; Eduardo et al., 2011; Kohn et al., 2008). Similarly, hypoechoic echotexture, increased scanning area, inflammatory changes, hepatomegaly, splenomegaly and gall bladder distention observed in USG examination in ehrlichiosis infected dogs were also reported by other workers (Kumar et al., 2006). Hepato-splenomegaly might have been due to multiplication of organism within circulating mononuclear cells and mononuclear phagocytic tissues of liver, spleen and lymph node (Hildebrandt et al., 1963). Splenomegaly is because of reactive lymphoid hyperplasia and concurrent extramedullary hematopoiesis (Egenvall et al., 1997). The sonographic changes in gall bladder with distention in the present study might be due to anorexia.

In ehrlichiosis, anaemia, thrombocytopenia, monocytosis and increase clotting time were the main abnormalities in the present study similar to other reports (Kohn et al., 2008). Anemia is typically in babesiosis might be due to both intravascular and extra vascular haemolysis. The most consistent laboratory abnormality of thrombocytopenia in ehrlichiosis and babesiosis infected dogs (Mathe al., 2006), anaplasmosis (Kohn et al., 2008) and hepatozoonosis (Mundim et al., 2008) were in agreement with the present study. The thrombocytopenia can be explained by the immune mediate destruction, sequestration or by decreased production, vasculitis and platelet function abnormalities (Lappin, 2010). The neutrophilia and leukocytosis in hepatozoonosis affected dog of this study are consistent with the findings of other researcher (Voyvoda et al., 2006). These alterations might be due to the parasite's invasion and multiplication in the animal's tissues and organs leading to an inflammatory response exacerbated by secondary bacterial infections, inter current with other hemoprotozoa. Increased monocyte in ehrlichiosis infected dog of the present study was also reported by other researchers (Poitout et al., 2005). Increase clotting time in various TBICDs observed in the present study might be due to thrombocytopenia.

Table 4: Differential leukocyte counts of dogs with various intracellular TBDs

Groups	N (%)	L (%)	M (%)	E (%)	B (%)
Healthy (n=6)	76.50±1.48^{ab}	21.0±1.70	1.00±0.40^{a}	1.00±0.0	0.00±0.0
Ehrlichiosis (n=60)	73.12±1.11^{a}	18.78±1.10	6.58±0.37^{c}	1.25±0.16	0.38±0.14
Babesiosis (n=10)	74.20±4.02^{a}	20.30±3.91	3.10±0.71^{ab}	1.60±0.34	0.40±0.22
Anaplasmosis (n=5)	78.8±2.85^{ab}	16.00±2.10	3.8±0.80^{bc}	1.00±1.0	0.00±0.0
Hepatozoonosis (n=4)	81.25±3.15^{b}	13.75±2.50	3.0±0.71^{ab}	2.00±0.41	0.00
Mixed infection (n=22)	71.77±2.10^{a}	20.82±2.28	5.73±0.61^{c}	1.41±0.26	0.24±0.11

Values are Mean ±SE; Values in the same column with the different superscripts are significantly different at (P<0.05).

Table 5. Serum biochemical profile of dogs with various intracellular TBDs Mean ±SE

Groups	T protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio	BUN (mg/dl)	Creatine (mg/dl)	T.bilirubin (mg/dl)
Healthy (n=6)	6.57±0.13^{c}	4.56±0.07^{c}	2.01±0.15^{b}	2.26±0.03^{a}	34.45±8.36	0.62±0.15^{a}	0.68±0.21^{a}
Ehrlichiosis (n=60)	5.10±0.23^{b}	1.73±0.13^{ab}	3.37±0.17^{c}	0.51±0.25^{b}	32.50±2.18	1.30±0.12^{ab}	1.37±0.07^{bcd}
Babesiosis (n=10)	4.67±0.77^{ab}	1.88±0.30^{ab}	2.79±0.52^{ac}	0.67±2.83^{b}	39.14±6.55	1.50±0.07^{b}	1.81±0.13^{d}
Anaplasmosis (n=5)	5.11±0.29^{b}	1.69±0.14^{ab}	3.42±0.28^{ac}	0.49±0.20^{b}	33.86±4.33	1.01±0.04^{ab}	1.04±0.05^{ab}
Hepatozoonosis (n=4)	5.10±0.19^{b}	2.33±0.25^{b}	2.77±0.27^{ac}	0.84±0.18^{b}	27.40±5.62	1.24±0.08^{ab}	1.27±0.06^{bc}
Mixed infection (n=22)	4.09±0.23^{a}	1.21±0.12^{a}	2.88±0.15^{ac}	0.42±0.08^{b}	35.88±2.42	1.16±0.04^{ab}	1.54±0.06^{cd}

Values are Mean ±SE; Values in the same column with the different superscripts are significantly different at (P<0.05).

Hypoproteinemia along with hypoalbuminemia, hyperglobulinemia and hyperbilirubinemia in different TBICDs in dogs were in agreement with previous observation (Mylonakis et al., 2010) might be due to a chronic inflammatory disease, anorexia or decreased protein intake (Poitout et al., 2005). Significantly (P<0.05) higher bilirubin level in dogs with ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infection observed as compared to healthy ones, can probably be attributed to a chronic state of the disease (Zygner et al., 2007). The bilirubin has been increased due altered hepatic function (Tennant and Center, 2008). Higher levels of ALT, ALP and GGT in dogs with ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis and mixed infection of the present study are in agreement with previous observations (Mylonakis et al., 2010) Increase activity of ALT and GGT in dogs of various tick borne diseases might be due to hepatic dysfunction.

Conclusions

Incidence of tick borne intracellular diseases in dogs revealed that ehrlichiosis is the most common tick borne intracellular disease in dog followed by mixed infection, babesiosis, anaplasmosis than hepatozoonosis. TBICDs were manifested by wide range of clinical signs and symptom with overall mean clinical score of 8.39±0.18. USG examination revealed hypo echogenicity of liver, gall bladder distension, splenomegaly, hepato-splenomegaly and ascites. Hb, PCV, TEC and platelet count were decreased whereas clotting time was increased. Hypoproteinemia, hypoalbuminemia, hyperglobulinemia and hyperbilirubinemia with increased ALT, ALP and GGT values were observed in dog with TBICDs.

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Table 6. Liver enzyme profile of dogs with various intracellular TBDs

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)
Healthy (n=6)	43.79±8.76^{a}	21.06±4.21	42.49±9.40^{a}	7.45±1.04^{a}
Ehrlichiosis (n=60)	142.97±8.28^{b}	46.69±5.37	184.34±12.06^{b}	20.57± 1.53^{a}
Babesiosis (n=10)	155.71±9.30^{b}	25.48±5.86	180.57±14.63^{b}	22.09±4.27^{a}
Anaplasmosis (n=5)	137.20±7.96^{b}	24.60±3.28	151.54±16.42^{b}	36.48±2.34^{b}
Hepatozoonosis (n=4)	184.20±6.07^{bc}	29.07±7.78	243.24±39.72^{b}	32.52±11.67^{b}
Mixed infection (n=22)	247.16±17.26^{c}	31.71±2.78	224.55±18.26^{b}	20.92±2.86^{a}

Values are Mean ±SE; Values in the same column with the different superscripts are significantly different at (P<0.05).

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Occurrence of *Ehrlichia canis* and *Anaplasma platys* infections in dogs in and around Bengaluru

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Abstract

The objective of this study was to detect occurrence of *Ehrlichia canis* and Anaplasma platys infection in dogs in and around Bengaluru. For initial screening, ELISA rapid assay test kit (IDEXX laboratories, Westbrook, USA) was used. Of the 50 clinical cases suspected, 11 cases were found positive for canine ehrlichiosis, seven (14%) cases were found positive for canine anaplasmosis by this kit. Further, on PCR which was used as a confirmatory diagnostic technique, of the 50 clinical cases suspected, 41(82%) were positive for the presence of ehrlichia DNA with genus specific and species specific primers, 43 (86%) cases were diagnosed positive for canine anaplasmosis (*A.platys*) and mixed infection with ehrlichia and anaplasma was found in 39 (78%) cases on PCR. This study has provided evidence of the presence of a high level of *E. canis* ans *A. platys* in community dogs in Bengaluru.

Keywords: *Ehrlichia canis, Anaplasma platys,* Dogs

Ehrlichiae were first discovered at the Pasteur Institute in Algeria by Donatien and Lestoquard (1935). They observed that experimental dogs infested with ticks, *Rhipicephalus sanguineus,* occasionally developed severe illness characterized by anemia and the blood smears of the infected dogs stained by Giemsa technique, showed small rickettsia like organisms inside the monocytes which they named as *Rickettsia canis*. Mudaliar (1944) reported ehrlichiosis for the first time in India from Chennai.

In 1978, an ehrlichial infection that affected platelets was first identified in the US. It was caused by *Anaplasma platys* (initially identified as *Ehrlichia platys*), and it caused a clinical syndrome known as canine infectious cyclic thrombocytopenia (Harvey *et al*., 1978).

Canine monocytic ehrlichiosis (CME) and canine cyclic thrombocytopenia (CCT) are infectious diseases caused by gram-negative bacteria of the order Rickettsiales, family Anaplasmataceae, genera Ehrlichia and Anaplasma (Dumler *et al*., 2001).

Ehrlichia canis and *Anaplasma platys* are obligatory intracellular organisms organized in clusters, called morulae, and frequently observed in leukocytes and platelets, respectively, with the possibility of concomitant infections (McBride *et al*., 1996; Cohn, 2003; Suksawat *et al*., 2001a). These infectious diseases are of great importance for small animal clinics and public health, since they are increasingly prevalent in dogs and because there is evidence that these pathogens can also affect humans (Dagnone *et al*., 2001; Tamí and Tamí-Maury, 2004; Neer and Harrus, 2006).

Routine diagnoses of CME and CCT are based on characteristic clinical and hematological findings. The identification of hemoparasites in blood smears is the most widely used technique in clinical practice to characterize morulae in leukocytes and platelets, but this method has low diagnostic sensitivity and specificity and must be supplemented with the use of molecular techniques, such as PCR (Nakaghi *et al*., 2008; Dagnone *et al*., 2009; Ramos *et al*., 2010).

Despite the fact that tick borne diseases are of great concern worldwide, in India there is only limited information regarding the prevalence of vector-borne pathogens in dogs. Further, there are few published reports on canine anaplasma in India (Megat Abd Rani *et al*., 2010).

To our knowledge, there are no reports on the occurrence of CCT in community dogs in and around Bengaluru

Materials and Methods

The source of the animals for the present study were clinical cases of dogs with signs suggestive of tick borne diseases presented to Veterinary College Hospital, Hebbal, Bengaluru. A total of 50 clinical cases were selected based on the history and clinical signs such as high temperature, lethargy, anorexia, presence or history of tick exposure, anemia, hematuria, epistaxis, congested/pale/icteric mucous membranes, lymphadenopathy, lameness and weakness.

Blood smears and buffy coat smears were made from blood of affected dogs and stained with Giemsa stain (Himedia) as per the standard method for demonstration of hemoprotozoan organisms (Schalm *et al.,*1975).

For screening for Ehrlichiosis and Anaplasmosis the ELISA rapid assay test kit was used. (IDEXX laboratories, Westbrook, USA)

DNA was extracted from all the blood samples using the kit procured from QIA amp DNA blood mini kit (QIAGEN, GmbH, Germany) adhering to the manufacturer's protocol.

The PCR for the detection of *E. canis* in the blood samples was carried out based on the method of Murphy *et al*. (1998) with some modifications. Primers were selected based on the study of Murphy *et al*. (1998). Genus specific primers used for the amplification of ehrlichial DNA were ECC (5' -AGA ACG AAC GCT GGC GGC AAG C- 3') and ECB (5'- CGT ATT ACC GCG GCT GCT GGC A -3').

Extracted DNA (4.0 µL) was used as a template to amplify a fragment of the 16S rRNA gene in 25 µL of reaction mixture containing 12.0µl of *Taq* DNA Polymerase Master Mix RED (2X), 1.0µl of primer Ehrlichia F (20 pmol/µl) , 1.0µl of primer Ehrlichia R (20 pmol/µl) and 7.0µl of sterile Nuclease free distilled water.

PCR was carried out in a thermal cycler (Eppendorf, Germany). The thermocycle profile consisted of initial denaturation at 94°C for one min, followed by 29 cycles of denaturation at 94°C for one min., annealing at 65°C for 2 min. and extension at 72°C for 2 min. This was followed by a final extension at 72 °C for 5 min. The amplicons obtained were subjected to nested PCR for confirmation of the species of ehrlichia. Nested reactions were performed using 1 µL of this amplicon as a template with species specific primers of *E. canis*, namely, E.canis-F (20 pmol/µl) (5'- CAA TAA TTT ATA GCC TCT GGC TAT AGG A- 3') and E.canis-R (20 pmol/µl) (5'- TAT AGG TAC CGT CAT TAT CTT CCC TAT - 3') under the reaction conditions described above.

For species- specific amplification of *A. platys*, primers were selected as suggested by Inokuma *et al.* (2001) and Beaufils *et al.* (2002), i.e PLATYS-F (5'-AAG-TCG-AAC-GGA-TTT-TTG- TC-3') and PLATYS-R (5'-CTT-TAA-CTT-ACC-GAA-CC-3')

The conventional PCR for the amplification of *Anaplasma platys* in the blood samples was carried out based on the method of Inokuma *et al.* (2001) and Beaufils *et al.* (2002) with some modifications instead of nested PCR. Different buffer, enzyme and master mix have been used in this study.

Extracted DNA (4.0 µL) was used as a template to amplify *A. platys* DNA in 25 µL of reaction mixture containing 12.0µl of *Taq* DNA Polymerase Master Mix RED (2X) procured from Ampliqon, 1.0µl of primer PLATYS-F (20 pmol/µl), 1.0µl of primer PLATYS-R (20 pmol/µl) and 7.0µl of sterile Nuclease free distilled water.

Template DNA was added after the nuclease free water, master mix, forward and reverse primers were distributed to individually marked 0.2 ml PCR tubes. Contents were mixed by flicking the tubes. Appropriate known positive controls, negative controls and no template controls were included with every run. Conventional PCR was carried out in a thermal cycler (Eppendorf, Germany). The thermocycle profile consisted of initial denaturation at 94°C for one min, followed by 29 cycles of denaturation at 94°C for one min., annealing at 55°C for 2 min. and extension at 72°C for 2 min. This was followed by a final extension at 72 °C for 5 min.

A total of 50 samples were subjected to PCR. All collected samples were subjected to PCR and *Anaplasma platys* species was confirmed by an expected PCR product of 504 bp amplicon band size.

PCR amplicons, known positive and negative controls and a molecular weight marker (100 bp ladder) were electrophoresed in 1.5% agarose gel stained with ethidium bromide using submarine gel electrophoresis. The products were visualized using the UV transilluminater and photographed using a video documentation system (ms-major science).

Results and Discussion

None of the cases were found positive for ehrlichia and anaplasma organisms in direct blood smear as well as buffy coat smear examination.

ELISA Rapid Assay Test Kit was used as the initial screening test to detect the occurrence of tick borne diseases in dogs in the present study. Whole blood was used for the purpose. Of the 50 clinical cases suspected, 11(22%) cases were found positive

for canine ehrlichiosis, seven (14%) cases were found positive for Canine Anaplasmosis (*A.platys*) and two (4%) of the cases had mixed infection with ehrlichiosis and anaplasmosis (*A.platys*). (Plate – 1, 2, 3 and 4)

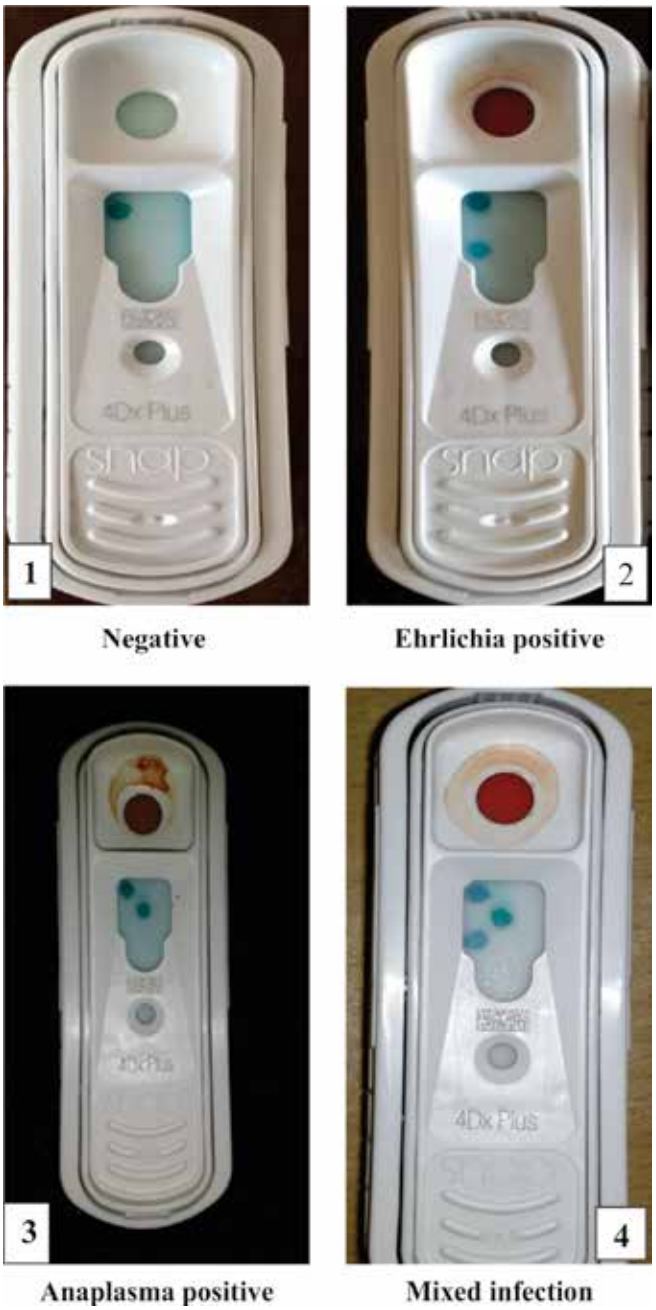

Plate 1,2, 3 and 4: ELISA rapid assay test kit results (SNAP-4DX test kit)

Further, on PCR which was used as a confirmatory diagnostic technique, of the 50 clinical cases suspected, 41(82%) were positive for the presence of ehrlichia DNA with genus specific primers which produced a 477 bp band in accordance with the known positive control.. All the 41 (82%) samples were positive for *E. canis* when subjected to nested PCR with *E. canis* specific primers, which produced a 387 bp band in the DNA ladder. (Plate – 5 and 6)

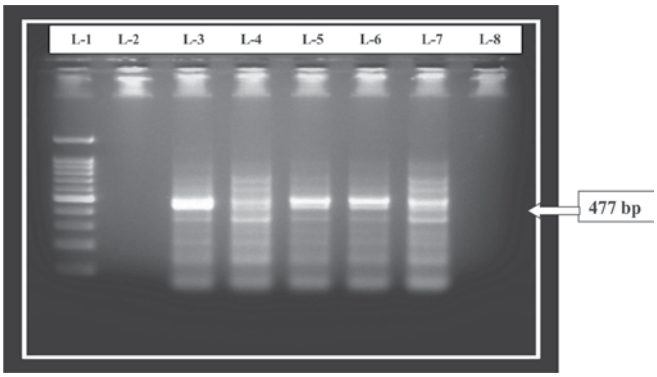

Plate 5: Genus specific PCR product positive for ehrlichia genus (477 bp)

Lane 1	100 bp DNA marker
Lane 3, 4, 5, 6 and 7	Positive test samples
Lane 2 and 8	Negative test samples

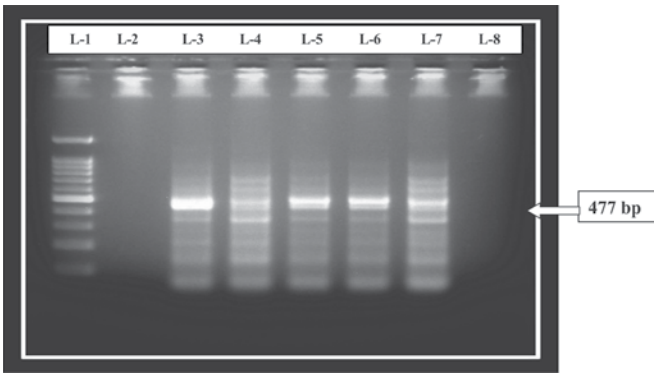

Plate 6: Species specific PCR product positive for *Ehrlichia canis* (387 bp)

Lane 1	100 bp DNA marker
Lane 2	Known positive sample
Lane 3	Known negative sample
Lane 4,5, 6, 7, 8,9, 10, 11, 12, 13, 14 and 15	Positive test samples

Forty three (86%) cases were diagnosed positive for canine Anaplasmosis (*A.platys*). *Anaplasma platys* species was identified based on 504 bp band produced in accordance with positive control using species specific primers platys-F and platys-R. (Plate – 7)

Mixed infection with ehrlichia and anaplasma was found in 39 (78%) cases on PCR.

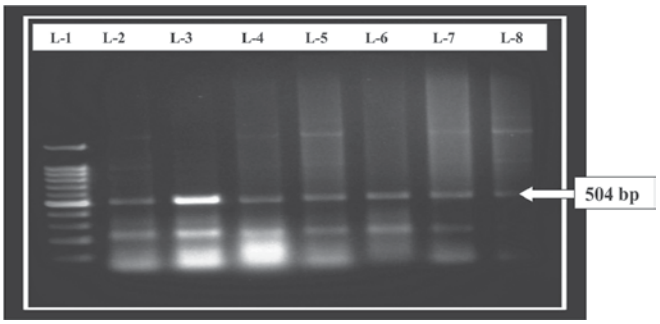

Plate 7: Species specific PCR product positive for *Anaplasma platys* (504 bp)

Lane 1	100 bp DNA marker
Lane 2, 3, 4, 5, 6, 7 and 8	Positive test samples

The study was taken up to detect the occurrence of E.canis and A.platys infection in dogs in and around bengaluru. parameters such as blood smear, buffy coat smear, ELISA rapid Assay test kit and PCR were need for diagnosis. None of the cases in the present study were positive for ehrlichia and anaplasma organisms in direct blood smear as well as buffy coat smear examination. This might be due to the fact that the percentage of *E. canis* infected cells in the peripheral blood is low as indicated by Iqbal *et al*. 1994 and Harrus *et al*. 1998.

The stage of infection and level of parasitemia is important while demonstrating the organisms by blood smear examination and it requires more number of blood smears to be examined. Further, faster the processing and examination of the smears, greater are the chances of getting positive results as there are chances of disintegration of parasites in the collected blood samples and this could be the reason for the lack of success in finding morula in blood and buffy coat smears.

According to Shaw *et al*. (2005), the method of detecting morulae in platelets during an *A. platys* infection has a low sensitivity. Besides, Harvey (2012) stated that this is not a reliable method of diagnosis as the parasites are either absent or present in very low numbers.

Of the 50 clinical cases suspected, 11(22%) cases were found positive for canine ehrlichiosis, seven (14%) cases were found positive for Canine Anaplasmosis (*A. platys*) and 4% of the cases revealed mixed infection with ehrlichiosis and anaplasmosis (*A. platys*) by the ELISA Rapis Assay Test kit. This is in accordance with the findings of Wise and Tarlinton (2011) who reported 19% cases as ehrlichiosis, 21% cases as anaplasmosis (*A. platys*) and 5% cases as co-infection between these two species, in a study of vectorborne diseases in free roaming dogs in Goa.

Of the 50 samples, 41 (82%) were positive for the presence of ehrlichia DNA with genus specific primers and all 41 (82%) samples were also positive for *E. canis* species with nested PCR. This is similar to the findings of Harrus *et al.* (1998), Murphy *et al*. (1998), Asha *et al.* (2004), Bindu *et al.* (2007b), Carvalho *et al.* (2008), Nakaghi *et al.* (2008), Saira Banu *et al*. (2009), Sunita Choudhary (2009) and Arun (2015) who had detected ehrlichia organisms to the genus and species levels.

Of the 50 samples, 43 (86%) were positive for the presence of *Anaplasma platys* DNA with species specific primers by conventional PCR. This correlates with the findings of Martin *et al*. (2005) who observed 54.54% positive cases by nested PCR and 50% by conventional PCR. Similar findings with lower occurrence was reported by Lasta *et al*. (2013) and Ferreira *et al*. (2007) who reported 14.07% and 15.84% occurrence of *A. platys* in Brazil respectively. Megat Abd Rani *et al*. (2011) observed 6.5% cases positive for *A. platys* in India.

Co-infection of *E.canis* and *A. platys* was observed in 39 (78%) cases by PCR which is in agreement with the findings of Brietschwerdt (1995), Greig and Armstrong (2006) and Sainz (2011) who reported that co-infection with *E.canis* and *A. platys* is common. This could probably be due to transmission of both the organisms by a common vector, most likely *Rhipicephalus sanguineus*, which is known to be the vector for *E. canis* (Nicholson *et al*., 2010) and also *A. platys* (Yabsley *et al*., 2008). Rhipicephalus species ticks are also known to comprise almost 80 per cent of the tick infestations in street dogs in the urban areas of India (Megat Abd Rani *et al*., 2011).

Conclusions

This study has provided evidence of the presence of a high level of *E. canis* in community dogs in Bengaluru and it has also provided evidence of a high level of co-infection with *A. platys* probably because of the fact that both the conditions are transmitted by the same tick vector. Further the study has thrown light to the fact that there is also a high level of *A. platys* infection occurring in dogs in and around Bengaluru, which had

not been reported till date. The high occurrence of these parasites would indicate a significant risk of tickborne diseases in both the human and dog populations in this area, though the identification of the exact vector and pathogen systems involved would require further work.

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Short Communications

Epidemiology of Bovine Mastitis in eastern plain zone (UP-9) of middle Gangetic Plains

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Abstract

The present study was planned with a primary aim to assess the present situation of mastitis in this region of eastern plain zone. Diagnosis was made on the basis of physical examination and screening of milk through battery of tests like California mastitis test, white side test and Mastrip test. A total of 3216 dairy animals (1066 cattle and 2150 buffaloes) presented to the hospital from different districts of middle gangetic plains namely Faizabad, Sultan Pur, Amedkar Nagar etc. An overall incidence of 12.78% of clinical udder infection was recorded. Maximum cases were reported in cows (13.88%) followed by buffaloes (12.23%). Lactation wise cows in their third lactation were mostly affected. Buffaloes in fourth lactation exhibited highest incidence of mastitis. Season wise maximum total incidence was seen in december (16.67%) and least no. of udder affections were reported in may and june (7.17 % each). Overall, highest prevalent problem was acute mastitis seen in 24.82% animal and lowest was udder abscess and burn, each recorded only in 0.49% animals.

Keywords: Mastitis, Bovine, Udder, Incidence and Lactation

Udder is a productive unit of a milch animal, with even a minute defect drastically reducing the production status. Owing to its location, udder is exposed to variety of insults like cascade of micro organisms, manage mental and environmental factors. A slight imbalance in any one may cause microbes to surpass all defence mechanisms of bovine udder resulting in proliferation of the otherwise dormant microbes leading to the development of mastitis and other udder affections affecting the quality and quantity of milk. Once the udder gets affected it not only drastically reduces the production potential of lactating animals but sometimes causes irreparable damage to the affected quarter also thereby causing heavy economic losses to the farmers. It is therefore one of the most economically significant diseases for the dairy industry for backyard farmers in developing countries and high producing herds worldwide, with different levels of economic losses reported by different countries (Tiwari *et. al*., 2013). Conducive environmental factors favour the growth and proliferation of organisms both outside and inside the udder. Morbidity as high as 40% can be recorded in a herd, if it lacks an effective mastitis control programme. Of the various clinical a manifestation, subclinical mastitis is economically the most important due to its long term effects on milk yields (Zafalon *et. al.* 2007). The proper knowledge of the disease, its association with age, breed and season, antibiotic sensitivity pattern is of utmost importance to curtail curb this menace ruining the lactation potential of our milch animals. So the present study was planned with a primary aim to assess the present situation of mastitis in this region of eastern plain zone.

The animals brought to college's Teaching Veterinary Clinical Complex were examined thoroughly and animals with different udder affections formed the subject of the study. Diagnosis was made on the basis of physical examination and screening of milk through battery of tests. Udder was examined for abnormalities like change in size, consistency and contour, presence of any lesions. The size and shape of the udder, individual quarters and the teats was inspected by viewing it from front, on each side and from behind. Milk from animals were examined quarter wise as per number of quarters affected with series of milk tests as by California Mastitis Test, White Side Test, and Mastrip Test. A total of 3216 dairy animals (1066 cattle and 2150 buffaloes) presented to the hospital from different districts of middle gangetic plains namely Faizabad, Sultanpur, Amedkarnagar etc. were examined. On the basis of all these examinations the udder complications were categorised as normal udder, acute mastitis, chronic mastitis, gangrenous mastitis, udder abscess, agalactia, papillomatosis and blood in milk, udder edema, udder fibrosis, teat obstructions and thelitis.

Out of a total 3216 dairy animals (1066 cattle and 2150 buffaloes) presented to the hospital, 12.78%

of animals had clinical udder infection. Maximum cases were reported in cows (13.88%) followed by buffaloes (12.23%).

Analysis of the data revealed higher prevalence in right quarters (54.01%) with 54.05% in cows and 53.99% in buffaloes. Collectively rate of infection was greater in rear quarters (60.83%) than fore quarters 39.17%. Involvement of left fore quarter was recorded in 17.27% whereas right fore, left rear and right rear showed 21.90%, 28.71% and 32.12% involvement respectively. In cows 27.7% right rear were affected followed by right fore (26.35%), left hind (24.63%) and left fore (21.62%). In buffaloes the prevalence was highest in right hind (34.6%) followed in decreasing order by left hind (31.18%), right fore (19.39%) and left fore (14.83%).

Lactation wise cows in their third lactation were mostly affected. Buffaloes in fourth lactation exhibited highest incidence of mastitis.

Season wise maximum total incidence was seen in december (16.67%) and least no. of udder affections were reported in may and june (7.17 % each). In cattle incidence was highest in September (21.81%) and least in June (9.375%). In buffaloes October month recorded the maximum incidence (19.44%) and the month of May had least incidence (2.76%). Season wise incidence is given in table 1.

Out of the total 411 animals examined for clinical udder affections, maximum cases were reported in buffaloes (263/411, 63.99%) followed by cows (148/411, 36.01%). Overall, highest prevalent problem was acute mastitis seen in 24.82% animal and lowest was udder abscess and burn, each recorded only in 0.49% animals. Second most prevalent udder affection was agalactia, reported in 14.60% followed in descending order by chronic mastitis (13.63%) and blood in milk (12.90%), teat obstruction (10.46%), thelitis (11.92%), udder edema (4.87%), gangrenous mastitis (4.62%), udder papillomatosis (1.22%).

Highest problem was acute mastitis found in 23.19% buffaloes followed by thelitis (18.63%), teat obstruction (14.45%), chronic mastitis (13.69%) animals and agalactia (12.17%) buffaloes. Blood in milk was recorded in 9.13% buffaloes, gangrenous mastitis in 7.22%, udder abscess and burn found in 0.76% buffaloes.

In cattle maximum prevalence was of acute mastitis observed in 27.70% cows followed by blood in milk (19.59%), agalactia (18.92%) cows. Chronic mastitis and udder edema each were reported 13.51% cases. In cows papilloma on udder was reported in 3.38% cases. Another 3.38% cows were found suffering from teat obstruction.

The findings are in relation to the earlier findings of Khate and Yadav (2009) who reported 26.43 and 18.91% mastitis in Sahiwal cows and Murrah buffaloes. An alarmingly high incidence was reported by Rani *et al.* (2008) in buffaloes, who recorded mastitis in 58.95% animals, where 22.12% and 77.87% were clinically and sub clinically infected, respectively. The incidence of single quarter infection was 70.04%. The prevalence was higher in rear compared to fore, and right compare

Table: Season wise incidence of udder affections

Month	Cattle (%)	Buffalo (%)	Total (%)
January	16.67	15.44	15.84
February	16.67	6.92	10.2
March	16.28	8.4	11.7
April	10.11	6.0	7.93
May	14.13	**2.76**	**7.17**
June	**9.375**	5.67	**7.17**
July	10.75	7.32	8.38
August	13.26	16.97	15.99
September	**21.81**	13.58	15.57
October	10.81	**19.44**	16.57
November	11.57	15.89	14.48
December	17.18	16.43	**16.67**

to left udder quarters. Mastitis was highest in the right hind quarter (37.9%), followed by the left hind (33.97%), right fore (15.98%) and left fore (11.96%) quarters. Sharma *et al.* (2012) also observed a higher incidence (64.44%) of mastitis of right sided quarters and were of the view that cows mostly sit on right-side with the result right-side quarters are frequently exposed to dung and soil moreover due to pressure of the body of animal the milk dribbles out through the teats of high yielders and thus increasing their susceptibility. Contrary to present findings, Khan and Muhammad (2005) reported higher prevalence of mastitis in left sided quarters. A relatively less prevalence of clinical and subclinical mastitis, 5.5% and 15.75%, respectively, was recorded by Bhatt *et. al.* (2011) in and around Anand. Kumar *et al*., (2010) observed the overall mastitis incidence of 9.28, 3.59 and 4.10% for crossbred cows, indigenous cows and buffaloes, respectively with highest incidence during the rainy season, followed by winter and summer. Singh and Pachauri (2004) and Pachauri *et. al.,*(2001) also recorded higher incidence in monsoons when the rainfall and relative humidity was highest. Rainfall and humidity makes the environment suitable for the growth of pathogenic organisms (Radostitis *et al.,* 2007). The floor too remains wet for longer a period which also favors the microbial growth and colonisation (Deore, 2001). Similarly Doraisamy and Elango (2008) also observed higher prevalence during monsoon followed by winter and summer with higher incidence in right hind quarter followed by left hind, right fore and left fore quarter in both cows and buffaloes. A significant difference ($P<0.05$) of the incidence of clinical mastitis was observed between fore- and hind udder quarters and between right (43%) and left (57%) side quarters by Ulema *et al.,* 2007. the higher incidence of mastitis in hind quarters can be attributed to the greater chances of hind quarters being soiled with urine or from the tail (Kavitha *et al.*, 2009).

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Incidence of cardiac diseases in dogs: A retrospective study

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Abstract

A retrospective study was carried out to ascertain the incidence of cardiac diseases in dogs which were presented at Veterinary Polyclinic, Indian Veterinary Research Institute (IVRI), Izatnagar,Bareilly during past five years. Records were used to obtain data regarding diagnosed cases of cardiac in dogs. An increasing trend was noticed in all cardiac diseases. Males were found to have affected more than females and highest incidence was seen in spitz breed with lowest in Great Dane. An increasing trend of cardiac disease incidence was also seen with increasing age group with highest in geriatric dogs.

Keywords: Incidence, Cardiac diseases, Dogs

Abnormality of any kind in the heart is referred as Cardiac disease. A specific term "Heart failure" refers to a condition when heart is no longer able to pump sufficient quantity of blood to the body at normal filling pressure (Fukuta *et al.,* 2008). Age is the greatest risk factor for heart disease in both people and dogs (Urfer *et al.*, 2017). As much as 15% of all dogs suffer from heart disease and the incidence of which increases to 60% or more for dogs over 7 years of age. Young dogs less than one year of age are in contrast susceptible for congenital malformation which presents a considerate reason of diseased heart and subsequent mortality among them (Buchanan, 1999). Whether congenital or acquired Cardiac diseases may ends into clinical syndrome of systolic or diastolic dysfunction. Almost 95% of heart diseases in canine are acquired of which 75% are valvular diseases and Dilated Cardiomyopathy (Rush, 2002). Being the most common cause of heart failure in dogs Chronic mitral valve disease has the highest occurrence (Borgarelli *et al*., 2008; Schober *et al*., 2010) and accounts for 75-80% of cardiac diseases in dogs (Borgarelli *et al*., 2008). Oliveira *et al*. (2011) reported breed and sex predilection of different congenital cardiac conditions. Breed predilection has been reported for some specific cardiac diseases with increased risk for occurrence in them (Urfer *et al*., 2017). Dilated cardiomyopathy (DCM) is the most common cardiac disease in large breed dogs and is reportedly inherited in Doberman Pinschers with a high prevalence (58%) as supported by Wess *et al*. (2017). Degenerative valve disease is found more commonly occur in small breeds than large dogs, with high risk on male dogs (Olsen *et al*., 1999). It has been reported that Cavalier King Charles Spaniels, Miniature Poodles, Miniature Schnauzers, Chihuahuas, Pomeranians, Fox Terriers, Cocker Spaniels, and Pekingese breeds tend to be particularly prone to Myxomatous mitral valve disease (Kim *et al*., 2017). However, cardiac diseases are common among the dogs the information regarding the specificity of different cardiac diseases with respect to age, sex and breed is meager. Thus, the present study was carried to investigate and record cardiac diseases IVRI-RVP incidence in order to specify the distribution trend of different cardiac diseases with respect to age, sex and breed in dogs.

The study was undertaken in Division of Surgery, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. A retrospective analysis was performed in order to estimate prevalence of cardiac disease among dogs on the basis of hospital incidence during past five years. The recorded data were reviewed to retrieve information regarding age, sex, breed and cardiac dysfunction. Cardiac diseases only with confirmed diagnosis on the basis of radiographic, electrocardiographic and echocardiographic examinations were included in the study. The data were analyzed to draw out trend and incidence of occurrence.

In the present study of cardiac disease was quiet less against total recorded diseases with a total of 131 confirmed diagnosed cardiac cases alone from IVRI-RVP during last five years. Still we cannot push aside its significant incidence and lethal outcome. Analysis exhibited an increasing trend for recorded various and total cardiac disease from 2013-14 to 2017-18. It was found that there is 54% increase in the incidence of

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cardiac diseases from 2013-14 to 2017-18, and systolic dysfunction was highest among the different cardiac diseases during the entire period (Fig.1).

Similar trend has also been observed with increasing age (Fig.2). We recorded increased vulnerability of geriatric dog for cardiac diseases especially systolic dysfunction and dilated cardiomyopathy (Fig.2). It has been accepted that the frequency of cardiovascular diseases increases with ageing in dogs (Hamlin, 2005). As reported by authors cardiac disease tend to be age dependent in dogs as the prevalence increase in 4-5 yrs and above (Borgarelli *et al*., 2004). Numerous changes of the cardio-vascular system that is manifested as morphological, functional, endocrinological, genetic and biochemical points could result from aging. However, the actual negative correlation between cardiovascular function with ageing which reflect specific cardiovascular degradation is difficult to ascertain (Kosić *et al*., 2017).

Cardiac diseases including Diastolic dysfunction, Systolic dysfunction, Ventricular septal defect, Ventricular hypertrophy, Mitral valve disease (MVD), Dilated cardiomyopathy (DCM) and Congestive heart failure (CHF) were recorded during the specified period with varied incidence (Fig.1). Maximum incidence was seen for systolic dysfunction followed by dilated cardiomyopathy (Figure 1). Rush (2002) also reported that 95% of heart diseases in canine are acquired, of which 75% are valvular diseases (atrioventricular valvular insufficiency) and Dilated Cardiomyopathy. They both often contribute to successive Congestive heart failure in dogs (Serres *et al*., 2007; Schober *et al*., 2010). Irrespective of mentioned two preceding events i.e. MVD or DCM for CHF we recorded a high number of Congestive heart failure cases. Their occurrence has been reported in dog affecting many breeds (Dambach *et al*., 1999,Calvert, 1986, Goodling *et al*., 1882). We recorded occurrence

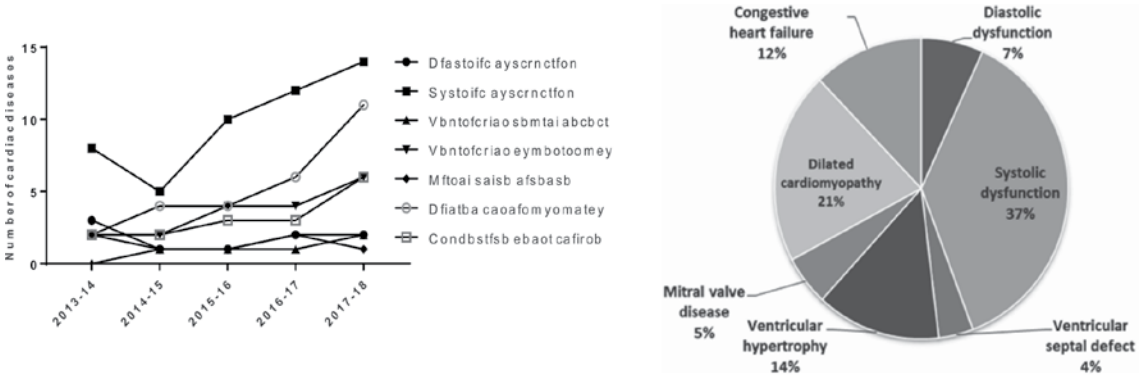

Fig. 1. Trends and percentage of different cardiac diseases in dogs diagnosed in Division of Surgery, IVRI, Izatnagar during the period of 2013-2018.

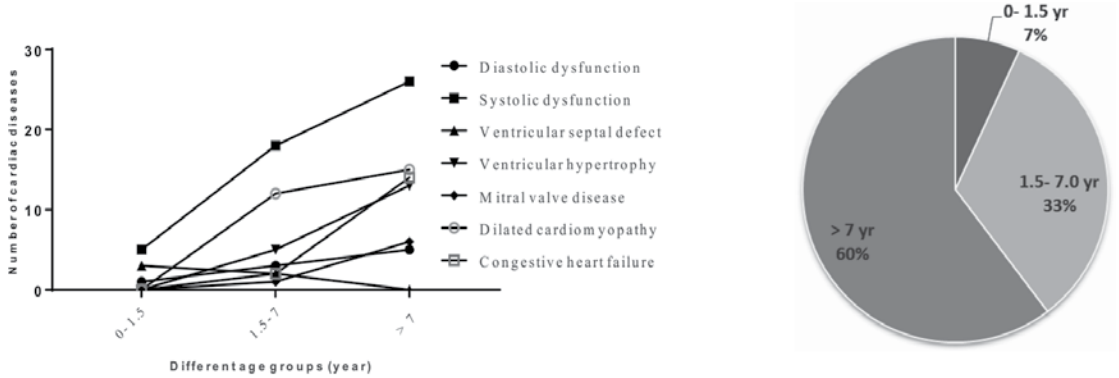

Fig. 2. Trend and percentage of cardiac disease incidence in different age groups of dogs diagnosed in Division of Surgery, IVRI, Izatnagar during the period of 2013-2018

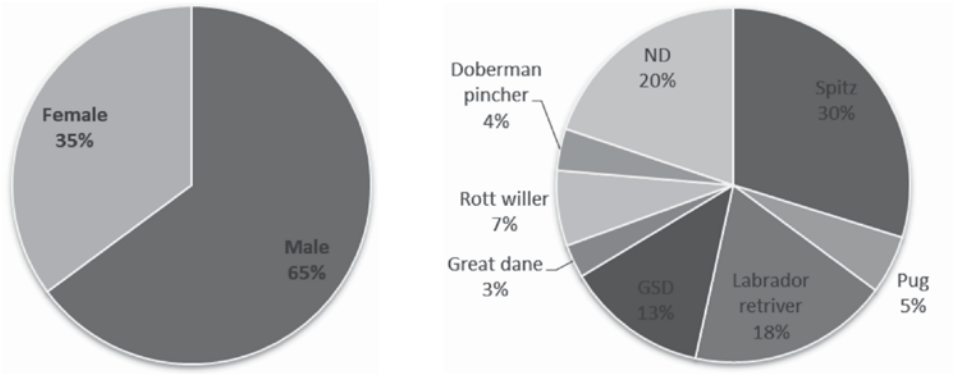

Fig. 3. Percentage of sex and breed wise distribution of cardiac disease in dogs diagnosed at Division of Surgery, IVRI during the period of 2013-2018

in various breeds including Spitz, Pug, Labrador Retriever, German Shepherd, Great Dane, RottWeiler, Doberman Pinchner and non descript breeds suffering from the ailment. However incidence among each breed was varied to a great extent. Incidence was recorded highest (30%) in spitz and lowest (3%) in Great Dane (Fig.3). Previously many authors have reported about the greater incidence of Chronic valvular disease in smaller breed in comparison to medium and large size breeds (Borgarelli *et al*., 2004). However, diseases like dilated cardiomyopathy are most often associated with large and giant breed dogs (Dambach *et al*., 1999). We recorded high incidence in Labrador Retriever, next to spitz this might be explained in light of obesity and lethargy related to this particular breed. Glickman *et al*. (1989) have documented the prevalence of combined overweight and obesity in domestic canine populations range from 23% to 41%. High blood pressure and heart rates resulted from high fat diet induced abdominal obesity ultimately end up to heart disease due to atrial hypertension and left ventricular hypertrophy (Verwaerde *et al.,* 1999). A sex predilection was also spotted as more number of males (65%) was found affected compared to females (35%) (Figure3). Sisson and Thomas (1995) also reported 2:1 male predilection for DCM in the affected breeds. Chronic valvular heart disease is approximately 1.5 times more common in males than in females (Atkins *et al*.,1999). Whereas some authors also reported no such sex predilection in dogs for congenital heart diseases (Tidholm, 1997).

In conclusion we observed varied incidence of cardiac diseases in dogs which exhibited specific trend of occurrence in the population and support the existence of its correlation with age and sex.

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Brainstem Auditory Evoked Response (BAER) testing with disc electrodes in dogs

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Abstract

Brainstem auditory evoked response (BAER) is widely usedto detect deafness in human beings for many years. It is increasingly being used for dogs. Canine studies with needle electrodes are documented so far. Use of needle electrodes are painful and requires sedation or anesthesia. Disc electrodes allows non-sedation assessments. However not much BAER studies are done in India. In this study a total of 6 dogs were evaluated for brainstem auditory evoked response (BAER) using computerized BAER system with disc electrodes used in human medical practice. Apparently healthy dogs were selected.The mean latency for I, II, III, IV and waves V were 1.78±0.18, 2.68±0.11, 3.69±0.19, 4.71±0.08, and 5.73±0.20 ms in left ear, and 1.78±0.17, 2.79±0.07, 3.72±0.21, 4.67±0.14, and 5.77±0.09 ms in right ear, respectively. The mean inter peak latencies for the I-III, III-V and I-V intervals were 1.89±0.32, 2.00±0.42, and 3.95±0.26 ms in left ear and 1.92±0.25, 4.03±0.19, and 2.09±0.31 ms in right ear, respectively. On BAER analysis all the dogs were found to apparently healthy and free from deafness. This study also highlighted that the computerized BAER system with disc electrodes can be effectively used to assess BAER in dogs and to diagnose the deafness if any.

Keywords: BAER,Disc Electrodes**,** Dogs

The brainstem auditory evoked response (BAER) testing is an electrodiagnostic test which iscommonly employed for assessing auditory function in humans and dogs.The test is objective, reasonably easy to perform, noninvasive, safe, and cost-effective, compared with other objective measures of auditory function (Wilson, 2005).The testing apparatus is portable, and test time is brief. Results are reliable, sensitive, anatomically specific, generally independent of the level of consciousness, and resistant to the influence of drugs and yield a comprehensive index of neurologic status(Hall, 1992).

Modern BAER equipment is typically personal computer based and can be divided into stimulus components and recording components. The stimulus components include a stimulus generator (clicks, pure tones) or bone conduction stimuli (applying vibrations to the mastoid region of the skull) being delivered to theear. The recording components include recording electrodes and amplifier, signal averager, and display screen (Wilson, 2005; Strain et al., 1993 and 1997).Today, all of these components, including medicalsoftwares, can be acquired as asingle package and is supplied by companies specializing in clinical electrodiagnostic equipment. The high cost and specialized nature of electrodiagnostic equipment precludes them from being purchased for general veterinary use.

To record BAER, a noise (usually a multitonal click in veterinary medicine) is delivered to the ear via earphones, to the ear being stimulated. Alternatively, the cochlea can be stimulated directly by vibrating the bone of the mastoid region (with a bone stimulator) on the same side as the ear of interest (Munro et al., 1997). In a clinically normal animal, the BAER recorded from each ear is symmetrical. It should be noted, however, that performing and interpretation of BAERs should only be done by specially trained and experienced clinicians as many factors must be considered when interpreting the BAER.During 19th century BAER has occasionally been utilized to diagnose the brain stem lesions. Nowadays many veterinarians and researchers have turned to BAER, detecting electrical activity in the cochlea and auditory pathways in brain.

The study was conducted at the Canine Cognition Lab of Department of Veterinary Medicine, Veterinary College and Research Institute, Orathanadu, Thanjavur. A total of 6 dogs were included in this studyof brainstem auditory evoked response (BAER).

BAER measurements were recorded by using a standard computerized electrodiagnostic machine (RMS Saluts 4C, Electromyography) used very commonly in human medical practice. Instead of needle electrodes, disc electrodes were used, as they are non-painful to animal subjects / patients.For a two channel recording

we use Cz which is the top of the fore head, A1 for the left ear and A2 for the right ear. Eachun-anaesthetized dogwaspositioned in the sternal recumbency and three non invasive electrodes were placed for the BAER recording. The recording electrode were placed in respective place (Ground: mastoid; Reference: fore head; Active: contra lateral mastoid) and earphone positioned over the dogs ear.The ear canals were examined and cleaned in order to deliver the stimulus correctly. The broad band frequencies wereset at 100 Hz and 3kHz, the sensitivity was set to 0.5µV/cm and the analysis time to 30 ms/cm. The headphone was positioned manually over the external auditory meatus of the dog. Rarefaction clicks were applied at 10 Hz, recording was made at 85 dB intensity. Contralateral ear noise masking done by using 40 dB. An average recording of 1000 sweeps for each ear of each dog was recorded and was stored for later measurement and analysis. In each test, the absolute latencies of waves I, II, III, IV and V, and the I-III, III-V and I-V intervals for each side were measured. Initially, the distribution of variables was analyzed and no abnormalities were found and statistics were produced to characterize the average latency of waves (ms) in the groups studied.

From the available Indian literatures on this subject this study appears to be the first study to describe the use of brainstem auditory evoked response (BAER) testing in dogs in India. The study included a total of 6 dogs. All the dogs were subjected to recording of parameters with digital BAER system. The mean latency for I, II, III, IV and waves V were 1.78±0.18, 2.68±0.11, 3.69±0.19, 4.71±0.08, and 5.73±0.20 ms in left ear, and 1.78±0.17, 2.79±0.07, 3.72±0.21, 4.67±0.14, and 5.77±0.09 ms in right ear, respectively. The mean inter peak latencies for the I-III, III-V and I-V intervals were 1.89±0.32, 2.00±0.42, and 3.95±0.26 ms in left ear and 1.92±0.25, 4.03±0.19, and 2.09±0.31 ms in right ear, respectively. Mean and standard deviation values of wave latencies and inter peak latencies are depicted in table 1. In the current assessment of BAER results all the dogs were found to be free from deafness. In contrast to our study in Brazil normative study conducted by Palumbo *et al.(*2014) reported that out of 40 boxer dogs were subjected to BAER examination of which 3 dogs were found deaf (1 unilateral deafness and 2 bilateral deafness). The prevalence of deafness in different breeds of dog reported by different researchers are Dalmation (21.8% unilateral and 8.0% bilateral), Bull terrier (10.3% unilateral and 0.8% bilateral), Australian cattle dogs (12.2% unilateral and 2.4% bilateral) (Strain, 2004), English Setters (12.7% unilateral and 2.4 bilateral) (Strain, 1996) and English Cocker Spaniels (7% unilateral and 1.8% bilateral) (Strain, 1996) and Border Collies (2.3% unilateral and 0.5% bilateral) (Platt *et al.,*2006).

These BAER parameters of healthy dogs will serve as base line reference values for further studies. This study also documented the computerized BAER system used in human medical practice can effectively be utilized for dogs and for clinical veterinary applications. Another advantage of modern BAER machines used in human medicine is that they have disc electrodes, which are non-painful and most patient friendly. Many documented canine studies used the Conventional BAER systems with needle electrodes, which are painful to dogs and hence they needto be sedated or anaesthetized for optimal recording. But such challenges are overcome with the modern digital BAER units, as they come with surface disc electrodes, which can be attached using special conductive gel. This is more convenient for usage in dogs.As neuro diagnostic testing is very essential in assessment of brain and other neurological disorders, these portable digital models of BAER can be very effective for veterinary application and for selection of deafness free animals for dog breeding purposes. Deafness free dogs are essential for security and military works. Hence evaluation of such dogs will be best, if BAER assessment is made an integral part of their selection, health care and performance assessments.

Table 1: mean and standard deviation values of wave latencies and inter peak latencies (ms) of left and right ear

Particulars	Wave I latency	Wave II latency	Wave III latency	Wave IV latency	Wave V latency	Wave I-III latency interval	Wave I-V latency interval	Wave III-V latency interval
Left ear	1.78±0.18	2.68±0.11	3.69±0.19	4.71±0.08	5.73±0.20	1.89±0.32	3.95±0.26	2.00±0.42
Right ear	1.78±0.17	2.79±0.07	3.72±0.21	4.67±0.14	5.77±0.09	1.92±0.25	4.03±0.19	2.09±0.31

Acknowledgement

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Skin and ocular irritancy studies of ozoomommy oil (intra mammary formulation) in rabbits

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Abstract

OzooMommy Oil is ozonized olive oil claimed as a 100% natural product. Present study was undertaken with an objective to test the formulation for skin and ocular irritancy in rabbits as an animal model, before its clinical use. Skin and ocular irritancy studies were undertaken in rabbits as per standard methodology. Results of present study demonstrated that the OzooMommy Oil caused non-significant skin and ocular irritation and found suitable for its clinical use as an intra-mammary formulation.)

Keywords: Mastitis, Antibiotic treatment, OzooMommy Oil, Skin And Ocular Irritancy

Mastitis is inflammatory reaction of the udder tissue. It causes heavy economic losses to dairy industry. Milk from cows suffering from mastitis showed marked physical, chemical and microbiological changes. In subclinical mastitis, pathogens are absent in milk, but it leads into increased somatic cell count, whereas, in clinical mastitis clinical signs of abnormal udder are variable (Galdhar *et al.,* 2005). Antibiotic treatment is commonly undertaken to treat bacterial mastitis but antibiotic residues are more concern with milk export quality and public health significance, this gives emphases on use of antibiotic free drugs for mastitis treatment. Ozone (O_3) is an unstable polymerized oxygen which is created by the passage of air or oxygen over high energy electrodes within an ozone generator system or by ultraviolet light . After a short period of exposure, bacteria, spores and viruses may be inactivated by ozone therapy. Ozone shows its efficacy with different mechanisms including the activation of erytrocytes and immune cells and it is a disinfectant against the anaerobic bacteria (Elvis and Ekta, 2011). Clinical use of ozone therapy in clinical mastitis and endometritis is well documented (Zobel *et al.*, 2014 and Enginler *et al.,* 2015). Present study was undertaken to test the OzooMommy Oil (Intra-mammary ozonized olive oil formulation) for skin and ocular irritancy in rabbits, before its clinical use in dairy animal mastitis.

Present work was carried out at Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Bombay Veterinary College, Parel, Mumbai. Present study was initiated after permission and approval from college level research project committee/PMC cell (Resolution No: 2/XIII/2015; Dated 28.11.2015) and Institutional Animal Ethics committee (IAEC), Bombay Veterinary College (Project Approval No: MVC/IAEC/34/2015; Dated: 17.10.2015).

OzooMommy Oil is ozonized olive oil claimed as a 100% natural product. Test samples of OzooMommy Oil (10 ml, intra-mammary formulation) were supplied by HKL Pharmaceuticals India Pvt. Ltd. (OPC), Kandavali, Mumbai (India).

Three rabbits each (02 males and 01 female) weighing 1.8-2.2 kg were used for skin and ocular irritancy study. The rabbits were fed conventional fodder and water ad libitum for 7 days before test and during the testing period.

A day before commencing the skin irritation testing, the rabbits were clinically evaluated for sound health and noticeable skin abnormality. The skin irritation study was performed on one rabbit initially; following remaining two rabbits. In all the rabbits, fur on both flanks was clipped (Approximately 6 cm^2). For asses skin irritation, 0.5 ml of OzooMommy Oil was directly applied on intact skin of right flank, whereas 0.5 ml of distilled water was directly applied on intact skin of left flank, respectively. Further, each site was covered with semi-occlusive patches for an exposure period of 4 hours. The individual evaluation of the sites was made as per the methods outlined by More *et al* (2013) at 4hrs, 24hrs, 48hrs and 72 hrs after the removal of patch to determine the score for primary irritation (SPI) for each rabbit.

A day before commencing the skin irritation

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testing, the rabbits were clinically evaluated for sound health and noticeable ocular abnormality. The ocular irritation study was performed on one rabbit initially; following remaining two rabbits. In all the rabbits, 0.1ml of OzooMommy Oil was administered by means of direct application on the bottom of the conjunctival sac of the right eye, while the left eye served as control. After one hours, test material was washed out with normal saline solution. All the rabbits were evaluated for ocular irritancy at 1 hrs, 24 hrs, 48 hrs and 72 hrs post administration as per the methods outlined by Draize *et al* (1994). Further, all the animals were monitored for any abnormal signs viz. erythema, edema, discharge from eye, response to light intensity and corneal cloudiness.

Results of score for primary irritation (SPI) are summarized in Table No: 01 and Plate No: 01, respectively. In the present study, there was nix evidence of Erythema and edema of the skin up to 72 hrs of post application of OzooMommy Oil in all the rabbits with zero score for primary irritation (SPI).

Results of ocular irritation study are summarized in Table No: 02 and Plate No: 02, respectively. After, the direct application of OzooMommy Oil on the bottom of the conjunctival sac of the right eye, all the rabbits expressed mild discomfort (evidence of inspection findings as shaking of the head and attempting movement of paw to right eye, however, the discomfort was subsided after 5 minutes and the animals resumed eating as usual, once placed in their cages. Present study demonstrated that, all the animals were free from ocular irritation up to 72 hours. There was no signs of erythema, edema, discharge from eye, response to light intensity and corneal cloudiness.

Results of present study demonstrated that the OzooMommy Oil caused non-significant skin and ocular irritation. However, a mild discomfort concern with ocular administration was recorded, which was subsided after 5 minutes and the animals resumed eating as usual, once placed in their cages. These findings are hopeful to use this formulation in clinical use. Enginler *et al.,* (2015) studied comparison of intramammary ozone administration doses in dairy cows with clinical mastitis in turkey. They reported that high doses ozone and high dose ozone in combination with antibiotic can be effectively used to treat clinical mastitis. However, in our case, use of ozone for treatment of mastitis is proposed, on the other hand, before its clinical use, present formulation was evaluated for skin and ocular irritation in rabbits, where OzooMommy Oil found suitable for its clinical use as an intra-mammary formulation.

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Authors are thankful to Associate Dean, Bombay Veterinary College for providing needful facilities to undertake the present research work. Authors are also thankful to HKL Pharmaceuticals India Pvt. Ltd. (OPC), Kandavali, Mumbai (India) for providing test samples and financial assistance.

Table 1: Score for primary irritation (SPI) after application of OzooMommy Oil and Distilled water

Rabbit	OBSERVATIONS							
	RIGHT FLANK (OzooMommy Oil)				LEFT FLANK (Control-Distilled water)			
Parameter:- Erythema								
	4hr	24 hr	48hr	72hr	4hr	24hr	48hr	72hr
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
Parameter –Edema								
	4hr	24 hr	48hr	72hr	4hr	24hr	48hr	72hr
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0

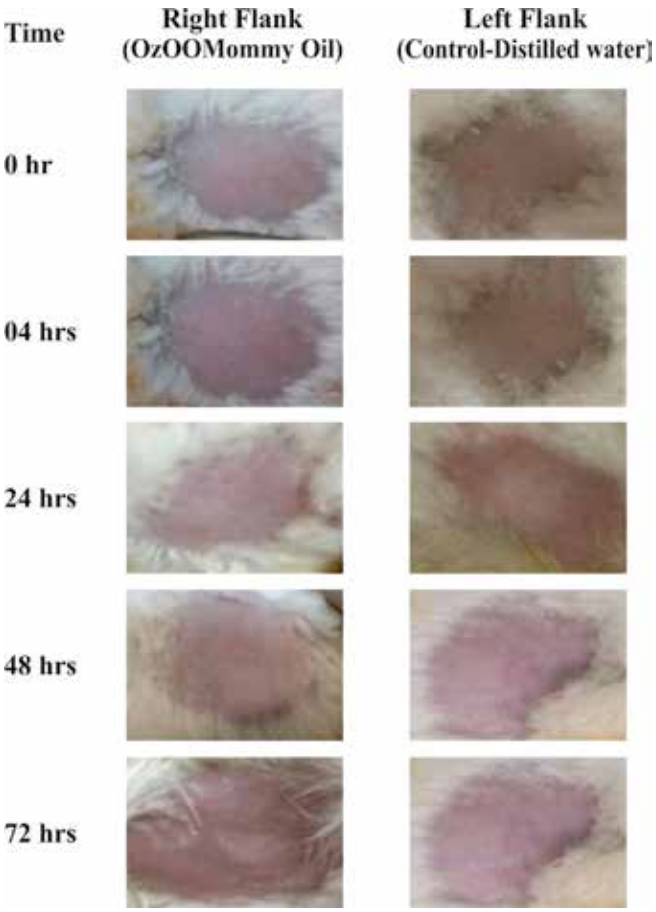

Plate 1: Skin irritation study after application of ozoomommy oil and distilled water

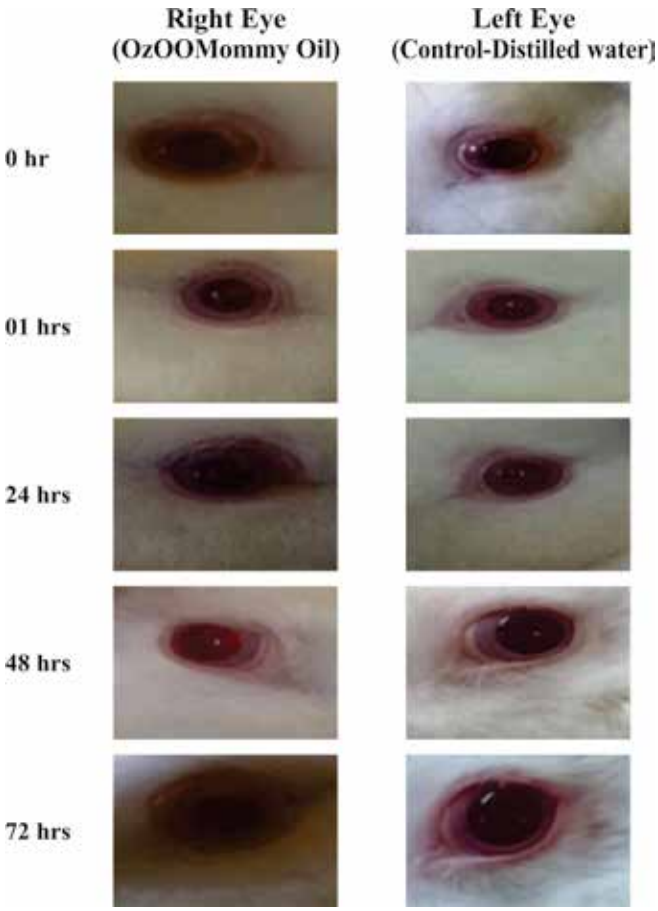

Plate 2: Ocular irritation after application of Ozoomommy oil

Table 2: Score of ocular irritation after application of OzooMommy Oil and Distilled water

Rabbit		Right eye (OzooMommy Oil)				Left eye (control)			
		cornea	iris	conjunctiva	chemosis	cornea	iris	conjunctiva	chemosis
R1	0 hr	0	0	0	0	0	0	0	0
	1 hr	0	0	0	0	0	0	0	0
	24 hr	0	0	0	0	0	0	0	0
	48 hr	0	0	0	0	0	0	0	0
	72 hr	0	0	0	0	0	0	0	0
R2	0 hr	0	0	0	0	0	0	0	0
	1 hr	0	0	0	0	0	0	0	0
	24 hr	0	0	0	0	0	0	0	0
	48 hr	0	0	0	0	0	0	0	0
	72 hr	0	0	0	0	0	0	0	0
R3	0 hr	0	0	0	0	0	0	0	0
	1 hr	0	0	0	0	0	0	0	0
	24 hr	0	0	0	0	0	0	0	0
	48 hr	0	0	0	0	0	0	0	0
	72 hr	0	0	0	0	0	0	0	0

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Analysis of environment around thermal power plant with respect to Cadmium

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Abstract

The study was taken to assess the impact of fly ash emitted from the coal fired Thermal power plant on the surrounding environment in terms of elevation of level of heavy metal particularly cadmium in the environment. Flyash contains number of heavy metals and cadmium is one of them.The level of cadmium in air around the power plant was 2.05±0.0015 ng/m³ which was within permissible range in urban areas. Samples of exposed water samples showed cadmium level of 0.000845±0.000098 ppm which was higher than control samples of water 0.0015±0.00036 ppm. Cadmium level in exposed samples of fodder was 1.2063±0.202 ppm while as the control samples showed a lower level of 0.1892±0.068 ppm. Exposed soil samples showed a cadmium level of was 1.2063±0.202 ppm which was higher than the level of cadmium in soil in control samples of soil (0.2342±0.006 ppm).The level of cadmium in exposed fodder 0.30775±0.067 ppm which was higher than control samples of fodder(0.1892±0.068 ppm). The level of cadmium in exposed blood and milk of lactating cattle was 0.047±0.014ppm, 0.002799± 0.00052 ppm respectively which was non significantly higher than control samples of blood (0.040 ±0.033ppm) and milk (0.001593 ±0.000107). Exposed urine samples of lactating cattle was 0.001616 ±0.0001ppm which was significantly lower than control samples of urine (0.001741±0.000053). The Haemato-biochemical parameters *viz.,* hemoglobin, AST, ALT, serum creatinine and BUN of exposed cows were insignificantly less than control cows.

Keywords: Thermal, Power, Cadmium, Cattle,

Coal fired Thermal power plants generate fly ash (Kanchan *et al,* 2015).It contains trace amounts of some heavy metals like Molybdenum, Mercury, Selenium and Cadmium etc. (Adriano *et al*, 1980). Koradi Thermal Power station is a coal based thermal power station located in Koradi village in Nagpur city of Maharashtra since1976 .The fly ash generated from the combustion of powdered coal in thermal power plant spreads around over large area and gets dispersed in air and finally deposited on the soil, water bodies and vegetation and finally enters in to animal food chains. Fly ash is one of the numerous substances that cause air, water and soil pollution, disrupt ecological cycles and set off environmental hazards (Kanchan *et al,* 2015). The Increasing amount of fly ash being generated from thermal power plants can pose a serious environmental threat (Nalawade *et al,* 2012).

The study was conducted to assess the impact of fly ash emitting from the thermal power station on the level of Cadmium in air, water, soil, fodder as well as in body fluids like blood, milk and urine of lactating cattle reared in the area around the Thermal power station and the level was compared with the unexposed control samples taken far away from Thermal power plant.

Glass vials of 5, 10, 20 ml capacity ,test tubes, plastic bottles and beakers were cleaned ,washed and sterilized in hot air oven and autoclave and stored till use. Dispovan syringes were used for the blood collection. Hot air oven, Inductively coupled plasma – optical emission spectrometer, Microwave Digester and high volume Respirable dust sampler were used.

Twenty lactating cows were randomly selected within a radius of 5km of Koradi Thermal Power Plant in villages namely Surradevi and Khapri for blood, milk and urine sampling and twenty lactating cattle were selectedfrom Agriculture Dairy farm, Deolapar Cattle Farm and Cattle Breeding Farm of Nagpur Veterinary as a healthy control group. Blood samples of 10 ml were collected from jugular vein in the 10 ml syringes, 5ml kept in Sodium citrate @ 0.2ml of 10% solution for cadmium estimationwhile as 5ml was collected in sterile EDTA for Haemato biochemical estimation. Urine samples from different age groups of cows were collected by manual compression of urinary bladder per rectal in clean and dry sterile vial fitted with rubber stopper and labelled properly. At the time of clinical examination the external genitalia of the cow was cleaned properly and 50ml urine from midstream was collected and stored in clean and dry sterile vial and stored at -20°C for selenium estimation.Milk samples from cows were collected from individual quarter in

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a clean and dry sterile vial fitted with rubber stopper and labelled properly. Before milking the udder and teats were cleaned properly. Firstly 2-3 streaks of milk were discarded and 50ml of milk was collected in sterile vial and stored at -20°Cfor selenium estimation. Environmental sampling like sampling of air, water, soil & fodder was done within the radius of 5km around Koradi Thermal Power plant in villages like Surradevi and Khapri and environmental sampling was also done at Agriculture Dairy Farm, Deolapar Cattle Farm and Cattle Breeding Farm of Nagpur Veterinary College as control. For soil sampling, the top soil was scrapped to remove the surface litter with the help of wooden plank. 'V' shaped pit was dug up to 22cm and thick uniform slice (2.5cm) of soil was taken from top to bottom. A total of 6 samples at different places around Koradi Power Plant were taken in clean and sterile polythene bags of 500 gm capacity each. For comparative analysis, 6 samples of soil were also taken from Agriculture Cattle Farm, Deolapar Cattle Farm and Cattle Breeding Farm of Nagpur Veterinary College. The soil samples were dried in hot air oven at 100±5 ℃ overnight and these were finally grinded.Fodder samples were taken from 6 different locations around Koradi Thermal Power Station. At selected areas fodder was taken in polythene bags and then kept for drying in hot air oven. Respirable dust sampler was kept at two different places within 5km range around Koradi thermal power plant overnight in villages Surradevi and Khapri. Water sampling was done in 500ml capacity sterile bottles at 6 different places within 5km range around thermal power plant. Bottles were first rinsed with water 4 to 5 times and then samples were taken.

Fig. 1: Respirable dust sampler

Cadmium estimation was done at National Environmental Engineering Research Institute (NEERI), Nagpur using INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETR (ICP-OES) (Model: THERMO ICAP 6300 DUO. Made by:THERMO FISCHER SCIENTIFIC,UK). All the samples of blood, milk, urine, fodder, water and soil were digested with conc.HNO3 and their volumes were made up to 100ml by adding distilled water and kept in clean and dry vial fitted with rubber stopper and and stored at -20^{0}c until estimation. Standards of 1%,5% and 10% were prepared for calibration. Before running the samples in ICP-OES, instrumental parameters of the system were adjusted according to the manufacturers manual. About 1hr prior to measurement, instrument was adjusted according to working condition. Initially standards were run to obtain the linear calibration function of the system and after obtaining the sensitivity and stability of the system firstly a blank reading were taken by running a distilled water sample in the system. After this one by one all samples were taken and distilled water was used for rinsing the instrument after each sample was taken.

The data which was obtained after compilation of the results were statistically analysed by standard method and technique as outlined by **Snedecor** and **Cochran** (1994).

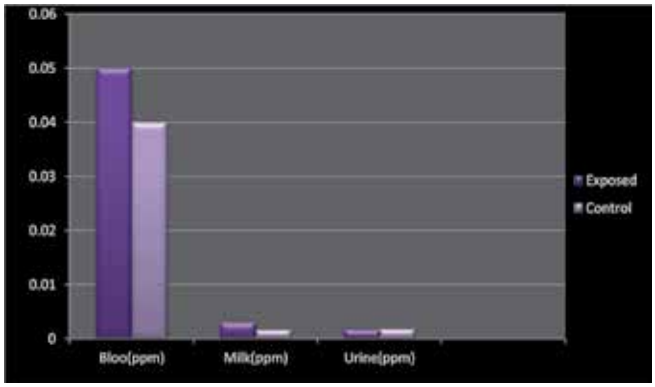
The study was conducted in 2012 in Nagpur Maharashtra. The level of cadmium in air around Thermal power Plant was 2.05ng/ m^3±0.0015.Ambient air cadmium concentrations have generally been estimated to range from 0.1 to 5 ng/m^3 in rural areas, from 2 to 15 ng/m^3 in urban areas, and from 15 to 150 ng/m^3 in industrialised areas (Elinder 1985, WHO 1992, OECD 1994).The level of cadmium in soil

Table 1. Cadmium Concentration in Blood, Milk and Urine of exposed and unexposed group of

Sr.No	Cadmium				
	Particulars		Average±S.E	f value	Significance
1	Blood	Exposed	0.05± 0.014	3.7	NS
		Control	0.040 ±0.033		
2	Milk	Exposed	0.003 ±0.00052	4.14	NS
		Control	0.001593 ±0.000107		
3	Urine	Exposed	0.001616 ± 0.0001	49.93	Significant
		Control	0.001741 ±0.000053		

NS=Non Significant

around the power plant was 1.2063±0.202 ppm Which was higher than the level of cadmium in control soil samples (0.2342±0.006 ppm) taken far away from Thermal Power station The average natural abundance of cadmium in the earth's crust has most often been reported from 0.1 to 0.5 ppm, but much higher and much lower values have also been cited depending on a large number of factors. Igneous and metamorphic rocks tend to show lower values, from 0.02 to 0.2 ppm whereas sedimentary rocks have much higher values, from 0.1 to 25 ppm (Sadeghi *et al*, 2014). The water samples taken around the power plant have cadmium level of 0.000845±0.000098 ppm while as control samples of water have shown cadmium level of 0.0015±0.00036 ppm. Kanchan *et al*, 2015 has recorded a cadmium level of 0.002ppm to 0.004 ppm at open dumping sites of wet Flyash slurry near Paricha Thermal Power plant, Jhansi. The level of cadmium in fodder around



Graph. 1. Graphical Representation of Selenium Concentration in Blood, Milk and Urine of exposed and unexposed cattle.

the power station was 0.30775±0.067 ppm while as control samples of fodder were having 0.1892±0.068 ppm. The normal amount of Cadmium in the rations of cows has been determined to be 0.1 to 0.2 ppm and its possible poisoning occurs above 50ppm (Sadeghi *etal*,2014). Samples of blood taken from exposed cattle around thermal power plant were having average value of 0.05± 0.014ppm which is non significantly($p< 0.01$) higher than control blood samples showing a cadmium level of 0.040 ±0.033ppm.0.047±0.014ppm. Milk samples of exposed cattle were having cadmium level0.003 ±0.00052ppm which is non significantly($p< 0.01$) higher than level of cadmium in control milk samples0.001593±0.000107ppm. Exposed urine samples registered a cadmium level of 0.001616± 0.0001ppm which was significantly ($p<0.01$) lower than control group level of 0.001741±0.000053ppm. These findings may be due to high biological half time of cadmium in the body of the animal as well as due to interaction between cadmium and other heavy and light metal toxicities which animals are susceptible near thermal power plant.

The mean haemoglobin level in lactating cows from exposed area was 10.98±1.03 g% compared to 11.04±0.16 g% in control group (Table 2). Among the exposed cows, mean serum aspartate aminotransferase (AST) level was 91.93±6.28 IU which is well within normal range. However, this value is insignificantly lower than 97.59±5.00 IU L^{-1} observed in control cows. The mean alanine aminotransferase (ALT) level in exposed cows was 25.24±1.63 IU L^{-1} which falls within normal range. However, it was insignificantly lower than control group (30.52±2.60 IU L^{-1}). Mean serum

Table 2. Haematological and biochemical estimates of exposed and control group of animals

Sr.No	Estimates of the Haemato-biochemical parameters in Exposed and Control group of animals.				
	Particulars		Average±S.E.	fcal	Significance
1.	Haemoglobin	Exposed	10.98±1.03	0.003296	NS
		control	11.04±0.16		
2.	AST	Exposed	91.93±6.28	0.496675	NS
		control	97.58±5.00		
3.	ALT	Exposed	25.24±1.63	2.941899	NS
		control	30.52±2.60		
4.	Creatinine	Exposed	1.43±0.08	3.924481	NS
		control	1.681±0.08		
5.	BUN	Exposed	11.14±.74	2.258618	NS
		Control	12.84±3.82		

creatinine value in exposed cows was 1.43±0.08 mg dL^{-1} which is within the normal range but is comparable with the value of 1.68±0.08 mg dL^{-1} in unexposed cattle and is insignificantly low. The mean blood urea nitrogen (BUN) level in lactating cattle from exposed area was 11.14±1.03 mg dL^{-1}. This was close to the estimated value of 12.84±0.16 mg dL^{-1} in control cows, though non-significantly on lower side. The haematological and biochemical parameters were within normal range and insignificantly lower than those of control cows. The study indicates that the haematobiochemical values of the cattle does not show any abnormal indication yet.

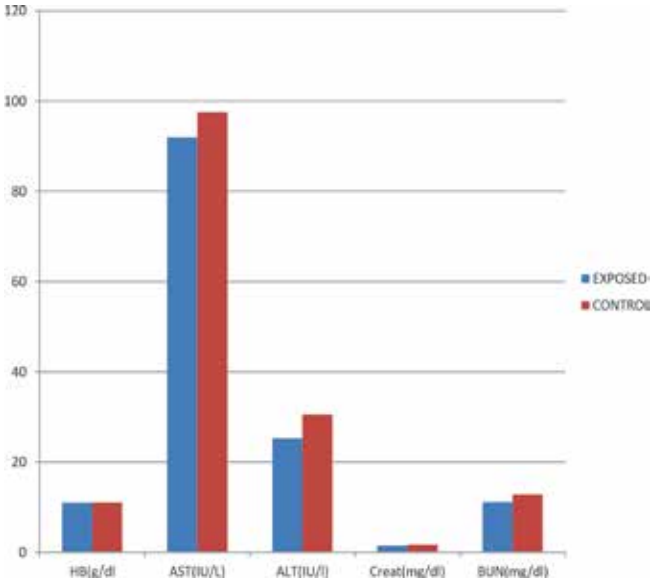


Fig. 2. Graphical representation of Haematobiochemical parameters of exposed and control group of animals.

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Intravenous Clindamycin in the management of *Babesia gibsoni* in a labrador with concurrent cholecystitis

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Abstract

Clinical case is a seven year old female Labrador dog presented to Small Animal Medicine unit of Teaching Veterinary Clinical Complex, Orathanadu. History was anorexia for past 5 days and voiding dark yellow colored urine. Previously she was treated for jaundice by a field Veterinarian and referred here for poor response. Physical examination revealed dullness and icterus of all visible mucus membrane. Peripheral blood smear revealed *Babesia gibsoni*. Hematology revealed anemia and thrombocytopenia. Serum biochemical analysis revealed elevated levels of alkaline phosphatase, alanine aminotransferase and hyperbilirubinemia. On abdominal ultrasound examination, the dog had hepato-splenomegaly, distended gall bladder with cholecystitis. The case was diagnosed as *Babesia gibsoni* with concurrent Cholecystitis and hepatitis in a Labrador and successfully treated with Intravenous Clindamycin along with supportive.

Keywords: *Babesia gibsoni,* Clindamycin, Labrador dog

Canine Babesiosis is the diseased state caused by the protozoal (single celled) parasites of the genus Babesia typically caused by *Babesia canis* or *Babesia gibsoni*. They infect the red blood cells of dog and typically cause hemolytic anemia. Infection with B. *gibsoni* has recently been recognized as an important pathogen than B. *canis* in the Middle East, Africa, and Asia. B. *gibsoni* resulted in more severe clinical manifestations in affected dogs which may leads to multiple organ dysfunction (Sunitha *et al*., 2011) Acute B. *gibsoni* infections are typically associated with fever, lethargy, thrombocytopenia, and anemia.

A seven year old Labrador female dog was presented to the Small Animal Medicine Unit of the Veterinary College and Research Institute, Orathanadu, Tamil Nadu, with a history of anorexia and voiding dark yellow colored urine. On physical examination, the dog had signs of depression, icteric mucous membranes, and was mildly febrile (rectal temperature, 38.7˚C). The abdominal palpation yielded no signs of pain, but the splenomegaly & hepatomegaly could be observed. Results of rectal and fundic examinations were within normal limits. Faecal sample examination did not reveal any ova of parasitic importance. Blood samples were subjected for laboratory assessment of hepatitis. 2ml of blood was collected in EDTA (1.5mg/ml) tube for complete blood count and 2ml of blood for biochemical analysis were collected along with peripheral smear to rule out heamoprotozoan diseases.

Hematologic abnormalities included moderate regenerative anemia (RBC-1.2×10^6/µl; PCV- 9.1%) leukocytosis (24.58×10^3/µl) and thrombocytopenia (10×10^3/µl) and erythrocyte morphologic characteristics were unremarkable (Fig.1). Small piroplasms consistent with *Babesia gibsoni* organisms were identified microscopically on blood smears stained with Giemsa stain. Similar findings were reported by Samradhni et al (2005). Results of serum biochemical analyses indicated moderate hypoalbuminemia (3.2 g/dl), high hyperbilurunemia (7.2 g/dl), moderate elevation in Alanine transaminase (86 U/L) and high alkaline phosphatase activity (268 IU/L). Similar were the findings observed by Johan Schoeman and Andrew Leisewitz (2006). A sample of urine was also collected. Urine specific gravity was 1.053; urinalysis revealed bilirubinuria (4+). No abnormalities were observed on thoracic radiographs, but abdominal radiography revealed splenomegaly, hepatomegaly. In order to investigate suspected liver damage, abdominal ultrasound was performed that showed gallbladder was oval in shape with symmetrically thickened wall (up to 4 mm) that was hyperechoeic when compared with the surrounding liver tissue. The gall bladder was filled with sludge which appeared as uniform echogenic sediment (Aissi and Slimani 2009). Based on the clinical signs, blood smear, haemato-biochemical changes and ultrasonographic findings this case was diagnosed as babesiosis due to Babesia gibsoni with concurrent Cholecystitis.

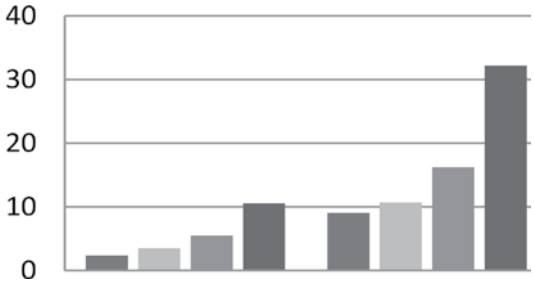

Fig. 1: Comparative diagram on heamatological changes in different treatment days of *Babesia gibsoni* infected dog

An uneventful recovery was recorded following treatment with Inj. Clindamycin @ 11mg/kg IV for two weeks and supportive care with Ursodeoxycholic acid @ 10mg/kg BID PO for 30 days, Silymarin based poly herbal syrup @ 15ml BID PO for 30 days and amino acid syrup @10ml SID PO for 30 days. On 20th day review, dog showed improvements in hematology as well as in serum biochemical values. Parenteral Clindamycin with oral Ursodeoxycholic acids resulted in a effective cure in this dog.

While *Babesia gibsoni* is common in clinical practice, non improvement to therapy may indicate severe infection and concurrent pathologies. Hyperbilirubinemia is uncommon in *B. gibsoni* and its presence in this case indicated severity of infection. Diminazene also seems effective against B. *canis* when administered IM as a single dose of 3.5 mg/kg. However, it does not have the same efficacy against B. *gibsoni*, although it does reduce parasitemia, morbidity and mortality. In this case, Intravenous Clindamycin along with supportive care lessening the severity of clinical signs. Because of the scarce scientific evidence regarding the efficacy of antibiotics in treating canine babesiosis, their use in this case restricted.

Clinical cure and a good therapeutic response are much more likely achieved for infections by large-sized Babesia species than infections by the small-sized species, the latter of which tend to be more refractory to conventional treatments (Laia *et al*.,2016). But in this case, Ultrasonography helped to diagnose the concurrent cholecystitis and helped in therapeutic decision making with intravenous Clindamycin to take care of both the clinical entities. After completion of therapy hemato-biochemical parameters returned to near normal to normal. Further, no recurrence was noticed after therapy. Always assess for concurrent diseases before drug selection for successful recovery in case of *Babesia gibsoni* infections.

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Clinical Articles

Successful therapeutic management of congenital goitre in goat kids- A report on two cases

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Abstract

The current study reports the successful diagnosis and thereafter management of the congenital goitre in two pashmina kids of a goat flock. The diagnosis of the condition made by biopsy smear examination and ultrasonography was followed by treatment with oral supplementation of the potassium iodide @ 20 mg/ kg b. wt. for 10 days. Both the kids had normal sized thyroid glands by 45th day post treatment and follow up of 10 months witnessed no relapse of the condition.

Keywords: Congenital-goitre, Goat, Potassium iodide, Ultrasonography

Case History , Diagnosis and Treatment

Two kids of the same flock, one-day-old female (pashmina boer cross) and one-month-old male (pure pashmina breed) were presented to the clinic with the history of swelling in the ventral aspect of neck region since birth. The size of the swollen part increased progressively in one-month-old kid (Fig. 1a). Both the animals were active with normal suckling. Rectal temperature, heart and respiration rates recorded were normal. Physical examination revealed mild alopecia with bright hair coat and grossly enlarged thyroid glands. On palpation painless swelling with bilateral enlargement (Fig. 1b) and on auscultation a fluid thrill was observed around enlarged area of the neck. Needle biopsy showed absence of any kind of pus/frank blood and/ cyst or proliferative changes. Ultrasonographic examination demonstrated thyroid gland with heterogenic echogenicity (diffuse hypo-echoic foci within hyper-echoic areas) as shown in Fig. 2. All such features including clinical, biopsy and ultrasonographic were suggestive of thyroid hyperplasia or goitre.

The kids were treated with oral supplementation of potassium iodide (KI) @ 20 mg/kg body weight daily for 10 days after which iodism, evident by hair fall and dandruff was observed. At 45th day, thyroid gland had normal size and follow up of 10 months revealed complete resolution of the swelling without any relapse in the condition (Fig. 3).

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Discussion

Goitre, a non-neoplastic and non-inflammatory enlargement of the thyroid gland, can develop in all domestic mammals, birds and other sub-mammalian vertebrates (Constable *et al.*, 2017). Congenital goitre is observed mainly in new-born animals to dams on low iodine intake or failure to get dietary iodine (Singh and Beigh, 2013, Constable *et al*., 2017). In addition, feeding of goitrogenic compounds (interference with thyroxigenesis), excess of dietary iodine and genetic defects in the enzymes (affect thyroid hormones biosynthesis) may also lead to the condition. Foetal thyroid is active in secretion as the dam thyroxine doesn't cross placental barrier. Thyroid hormones T_3 and T_4 are very important for maintaining control over metabolism (Jones *et al.*, 1997). Due to lack of vital ingredients, inadequate production of thyroxine in thyroid gland is sensed by thyroid stimulating hormone that promotes functional change and thereby hyperplasia of the organ (Capen, 1995, 2002).

Most cases of congenital hypothyroidism are associated with multiple late-term abortions, stillbirths or early postnatal deaths (Jones *et al.*, 1997). Animals that are born alive are weak, partly hairless with subcutaneous oedema of head and neck. Dam's deficient in dietary iodine give birth to susceptible kids to develop thyroid hyperplasia with clinical evidence of hypothyroidism (Capen, 2002). In most cases, the only gross lesion evident in aborted or neonatal animals is a bilateral enlargement of the thyroid glands. The

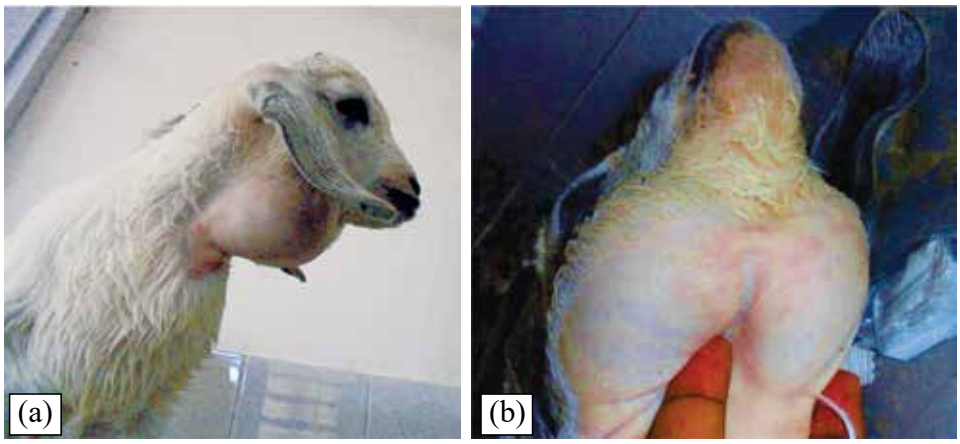

Fig. 1: (a) Gross appearance of enlarged thyroid gland (congenital goitre) in one month old male pashmina kid. (b) Gross appearance of bilobed thyroid gland (congenital goitre) in one month old pure breed pashmina male kid.

Fig. 3. Normal ventral neck appearance of the animals post medication indicating successful management of goitre in goats.

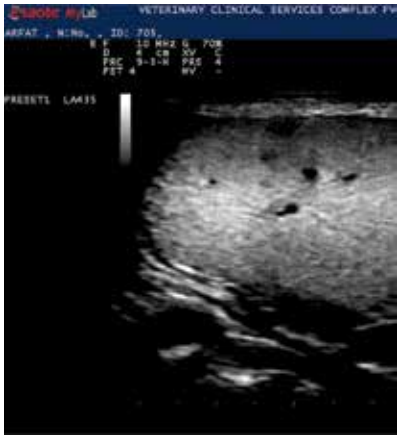

Fig. 2. Ultrasound of thyroid gland (congenital goitre) revealing diffuse enlarged hyper vascular thyroid.

enlargement may be due to simple gland hyperplasia (non-neoplastic and non-inflammatory) or its distended follicular lumens with increased colloid and can be confirmed by histopathology (Capen, 1995; Jones *et al.*, 1997). In addition, high-resolution ultrasonography is the most sensitive imaging modality available for examination of the thyroid gland and associated abnormalities and has been frequently reported in human literature (Chaudhary and Bano, 2013).

Goitre may be primary caused by deficient dietary iodine intake or secondary goitre caused by interference with dietary uptake of water with high content of calcium, nitrates, goitrogenic plants (*Brassica* sp. and some clovers), and, in rare cases, of excessive amount of iodine (Radostits *et al.*, 2007). The area of investigation being rich in clover and often grazed by the animals, might be the inciting cause of the disease.

In the present, the cases were successfully treated with chemical grade potassium iodide @ 20 mg/kg daily for 10 days, which is the cheapest and safest source of iodine. A possible treatment of kids, with deficiency of T_4 and FT_4, is Levothyroxine sodium, which is commonly used in man, has also been studied in dogs and kids by many workers (Ommaty, 2000, Ozmen *et al.*, 2005, Hassan *et al.*, 2013). However, lambs with goitre were treated successfully with 20 mg potassium iodide per os, once (Constable *et al.*, 2017). Ozmen *et al.* (2005) advised owners to add potassium iodate to the feed if the dams are fed cabbage during pregnancy and observed no congenital case of goitre. Wither (1997) also reported similar treatment in cattle.

Conclusions

It was concluded that the goitre in goats is very

rare in this geographical area as no previous published report could be found. The bilateral swelling in the neck region in goats may be suspected as goitre and can be confirmed by histopathology and ultrasonography. Congenital goitre in kid can be successfully treated by the use of oral administration of KI if diagnosed early.

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Therapeutic management of babesia associated jaundice and azotemia in dogs – a clinical report

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Abstract

A total of 3 (three) dogs of different breeds namely Labrador retriever (6 months), Great dane (6 years) and spitz (1 years) were presented to the clinical complex with the complaints of anorexia, vomiting, lethary and yellow discolouration of skin and urine for the last 3 to 5 days.Haematobiochemical examination revealed anaemia, thrombocytopenia, leukocytosis with neutrophilia and azotemia. Treatment was instituted with Imidocarb dipropionate along with supportive therapy and all the cases recovered gradually after day 0 of treatment.

Keywords: Anaemia, Babesia,imodocarb dipropionate, Jaundice

Babesiosis a tick borne haemoparasites continues to pose a threat to dogs population worldwide as a cause of death with clinical abnormalities associated with piroplasmosis is frequently comprised of lethargy, anorexia, icterus, pyrexia, haemolytic anaemia, haemoglobinuria and thrombocytopaenia (Kumar et a., 2013). Jaundice and haemoglobinuria is associated with haemolysis of erythrocytes in babesiosis. Haemolytic jaundice in babesiosis has an acute onset and is accompanied by depression, anorexia and discolouration of the urine in the event of intravascular haemolysis. The present clinical report deals with prompt diagnosis and intensive therapy of babesia associated icterus and azotemia in dogs from the north eastern state of Tripura.

Case History and Observations

A total of 3 (three) dogs of different breeds namely Labrador retriever (6 months), Great dane (6 years) and spitz (1 years) were presented to the clinical complex with the complaints of anorexia, vomiting, lethary and yellow discolouration of skin and urine for the last 3 to 5 days. Detailed clinical examination revealed elevated rectal temperature (102.8 to 103.8 0F), pale conjunctival mucous membrane with yellow discolouration of all visible mucous membrane and skin in addition to mild hepatomegaly and severe spleenomegaly on Ultrasonography. Blood samples were collected from the cephalic veins for the haematobiochemical examination viz., Hb, TLC, DLC, platelets, ALT, AST, serum creatinine, BUN, blood glucose (Random), total bilirubin and direct bilirubin. Blood smear was examined by using Giemsa stain for haemoprotozoan parasites. Treatment was instituted with inj. Imidodocarb dipropionate (Imicarb®) @ 6.6 mg/ kg b. wt. s/c and repeated at 14 days intervals, inj Dextrose 5% @ 10-15 ml/ kg b wt iv daily daily for 7 to 10 days, injection liver extract (Neohepatex®) 1 ml/ 20 kg b wt im for 5 days, injection iron dextran (Imferon ®) @ 1 ml/10 kg b. wt at 3 days intervals for four occasions, tab spironolactone and furosemide combination (Lasilactone®) @ 1tab/20 kg b wt daily for 7 days and tab silibin and SAM combination (Lisybin Medium®) @ 1 tab/15 kg b wt for 2 months.

Results

Giemsa stained blood smear revealed the presence of basophilic dots like Babesia gibsoni infection inside the erythrocytes. Haematology revealed Hb (5 - 7 gm/dl), HCT (16-21.8%), TEC (2.8 - 3.56 x 10^6/µl), TLC (16.2 - 23.5 x10^3/ µl), platelets (56-130 x10^3/ µl) and DLC revealed Neutrophils (74-77.1%), lymphocytes (20 - 14.9 %), Monocytes (4-5%) and eosinophils (2-3%). Biochemical values serum creatinine (6-8.91 mg/dl), BUN (46-67 mg/dl), total protein (5.2-6.3 gm/dl), albumin (3-4.1 gm/dl), blood glucose (80-88 mg/dl), total bilirubin (9-11.2 mg/dl), direct bilirubin (5.6 to 9.6 mg/dl). The haemato-biochemical analysis in our study revealed anaemia, leukocytosis with neutrophilia, thrombocytopaenia, hypoproteinemia and unconjugated hyperbilirubinemia in associated with babesiois (Ajith et al., 2017). Azotaemia associated with babesiosis is in agreement with Bradea et al. (2014)..Jaundice is one of the most common complications of canine babesiosis (Lobetti R.

G., 1998) and anaemia is typically due to intra-vascular and extra vascular haemolysis (Bozer and Macintire, 2005). Ultrasonography revealed mild hepatomegaly with severe splenomegaly. There was gradual improvement in the cases after day 0 of treatment and all the haematobiochemical parameters were found within normal reference values on day 15 of treatment. Imidocarb dipropionate which is a urea derivative developed as an antiprotozoan agent specifically for treatment of parasitic infections. Haematinics was given to overcome the anaemia, along with silibin in blend with S-adenosyl methionine which causes synthesis of glutathione acts as a potent antioxidant were administered to overcome the oxidative stress associated with liver disease. In conclusion authors opined that prompt diagnosis and thorough specific treatment of babesiosis with imidocab dipropionate along with supportive therapy is competent to save the life of critically complicated cases of babesiosis in dogs.

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Clinico-therapeutic management of mange infestation in a rabbit

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Abstract

A six month old, non descriptive, male rabbit was presented at TVCC, Mathura with the clinical picture of generalized alopecia, Pruritis, loss of skin texture and weak body condition. History, clinical examination and microscopic examination diagnosed the condition as sarcoptic mange infestation and treated by using subcutaneous injection of ivermectin once weekly for three week and single sponge bathing with Amitraz solution.

Keywords: Mange, ivermectin, Amitraz.

Among all the dermatological problems sarcoptic mange infestation is considered as the most common and major coerce in rabbit industry in India (Darzi *et al*., 2007, Ravindran and Subramanium 2000). The genus Sarcoptes includes one species i.e. S. scabiei, which is further divided into different variety on the basis of host species (Suckow *et al*., 2002). The term Sarcoptes scabiei is derived from the Greek words "sarx" means flesh and "koptein" means to cut and the Latin word "scabere" means to scratch (Hengge *et al*., 2006). Sarcoptes scabiei is categorized as burrowing mites which lives on epidermis of the skin and infest mammals and humans and complete its life cycle on a single host (Suckow *et al*., 2002). Induction of self limiting dermatitis in human due to handling of infected rabbit has also been reported (Hengge *et al*., 2006). A previous report suggested the incidence of sarcoptic mange in India as approximately 9.3% (Soundararajan and Iyue, 2005). The same report also revealed that White Giant and New Zealand White rabbits are free from mange infestation. Because of its contagious nature the disease it may spread from infected to non infected rabbit by direct skin contact (Panigrahi and Gupta 2013). Overcrowding and poor hygienic conditions are predisposing factors for S. scabiei infection (McCarthy *et al*., 2004). The untreated case of disease results into great morbidity and huge economic loss in livestock. Ivermectin (orally or parentally) can be used as effective therapeutic agent for the treatment of rabbits that are naturally infected with scabies (Kachhawa *et al*., 2013, Aulakh *et al*., 2003 and Erasian *et al*., 2010). The present case report proves the successful management of sarcoptic mange with subcutaneous injection of Ivermectin and tropical sponge bath with Amitraz solution.

Case History and Observations

A six month old, non descriptive, male rabbit was presented at TVCC, Mathura with the clinical symptoms of generalized alopecia, Pruritis, loss of skin texture and weak body condition (Fig. 1). As per owner history rabbit was showing the symptom of itching almost all over the body and rubbing its body with inanimate objects and wall. On detailed clinical examination, it was found that whole area of back, ear and nose were devoid of hairs with somewhat reddening of these areas. Rabbit was also showing the frequent itching over the body. Skin scrapping were taken from deeper part of 2-3 lesions, treated with 10% potassium hydroxide and observed under microscope which was found positive for the presence of mite. So, on the basis of history, clinical symptoms and microscopic examination the condition was diagnosed as sarcoptic mange infestation.

Treatment and Discussion

Treatment was started with subcutaneous injection of ivermectin @ 300µg/kg b wt once weekly for three week along with single tropical sponge bath with Amitraz solution (Ridd[a]) containing 3 ml per liter (Deshmukh *et al*., 2010). Use of Amitraz for the treatment of mange in dogs has been well approved (Ettinger and Feldman, 2000) so it was used in present case. The clinical symptoms were started subsiding from 14th day of start of treatment and the rabbit was fully recovered within 24 days after the start of treatment. Distribution of skin lesions in a specific

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pattern and the occurrence of clinical symptoms were in accordance with those described in various species (Muller *et al*., 1983; Quesenberry, 2000). Diagnosis of disease is usually made by skin scrapping but sometimes the result may become false negative and repeated deep skin scrapping should be taken for detection of mites in such cases to confirm the condition (Birchard and Sherding, 2000). Mange infestation has been reported as major skin diseases in young ones as well as adult rabbits (Siegmu 1979). Sarcoptic mange is a non seasonal and transmissible disease with very intense itching due to burrowing nature of mites (Quesenberry, 2000). Sarcoptes species produces their pathological effects by mechanical damage caused by the parasites during excavation, irritation action of their different body secretion, allergic reactions to some of their extracellular products and especially release of an inflammatory mediator inter-leukin-I (Wall and Shearer 1997). Ivermectin, which was given subcutaneously @300 µg / kg body weight selectively binds to glutamate gated and GABA gated chloride channels in the nervous system of mites, resulting in hyperpolarizations of cells, paralysis and finally death of mites (Aulakh *et al*., 2003). It is recommended to give the treatment of ivermectin in every six month interval to suppress the ear mange (Koopman *et al*., 1989). It was also reported that administration of 3 doses of Ivermectin @ 200-400 µg / kg body weight at an interval of 2 weeks is usually curative in rabbits affected with mange. Disinfection of the rabbit cages and their houses with a blow lamp may become effective in control of mange in rabbits (Darzi *et al*., 2007). Present study indicates the effective use of ivermectin along with tropical application of Amitraz in management of mange infection in rabbit.

Fig. 1. Mange infested rabbit

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Therapeutic management of chronic malasseziosis in a Pug

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Abstract

A 7 year old male pug dog was presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute with a complaint of generalized alopecia, foul smell emanating from body with severe pruritus since 2 months. The case was previously treated for general pyoderma elsewhere. On clinical examination, excoriation at the limbs, bilateral otitis externa, erythema, a rancid, musty or yeasty odor, seborrhea, scaling, alopecia, lichenification, hyperkeratosis and hyperpigmentation of skin was observed. Skin scraping was found to be negative and the simultaneous fungal culture showed heavy growth of *Malassezia* spp. organisms. An impression smear taken from face and body, stained with Giemsa stain and lactophenol cotton blue stain showed positive for yeast cells of *Malassezia pachydermatis*. The confirmatory diagnosis was done by using molecular technique PCR. The treatment was initiated with oral Ketaconazole @ 10 mg/Kg BW BID for one month, Ketaconazole lotion and combination of Ketaconazole plus Chlorhexidine shampoo for topical application, Tab. Cefadroxil @ 22 mg/kg BW, PO, BID for 15 days to control secondary bacterial infection along with supportive therapy using antihistamine, antioxidant and Omega 3 and Omega 6 fatty acids supplementation. After one month of therapy, the dog was completely recovered. Chronic malasseziosis in a dog was successfully diagnosed and treated.

Keywords: Ketoconazole, *Malassezia pachydermatis,* Polymerase chain reaction, Seborrhea, lichenification, hyperpigmentation.

The skin fungal genus, *Malassezia*, is considered to be responsible for development of seborrheic dermatitis (Hay, 2011). Malassezia dermatitis rarely occurs as a primary disease in dogs. It usually occurs concurrently with other disease conditions like atopic dermatitis, keratinization defects and endocrinopathies (Bond *et al.,* 2002). Presence of skin folds in some breeds of dogs may favor the yeast growth and predispose them for malasseziosis (McEwan, 2001). The disease can be observed in focal or generalised form. Commonly observed clinical signs include otitis externa, pruritus, erythema, a rancid, musty or yeasty odour, seborrhea, scaling, alopecia, lichenification, and hyperpigmentation (Nardoni *et al.,* 2008).

Commonly affected sites are ears, lips, muzzle, feet, ventral neck, axilla, medial limbs, and perineum. This case report depicts a confirmation of chronic infection of *Malassezia pachydermatis* by using molecular diagnostic techniques like PCR to find out the species of affecting organism and its successful therapeutic management in a Pug.

Case history and Observations

A seven year old male Pug dog was presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, Bareilly with a complaint of generalized alopecia, foul smell emanating from body with severe pruritus and greasy lesions since 2 months (Fig. 1). The case was previously treated for general pyoderma elsewhere. On clinical examination following skin lesions like excoriations, erythema, rancid odor, seborrhea, scaling, alopecia, lichenification, hyperkeratosis and hyperpigmentation were noticed on inner aspect of ear, limbs, medial thighs, ventral aspect of chin, neck and abdomen. Skin scraping examination was negative. Impression smear was obtained from lesions and was subjected to both Giemsa staining and lactophenol cotton blue staining. Numerous budding yeast cells were observed in both Giemsa staining (Fig. 2) and also in wet mount with lactophenol cotton blue staining (Fig. 3). Further skin scrapings were subjected for fungal culture in Saboraud Dextrose Agar (SDA) as per standard method (Merz and Roberts, 1995). Heavy growth of small, off-white, moist colonies of *Malassezia spp* were observed after 48 hours of incubation (Fig. 4). This was further confirmed by staining. PCR was done

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Fig. 1: Affected dog showing skin lesions

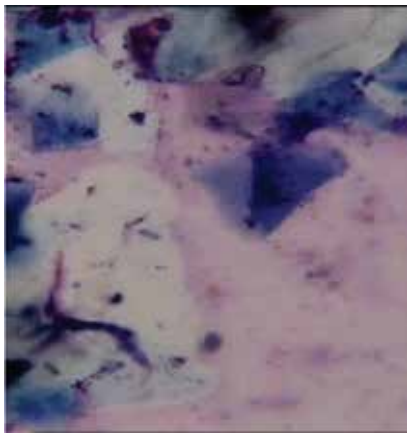

Fig. 2: Giemsa staining showing budding yeast cell

Fig. 3: Wet mount with lactophenol cotton blue showing yeast cells

Fig. 4: *Malassezia* spp. Colonies on SDA

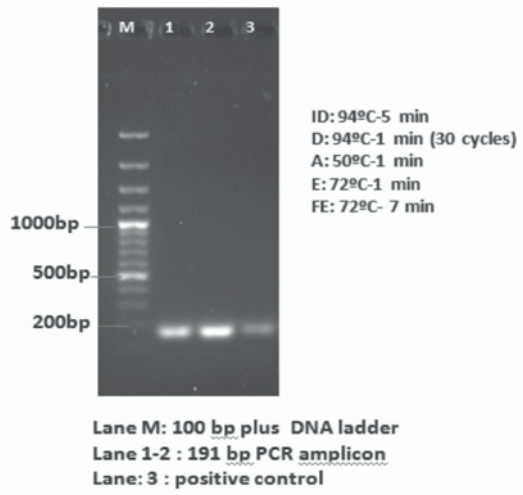

Fig. 5: PCR for *Malassezia pachydermatis*

from this culture by targeting 26s rRNA gene which amplifies 191bp product (Fig. 5).

Treatment and Discussion

The treatment was initiated with oral Ketaconazole @ 10 mg/Kg BW BID for one month, Ketaconazole lotion (2% w/v) for topical application and Ketochlor™ Shampoo (combination of Ketaconazole 1% w/w with Chlorhexidine 2.1 % w/w) for bathing once in five days, Tab. Cefadroxil @ 22 mg/kg BW, PO, BID for 15 days to control secondary bacterial infection along with supportive therapy using antihistamine (Cetrizine, 0.5mg/kg BID per orally for first 7 days), antioxidant (Ascorbic acid, 20 mg/kg OD per orally for 15 days) and Syr. Nutricoat Advance™ (Omega 3, Omega 6 fatty acids, EPA-Eicosapentaenoic acid and DHA-Docosahexaenoic acid supplementation) 1 tsp, PO, BID for one month. After one month of therapy, the dog was again presented in the polyclinic and it completely recovered with no report of pruritus and foul smelling seborrhic lesions in the body (Fig. 6).

Fig. 6: Recovery after 1.5 month of therapy

The skin fungal genus, *Malassezia*, is considered to be responsible for development of seborrheic dermatitis (Hay, 2011). *Malassezia* genus is divided into two groups based on their lipid dependency in culture media- lipid dependent and lipid independent (Cabanes and Theelen, 2007). *Malassezia pachydermatis* is unique within the genus in that it can be cultivated on routine mycologic media without lipid supplementation (Sugita *et al.,* 2005). Dogs are very frequently colonized by *M. pachydermatis* but very rarely colonized by lipid-dependent *Malassezia* spp (Crespo *et al.,* 2000). In dogs, *M. pachydermatis* is frequently isolated from the haired skin of the chin and lips, interdigital skin, and external ear canal and less often from axilla and groin. Clinical signs include localized or generalized (ear, muzzle, interdigital area, ventral neck, medial thigh, axilla) dermatitis with mild or intense pruritus, rancid smell, erythematous, greasy, crusty, hyperpigmented, lichenified, alopecic lesions (Scott, 2001). Otitis externa caused by *M. pachydermatis* is one of the most common diseases of dogs (Bond, 2010). For diagnosis of malasseziosis, positive yeast recovery and identification by cytologic examination (cotton swab smears, skin scrapings, direct impression smears, and acetate tape impressions), culturing, or histopathologic examination of samples collected from affected skin is done (Kennis, 1996). Direct impression smear obtained from lesions on different body areas stained with Giemsa stain will show numerous budding yeast cells (Carmen *et al.,* 2001) and wet mount from fungal culture with lactophenol cotton blue will show blue coloured yeast cells (Roman, 2016). Molecular methods like nested PCR, RT- PCR, PFGE, AFLP, RFLP etc have been used for identification of *Malassezia* (Sugita *et al.,* 2010; Eidi *et al.,* 2011). In this particular case also *Malassezia pachydermatis* infection was diagnosed by conventional techniques (culture, Giemsa staining and lactophenol cotton blue staining) and molecular method (PCR).

Topical therapy is generally the most cost effective and safest treatment for malasseziosis but treatment of severe infections may require systemic therapy with high doses of antifungal agents (e.g., Ketoconazole and Itraconazole) for prolonged periods (Negre *et al.,* 2009). So in this case along with topical application, oral supplementation of Ketoconazole was given as the severity of infection was more. Oral supplementation of Ketoconazole (5–10 mg/kg p/o) and Itraconazole (5 mg/kg p/o) are highly effective as systemic therapy against malasseziosis (Bensignor, 2001). In antifungal sensitivity testing, itraconazole, ketoconazole and posaconazole showed highest activity against *M. pachydermatis* strain, whereas miconazole, terbinafine and fluconazole the lowest as reported by Claudia *et al.,* (2012). Systemic terbinafine (30 mg/kg p/o q24h) is also effective and well tolerated, but is not licensed for dogs (Guillot *et al.,* 2003).

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Magnetic resonance image based diagnosis of brain tumor in a Labrador dog

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Abstract

An eight year old Labrador was presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, Bareilly with a history of anorexia, head tilt to right side with circling movement, fits and bilateral blindness. On clinical examination, there was no corneal opacity, negative blinking reflex, negative menace reflex and normal clinical indices was noticed. On laboratory examination, peripheral blood smear was negative for haemoprotozoa, blood parameters were within normal range with serum creatinine 1.2 mg/dL, blood urea nitrogen 11.6 mg/dL, SGPT 44 IU/L and blood glucose 102 mg/dL. Cerebro-spinal fluid examination revealed a mild mononuclear pleocytosis (20 cells/uL) and an elevated total protein (50.2 mg/dL). No etiologic agents or neoplastic cells were identified. On Magnetic Resonance Imaging, a growth was evident in the third ventricle of the brain with occlusion of the cerebral aqueduct. Based on the clinical and laboratory findings, it was diagnosed to be a case of brain tumor. Animal was given supportive therapy with Inj. Cefotaxime + Sulbactam (25 mg/kg OD IV for 14 days), Tab. Phenobarbital (4 mg/kg OD per orally for 21 days), Tab. Hydroxyurea (30 mg/kg per orally for 8 weeks), Inj. Pheniramine, Inj. Neurobion and steroids. Animal responded well to therapy and started to take food but after twelve weeks animal suddenly succumbed to death.

Keywords: Cerebro-spinal fluid, Tumor, Cefotaxime, Sulbactam, Hydroxyurea

Nervous system tumors are detected in 1-3% of necropsies in dogs. Older dogs are more predisposed for primary brain tumors and breeds at higher risk include the Boxer, Golden Retriever, Doberman Pinscher, Scottish Terrier, and Old English Sheepdog (Heidner *et al.*, 1991). Bagley *et al.* (1999) reported that primary central nervous system tumors arising from mesodermal origin (meningiomas) are the most common intracranial tumors in dogs, followed by neuroectodermal (glial) tumors (astrocytoma and oligodendroglioma).Choroid plexus tumors account for 7–13% of all primary brain tumors in dogs and may lead to obstructive hydrocephalus (Snyder *et al.*, 2006). Little data exist concerning survival of dogs with brain tumors. Increased protein concentration and a normal to mild increase in total nucleated cell count are typically found in intracranial neoplasms (Hugo *et al.*, 2016). Diagnosis of intracranial mass can be done by MRI or CT. Before the advent of advanced diagnostic imaging procedures, such as magnetic resonance imaging (MRI), the detection of a pleocytosis was historically a principal means for confirming central nervous system disease antemortem (Bohn *et al.*, 2006). Due to the inherent high resolution and contrast available with MRI, it is well suited for imaging intracranial neoplasia (Shores, 1993). Preliminary results suggest radiation therapy, when given alone or after surgery, can enhance survival of dogs with primary brain tumors. Treatment guidelines for specific type of brain tumor are lacking in veterinary medicine. Chemotherapy for treatment of brain tumor in veterinary medicine is primarily non-reliable.

Clinical History and Observations

A case of eight year old intact male Labrador Retriever dog was presented to Referral Veterinary Polyclinic, IVRI, Izatnagar, Bareilly with a history of inappetance for 15 days, followed by anorexia for next 3-4 days. Animal was having blindness of both eyes, previous history of *Babesia gibsoni* infection, serous otic discharge from right ear with proper vaccination and deworming history. Clinical examination revealed normal rectal temperature (100.6˚F), pink conjunctival mucous membrane, normal lymph node on palpation, respiration rate (52/minute), heart rate (80 beats/minute). Haematology examination revealed haemoglobin-10.2g%, PCV- 33%, TEC-5×10^6/mm^3, TLC-21.2×10^3/mm^3, DLC: neutrophils-82%, lymphocytes-14%, monocytes-3%, eosinophils-1%, basophils-0%, total platelet count-1.5 lacs/mm^3. Serum biochemistry revealed BUN-11.6 mg/dl, Creatinine-1.2

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mg/dl, SGPT-44 IU/L and random blood glucose-102mg/dl. Peripheral blood smear was found to be negative for haemoprotozoa. On opthalmologic examination, no corneal opacity, no blinking reflex, no menace reflex was noticed. Otic examination revealed redness of ear canal with thick serous discharge. On the basis of clinical signs and laboratory examination it was tentatively diagnosed to be a case of otitis media and treatment was started for the same. After four days, animal was again presented with history of head tilt towards right side and circling with episodes of seizures for two minutes. On neurological examination it was found that there was no nystagmus, no strabismus, no proprioceptive deficit and normal wheel-barrowing reflex. Cerebro-spinal fluid was collected from cisterna magna and examination revealed mild mononuclear pleocytosis (20 cells/µl), elevated total protein (50.2 mg/dl), but no etiological agents or neoplastic cells were found to be present. Blood sample was sent for blood culture but no bacterial growth was seen. For confirmatory diagnosis, Magnetic Resonance Imaging (MRI) was done under general anaesthesia with Xylazine-Ketamine which revealed, abnormal signal intensity mass lesion of size 19×19 mm in third ventricle region heterogeneously hyperintense on T2 and FLAIR, isointense to hypointense on T1 (Fig. 1a and 1b) with moderate dialatation of B/L lateral and third ventricle with mild periventricular ooze suggesting brain tumor likely intraventricular with obstructive hydrocephalus.

Rest of brain parenchyma revealed normal differentiation of the grey and white matter and signal intensity. No infarct/ Intra-cranial bleeding noted. Hippocampal and parahippocampal regions, B/L basal ganglia and thalami, corpus callosum were normal. Brain stem and cerebellum showed normal morphology, VII and VIII cranial nerves were normal. Visualization of inner ear revealed no significant abnormality.

Initial therapy was started with Inj. DNS (500ml IV for 1 day), Inj. Ceftriaxone + tazobactum (@ 25 mg/kg IM for 3 days), Inj. Meloxicam (@ 0.2 mg/kg IM for 5 days), Inj. Neurobion (2ml IV for 5 days), Cap. Retinol (@ 750 mg per orally on alternate day for 5 days), Multivitamin syrup for supportive therapy, Drops Ofloxacin+ Clotrimazole + Betamethsone + lignocaine hydrochloride (@10 drops tid for 7 days for ear cleaning). After diagnosis of brain tumor treatment was started with Inj. Cefotaxime + Sulbactam (@ 25 mg/kg OD IV for 14 days), Tab. Phenobarbital (@ 4 mg/kg OD PO for 21 days), Tab. Hydroxyurea (@ 30 mg/kg PO alternate day for 8 weeks), Inj. Prednisolone (@1 mg/kg IM for 21 days), Pheniramine and multivitamin given as supportive therapy for 21 days. Later, animal started to take feed with improvement in condition (less deviation of head) along with marked reduction in seizures and circling movements but after twelve weeks animal suddenly succumbed to death. Post-mortem and histo-pathological examination could not be attempted as owner was not willing for the same.

Discussion

Intracranial neoplasia is well described in the dog (Zaki, 1977) and is a common cause of neurological dysfunction in dogs. As per Snyder *et al*. (2006), choroid plexus tumors account for 7–13% of all primary brain tumors; third and fourth ventricle are commonly involved, but involvement of lateral ventricle in some cases is also reported. Secondary obstructive hydrocephalus is a common finding. Diagnostic imaging aids like plain-film radiography, contrast radiography, CT scan, MRI are available. MRI is a noninvasive imaging modality that constructs images of the brain using the magnetic resonance of protons under the influence of various radio wave pulses (Hecht *et al.*, 2010). MRI is best for soft tissue structures as it provides images in different plains. Magnetic resonance imaging results were similar to that of choroid plexus tumor with abnormal mass intensity, heterogeneously hyperintense on T2 and FLAIR, isointense to hypointense on T1. Treatment of brain is centered mainly on surgical resection, radiation therapy, chemotherapy but chemotherapy is anectodal as no specific treatment for specific tumor is present. Hydroxyurea with dose rate of 30mg/kg per orally has been used in case of brain tumor (Itoh *et al.*, 2016) in case of choroid plexus tumor.

Hydroxyurea is an antimetabolite that specifically affects the S stage of the cell cycle (Hoshino *et al.*, 1986). Acceptable and reversible toxicity of hydroxyurea in humans is the reason why it may be the optimal drug for treating slow-growing tumors with low mitotic indices. In the present case study cerebro-spinal fluid examination revealed increased total protein, mild mononuclear pleocytosis but no neoplastic cells were present, which is characteristic of brain tumor (Vernau, 2005). As hydroxyurea is having cytostatic as well tumor-shrinking effects and dexamethasone having antiedema effect which appeared to result in symptom

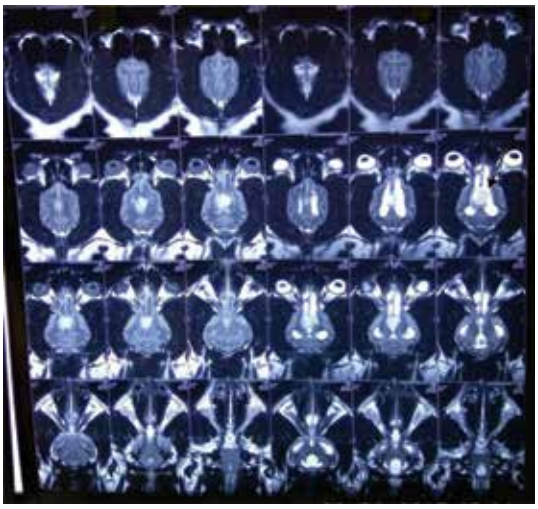

Fig. 1a

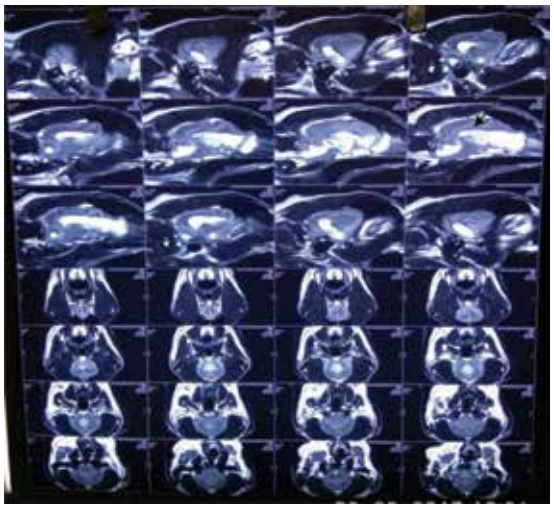

Fig. 1b

Fig. 1a and 1b. MRI images showing abnormal mass in third ventricle of brain

relief in the present case. Combination of steroid and hydroxyurea chemotherapy might have reduced the tumor size and lead to symptomatic relief to animal, which suggests that this combination therapy may be an effective approach for treating brain tumors in dogs. Long-term control of tumor with cytotoxic drugs alone is poor, so symptomatic treatment is required along with them.

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Medical management of cerebral babesiosis in a labrador retriever

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Abstract

A 2.5 years old Labrador retriever was presented at the university clinical complex with the history of anorexia, reduced water intake, not defecating, and sudden lateral recumbency. On general examination, fever, pale mucous membrane, reduced response to stimuli was observed. On orthopedic examination, no bony abnormalities noticed, loss of proprioception of the forelimbs was revealed. On palpation enlarged spleen could be felt. Ultrasonography and radiography revealed spleenomegly. Animal had low haemoglobin and thrombocytopenia in complete blood count. On serum estimation increased creatinine was noticed. *Babesia gibsoni* could be detected in blood smear. Animal was stabilized with isotonic fluids, administered polybion and then was treated with one dose of dimenazine aceturate. Next day epidural depomedarol(2mg/kg) was administered. Animal was advised with doxycycline tablets (10mg/kg) for 10days, neurobion tablets and depomedarol injection once in three days for 2 weeks. After 10 days recovery was noticed, animal could bear weight. Advised to continue doxycycline tablets for next 10days and tablet neurobion. Animal had uneventful recovery.

Keywords: Treatment, Cerebral babesiosis, Labrador

Canine babesiosis is the disease caused by protozoan of genus Babesia. Dogs get this infection through tick bite, blood transfusion and sometimes transplacental transmission. But the most common mode of transmission is through tick bite. Ticks of genus Rhipicephalus are mainly involved in the transmission of the disease.

The incubation period ranges from 10-14 days. Symptoms can be mild to severe including fever, in appetence, pale mucous membrane, dark coloured urine and weight loss. In the present case we could find some neurological changes associated with Babesiosis and successful recovery of the animal.

Case history and Observations

A two and half year old male Labrador retriever weighing 26kg was presented to small animal outpatient unit of Teaching Veterinary Clinical Complex (TVCC), COVAS, Pookode with a complaint of anorexia, reduced water intake, not defecating, and sudden lateral recumbency.

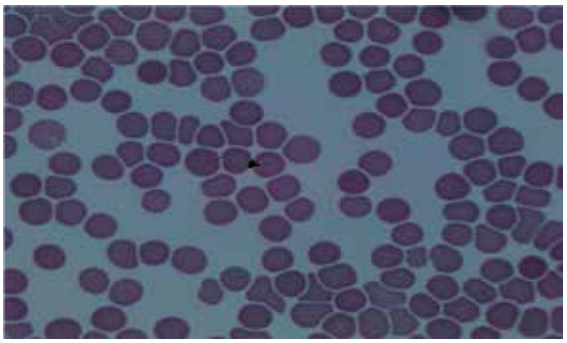
On clinical examination animal had fever (105.2˚F), pale mucous membrane, reduced response to pain stimuli with normal respiratory rate (30breaths/min) and heart rate (90 beats/min). Ticks were noticed on the dorsum of the body. Orthopedic examination, no bony abnormalities noticed, there was loss of proprioception of forlimbs. On palpation, spleen could be felt. For further examination animal underwent radiography and ultrasonography. Both revealed spleenomegaly. On complete blood count animal had low haemoglobin (7.2 g/dl) and low platelet count (6×10^{5}/cmm). On serum biochemistry high creatinine (3.2mg/dl) was noticed and all the other parameters were within the normal range. Simultaneously blood smear was examined which was positive for *Babesia gibsoni.* Haemoglobinuria was also noted.

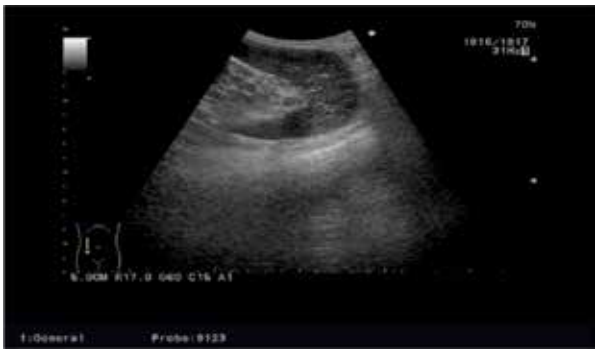
Treatment and Discussion

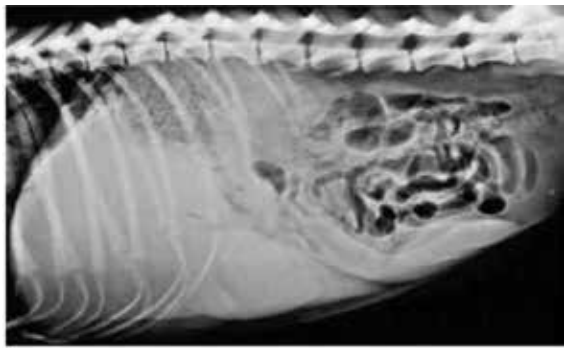
Animal was stabilized with isotonic fluids (NS 10ml/kg bwt), administered polybion intravenously and then was treated with one dose of dimenazine aceturate (3.5mg/kg bwt) intramuscular. Next day epidural depomedarol(2mg/kg) was administered. Animal was advised with doxycycline tablets (10mg/kg) for 10days, neurobion tablets and depomedarol injection once in three days for 2 weeks.

After 10 days of treatment, recovery was noticed, animal could bear weight. Advised to continue doxycycline tablets for next 10days and tablet neurobion.

Animal was presented one month post treatment and found negative for haemoprotozoa and showed uneventful recovery. Five months later all the blood parameters were within the normal range.







Canine Babesiosis is the worldwide distributed disease. It infects both wild and domestic canidae. It is the tickborne, protozoal, hemoparasitic disease which causes different degrees of haemolytic anemia, spleenomegaly, fever, and thrombocytopenia (Boozer and Macintire., 2003).

Sometimes nervous involvement is also seen which are called as cerebral babesiosis. Babesiosis with involvement of nervous system was also found in ten year old dog by Maele et al., 2008.

Piroplasms infect and replicate in red blood cells which later result in haemolytic anemia. Diagnosis primarily done by the clinical examination and blood smear examination. Molecular and serological diagnosis is also available. Treatment is same as standard treatment of babesiosis. Tick control plays an important role in prevention of the disease.

In this case of Babesiosis the manifestation of the disease was atypical and found neurological involvement.

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A case report on canine distemper with cardiac involvement

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Abstract

A three year old male canine non descript was brought to the Teaching Veterinary Clinical Complex (TVCC), COVAS, Pookode with the history of anorexia since 4 days, facial oedema since 2 days, and was showing periodic weakness. On general observation animal had temporal twitching, severe panting, and distended abdomen. Clinical investigation revealed respiratory distress, cardiac murmurs and high heart rate. Lateral flow test confirmed the case, positive for canine distemper. On thoracic radiography there was enlargement of heart indicative of dialated cardiomyopathy and abdominal radiograph revealed mild ascitis. Laboratory investigation was done with faecal sample, blood and serum. Anchylostome ova could be detected in the faecal sample. All the parameters of serum and complete blood count were within the normal range except slight neutrophilia. On ECG, T wave was high indicative of myocardial hypoxia. On Echocardiogram there was high fractional shortening and effusion fraction. Also there was hypertrophy of the cardiac wall. Animal underwent treatment with Frusamide 2mg/kg Bwt, Enrofloxacin 5 mg/kg Bwt and Meloxicam 0.2mg/ kg Bwt.

Keywords: Canine distemper, Temporal twitching, Dilated cardiomyopathy, Myocardial hypoxia

Canine distemper is a viral disease caused by canine distemper virus (CDV), member of the genus morbilli virus of the family paramyxoviridae. Susceptible to all carnivores. According to Deem et al., 2000 the disease is recognized as a worldwide problem of carnivores and after rabies this disease has the second highest fatality rate. Clinical signs of the disease vary depending on the virulence of the strain, environmental condition and immune status of the host. Systemic involvement, skin lesions, neurological signs, and bone lesions are the major signs seen in this disease.

Case History and Observations

A three year old male canine non descript weighing 11.4kg was presented to small animal outpatient unit of Teaching Veterinary Clinical Complex (TVCC), COVAS, Pookode with a complaint of anorexia since 4 days, facial oedema since 2 days, and was showing periodic weakness.

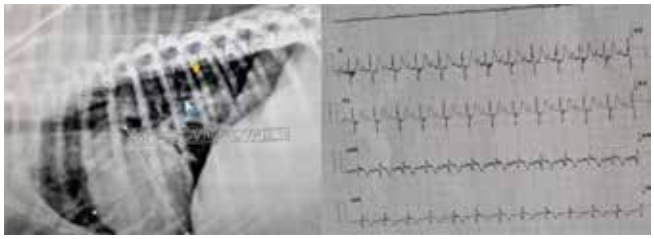
On general observation animal had temporal twitching, severe panting, and distended abdomen. On clinical examination there was subnormal temperature (100˚F), high pulse rate (160/min), murmurs on auscultation, tensed abdomen on palpation, and high respiratory rate with wheezing of trachea on auscultation. There was severe ocular and nasal discharge. Sample was collected from the ocular discharge and was subjected for lateral flow test.

The animal was subjected to detailed examination including Radiography, Electrocardiography, Echocardiography, Ultrasonography, Haemato-biochemical examination and faecal sample examination.

Lateral flow test gave positive result for canine distemper. On haematological evaluation there was leukocytosis (11.8×10^3/ µl). All the other parameters were within the normal range. On serum biochemical parameter like creatinie 0.9mg/dl, ALT 20.3 IU/L, total protein 6.3g/dl and albumin 2.4 g/dl.

On direct faecal sample examination, Ancylostma ova could be detected. Thoracic radiography revealed severe cardiomegaly (12.3v veterbral heart score) and mild generalised brochointestinal lung changes. On abdominal radiograph all the organs were intact, no abnormality noticed. Animal underwent ECG which revealed myocardial hypoxia by giving tall T wave. On echocardiography showed hypertrophy of the atrial and ventricular wall, mild regurgitation of tricuspid and aortic valve. There was high fractional shortening (42) and effusion fraction (82).

Animal underwent treatment with Frusamide 2mg/kg Bwt, Enrofloxacin 5 mg/kg Bwt and Meloxicam 0.2mg/ kg Bwt.



Discussion

Canine distemper virus (CDV) is the major pathogen of dog and wild carnivores worldwide. This virus mainly affects gastrointestinal, respiratory and central nervous system. Also it has a great effect on cardiac system.

Virus enters via inhalation, multiplies in tissue macrophages, and localize in lymphoid system. After the viremia stage virus enters central nervous system. Central nervous system lesions can lead to myocardial damage in dogs (Higgins et al., 1981). According to Gainer, 1974, Canine distemper virus can lead to cardiomyopathies in dogs.

The disease can be diagnosed by the clinical signs and ELISA kit. Cardiac or respiratory involvement can be revealed by radiography, ultrasonography, ECG and echocardiography.

Vaccination plays an important role in infectious disease. Proper vaccines should be administered at the right time in order to avoid such infectious condition among domestic and wild carnivores.

In conclusion, canine distemper is the disease which has a great systemic involvement leading to high fatality rate.

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Cardiomyopathy with supraventricular tachyarrhythmia in a crossbred dog and its management

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Abstract

A six years old male crossbred dog was presented to RVP with the history of chronic coughing, dullness, anorexia, lethargy, exercise intolerance and cardiac murmurs on auscultation. Atrial tachycardia was found in electrocardiography. The echocardiography showed systoloic dysfounction (FS: 14%, EF: 30%) and interventricular septal thickening. Serum biochemistry revealed severely elevated CK-MB levels (829IU/L). Based on clinical signs, ECG, Radiography, echocardiography and serum biochemistry the dog was diagnosed with cardiomyopathy with atrial tachycardia. Theraupetic management was initiated with combination of drugs *i.e.,* Digoxin, Enalapril, Lasilactone, Enrofloxacin and dextromethorphan HCl syrup. and case was successfully managed with the above combination therapy.

Keywords: Atrial tachycardia, Cardiomyopathy, ECG,

Cardiac arrhythmias are defined as variation of the cardiac rhythm from normal sinus rhythm. Arrhythmias involving sinus node, atrial tissue and AV junction without the involvement of ventricular conduction system are called as supraventricular tachyarrhythmia (Tilley *et al.,* 2008). The term supraventricular tachycardia (SVT) describes a pathologic rhythm disturbance characterized by a rapid, regular tachycardia that is initiated and/or propagated by myocardial structures above or within the atrioventricular (AV) node (Wright, 2004). The focus of origin may be located in the sinus node, atrial tissue, or the AV junction (Grubb and Muir, 1999). ECG findings of SVT frequently include a narrow QRS complex with a regular R-R interval. Persistent tachyarrhythmia can result in atrial enlargement and consequently tachycardia induced cardiomyopathy and heart failure. The present case was diagnosed at an initial stage and managed successfully.

Case History and Observations

A six years old male crossbred dog (Fig. 1) was presented with history of chronic coughing, anorexia, lethargy and exercise intolerance since one month. On clinical examination tachycardia (194), normal rectal temperature 102.3^{0}F, slightly congested conjuctival mucus membrane and tachypnea were recorded. The lung and cardiac auscultation revealed moist rales and grade II cardiac murmurs respectively. Electrocardiography was performed with BPL cardi art machine (paper speed 50 mm/sec), which revealed low QRS complexes and atrial tachycardia. Digital radiography of the left lateral and ventro-dorsal thoracic region was taken. Radiographic examination showed severe congestion of lungs and increased vertebral heart score (11.6).The dog was subjected to echocardiographic examination. The echocardiography was done with () 2D B mode examination revealed LVWd: 13.6 mm (Normal: 7.9-8.7 mm), LVWs: 14.1 mm (Normal: 12.7-13.8 mm) and interventricular septal thickening IVSd: 10 mm (Normal: 9.8-10.8 mm), IVSs: 14.6 mm (Normal: 14.8-15.9 mm). The FS: 14 % (Normal: 33.6 - 38.92%), EF: 30% (Normal: 70- 75%) suggestive of systolic dysfunction. Blood sample was collected for haematobiochemical examination. Hematological findings revealed Hb- 14.7 gm/dl, leukocytosis (TLC-47300 cells/cmm), DLC (Neutrophils- 90.0%, Lymphocytes- 6%, Monocytes-1%, Eosinophils- 3%) and total platelet count-228×10^3/µl. Serum biochemistry showed significantly elevated serum CK- MB level (829IU/L) and ALT (225 IU/L), but other parameters were within normal range (BUN- 9 mg/dl, serum creatinine- 0.8 mg/dl, serum calcium- 9.6 mg/dL and serum potassium- 4.6 mEq/L). Based on all the above findings case was diagnosed as cardiomyopathy with supraventricular tachyarrhythmia.

Treatment and Discussion

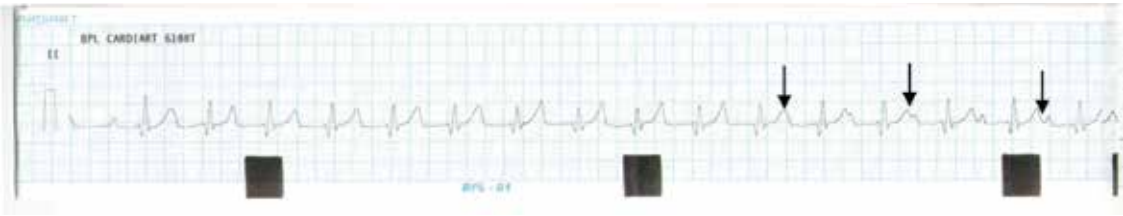
The therapy was started with Tab. Amoxicillin-potassium clavulunate @ 12.5mg/kg b.wt bid orally, Inj. Deriphylline 2ml i/m, Tab Digoxin @ 0.005 mg/kg b.wt

bid orally, Tab Enalapril @ 0.5mg/kg b.wt bid orally, Tab Lasilactone 50mg bid orally and Syrup Alex bid orally (dextromethorphan HCl) continued for one month. The dog was re-examined after 15 days of initiation of therapy then ECG findings revealed normal PQRST complexes and the dog showed marked improvement in condition after one month of therapy with normal ECG findings. The case was further monitored once in a month with the same line of therapy.

Cardiac arrhythmias are one of the frequently identified disorders in canines which can even lead to cardiac arrest and also death. Arrhythmias can arise either due to disturbances in impulse formation or impulse conduction alone or in combination and leads to abnormalities in heart rate and rhythm (Nelson and Couto, 2014). It has been suggested that disturbances of excitability and impulse formation are the most common causes of arrhythmias in dogs (Patterson *et al.,* 1960; Aptekmann *et al.,* 2010). In a hospital based study conducted in Brazil during 2003-2007 showed a prevalence of arrhythmia among dogs was 27% of which 4.8% had supraventricular tachycardia (Aptekmann *et al.,* 2010). The disturbance in supra ventricular impulse formation can results in supraventricular tachycardia (SVT) - atrial or junctional. These abnormal rhythms in canines are frequently found associated with structural lesions of atria and cardiomyopathy. In case of atrial tachycardia cardiac impulses arises from ectopic pacemaker located in the atria of heart unlike SA node in normal sinus rhythm. Clinical manifestation of atrial tachycardia includes rapid but regular pulse except in multifocal tachycardia and in rapid atrial tachycardias with variable AV conduction. Other symptoms include dyspnea, dizziness, fatigue, exercise intolerance and signs of heart failure. SVT is characterized by tachycardia where the P wave configuration differs from the sinus P waves with rapid heart rate (200-350 bpm dogs) (Borde, 2005). The P–R interval may differ from a normal sinus complex. Irregular rhythm is observed in case of multifocal tachycardia. Usually QRS complexes are normal but widened or electrical alternans can also be observed. Supraventricular tachycardia could be observed in case of atrial enlargement due to AV insufficiency, cardiomyopathy, congential heart disease and digitalis toxicity.

Thoracic radiography and echocardiography can be used as confirmatory diagnostic tool as alterations in lung field and cardiac silhouette in thoracic radiograph could be noticed. Elevation of trachea, straightening caudal border of heart, widened cardiac silhouette, displacement of cardiac apex, rounding of cardiac borders and displacement of caudal lobe of lung, caudal venacava and pulmonary artery could be detected in cardiac enlargement. Vertebral heart score (normal 8.5-10.5) obtained from lateral thoracic radiograph is used for detecting the presence and quantifying the degree of cardiomegaly (Nelson and Couto, 2014). Pulmonary edema is frequently found associated with bilateral enlargement of heart. The elevation of trachea often results in chronic cough. Biochemical marker cardiac specific creatine kinase CK- MB has been found to be elevated in the serum suggesting myocardial damage (Tharwat *et al*., 2013). The elevatedserum enzymes ALT is attributed to severe congestion of liver in present case due to low cardiac output in cardiomyopathy (Sisson *et al.,* 1999*)*. Liver enzymes normalized after the treatment was discontinued made this less likely (Jacobs *et al*., 2000).

Digoxin is a positive ionotropic agent which increases contractility of the myocardial muscle there by increases the QRS amplitude. It increases contractility by competitively binding and inhibiting Na^{+}-K^{+} ATPase pump at myocardial cell membrane. Intracellular Na^{+} accumulation then promotes Ca^{++} entry *via* the sodium calcium exchange. It sif found to be more effective for the treatment of cardiomyopathy (Srinivasan and Maheshkrishnan, 2008). Enalapril is an angiotensin converting enzyme inhibitor which causes vasodialaton by preventing conversion of angiotensin-I to angiotensin-II. In addition it has got some diuretic effect by decreased aldosterone synthesis and secretion. Lasilactone is a combination diuretic comprising furosemide and spironolactone reducing edema and lung congestion. Dextromethorphan, antagonists of NMDA-type glutamate receptors, has been found to be an effective and safe antitussive. Oral administration of dextromethorphan to the animal could successfully manage the cough (Brown *et al*., 2004). By administration of this combination therapy along with low salt diet, restricted exercise and regular health checkup the patient can be managed for a longer period of time without lot much complications.



Picture 1: Electrocardiogram on day one

Picture 2: Electrocardiogram after 15 days of therapy

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Clinico-pathological changes in dogs infected with *Babesia gibsoni*

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Abstract

Babesiosis is a life threatening tick borne disease of dogs caused by *Babesia gibsoni* and *Babesia canis*. Present report describes the clinico-pathological changes occurring in three canines infected with *Babesia gibsoni* presented to Teaching Veterinary Clinical Complex, with history of anorexia, emaciation and dullness from 7 days.

Keywords: *Babesiosis, Canine, Babesia gibsoni*

Babesiosis is one of the important tick borne diseases of domestic and wild canidae, caused by intra-erythrocyte piroplasms of the genus Babesia (Azaziah *et. al.,* 2010). The disease is caused by *Babesia gibsoni* and *Babesia canis* (Casapulla *et al.,* 1998) and is transmitted by brown dog tick *Rhipicephalus sanguineus, Dermacentor reticularus, Dermacentor marginatus* and *Haemaphysalis leachi* (Filipe, 2010). In India both *B. canis* (Varshney *et. al.,* 2004) and *B. gibsoni* (Varshney *et. al.,* 2003) are prevalent. Babesiosis has multisystemic effets that are responsible for various symptoms. Disease in dogs caused by *B. gibsoni* is manifested in the form of variable clinical signs and characterized by anaemia (Groves and Dennis, 1972), remittent fever, lethargy, anorexia and splenomegaly (Yamane *et al.,* 1993). Electrocardiographic changes have been documented in *B. canis* infection recorded in 40% of the cases (Dvir *et al.,* 2004). The present paper describes clinico-pathological changes in dogs suffering from *B. gibsoni* infection.

Case History and Observations

A 7 month, 10 month and 1 year old, male Pomeranian dog were presented to Teaching Veterinary Clinical Complex, with history of anorexia, emaciation and dullness from 7 days. Detailed clinical examination of the dogs revealed hyperthermia (104.4ºF), dehydration (skin tenting test > 3 sec), tachycardia (122/min), pulse deficit, pale eye mucus membrane, depression and presence of ticks over the body. In dog aged 7 month congested eye mucus membrane was noticed as an additional finding. Peripheral blood smear examination revealed presence of intraerythrocytic piroplasmic organisms (Fig. 1). Haematological examination revealed decreased haemoglobin and TEC values, leucocytosis with neutrophilia and lymphocytopenia (Table I). Electrocardiographic abnormalities evident in dogs were sinus tachycardia and sinus arrhythmia (Fig 2). Cardiac troponin I tested found positive in one case (7month old dog) (Fig. 3). The cases were treated with Diaminizine aceturate (@ 3.5 mg/kg b wt single dose) and clindamycin (@ 25 mg/kg b wt P.O. B.I.D. X 14 days) as a combinatory protocol. Supportive therapy with hematinics and liver protective syrups were also given. One dog died during the treatment.

Based on the clinical signs, and laboratory examination, dogs were found to be infected with *B. gibsoni*. Canine babesiosis, a tick borne disease, has a world-wide distribution and is a well established clinical entity in the tropical and subtropical world (Soulsby, 1982) including India and is emerging as an important clinical disease in cosmopolitan cities (Varshney *et al.,* 2008).

Complete blood count analysis is one of the most frequently used method enabling veterinarian to assess the condition of sick animal. It helps in diagnosing and differentiating various pathological processes, and thus enabling in rationale treatment (Cardoso *et al.,* 2010; Lobetti, 2010). Decreased haemoglobin and TEC values, neutrophilic leukocytosis, lymphocytopenia were noticed on clinico-pathological examination. Similar haematological findings including low haemoglobin, PCV, leukocytosis with neutrophillia and lymphopenia were reported by Samradhni *et al.* (2005).

Peripheral blood smear examination revealed presence of intraerythrocytic piroplasmic organisms. Microscopic examination remains the simplest and most accessible diagnostic test and is still the only

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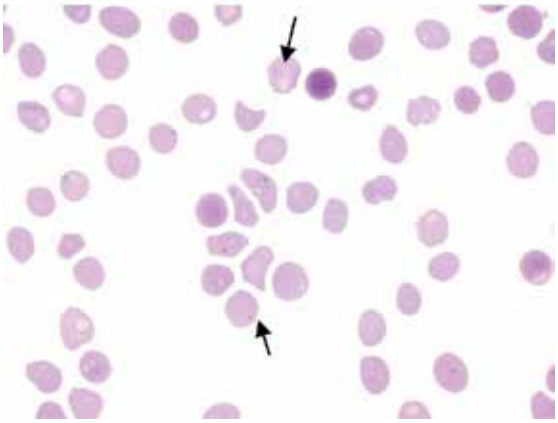

Fig. 1: Blood smear revealing presence of intraerythrocytic *B. gibsoni* organisms

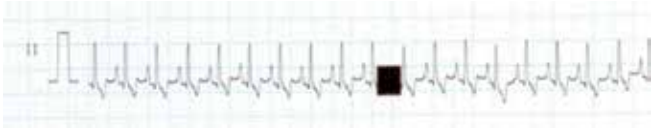

Fig. 2: ECG of a dog suffering from babesiosis show sinus tachycardia (Heart rate >200 bpm)

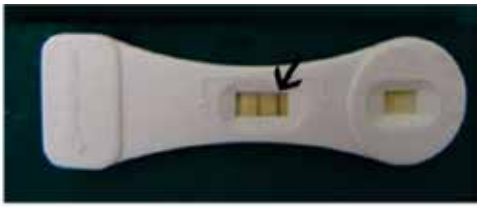

Fig. 3: Positive cardiac troponin I test

Table 1: Hematological changes in dogs suffering from babesiosis

Parameter	Case 1	Case 2	Case 3
WBC (10^3µ/L)	21.0	32.9	23.2
Lymphocytes (10^3µ/L)	2.6	3.3	3.8
Monocytes (10^3µ/L)	4.3	3.2	3.5
Neutrophils (10^3µ/L)	14.1	26.4	15.2
HB (g/dL)	3.6	20.4	4.5
HCT (%)	8.4	40.9	10.2
RBC (10^6µ/L)	1.74	8.24	1.93
PLT (10^3µ/L)	197.0	269.0	150.0

viable option available in many parts of the developing world where babesiosis is endemic (Bohm *et al.,* 2006). Probability of finding large Babesia species could be improved by sampling of blood from capillary beds (ear tip, toe nail) or examination of cells from beneath the buffy coat of a haematocrit tube (Irwin 2009).

Discussion

The clinical presentation in naturally occurring cases of babesiosis was very diverse and wide variability in clinical signs was noticed. Dogs with babesiosis showed the signs of fever, anorexia, depression, dehydration and these findings in accordance with the earlier workers (Irwin, 2010). It is thought that the clinical signs are the result of tissue hypoxia following the hemolytic anaemia and a concomitant systemic inflammatory response syndrome caused by marked cytokine release (Lobetti, 2006).

Electrocardiographic abnormalities evident were sinus tachycardia and sinus arrhythmia which were in accordance with Chaudhari (2006) who reported a prevalence of 51.32% of electrocardiographic disturbances in canine babesiosis with tachycardia, low voltage complex, sinus arrhythmia, tachyarrhythmia, atrial fibrillation and sinus arrest as the common ECG changes recorded.

Cardiac troponins (I or T) are the biomarker of choice to detect microscopic zones of myocardial necrosis with absolute specificity and sensitivity (Thysen *et al.,* 2000). Estimation of cardiac troponin in cases of babesiosis could thus help in management of critically ill patients and could also serve as a prognostic indicator (Reynolds and Oyama, 2008). Wulansari *et al.* (2003) reported that use of clindamycin (25 mg/kg body weight, per os) for 14 days gradually reduced parasitemia levels in the affected dogs.

From the above study it is concluded that accurate diagnosis of canine babesiosis is of paramount importance to treat clinical cases and in turn preventing loss of our beloved pets.

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Medical management of soft tissue sarcoma in a male Labrador retriever

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Abstract

Soft tissue sarcomas (STS) refer to a class of malignant growth in the mesenchymal tissues, observed most frequently in subcutaneous structures and the extremities. Metastasis may occur in nearly 20% STS cases. Treatment options are surgery, radiation therapy and chemotherapy, or a judicious combination of these remedial strategies. In the client-owned 10 year old male Labrador retriever dog, named Bonzo, a large tennis ball sized mass in the palmaro-medial aspect of the right antebrachium was observed on presentation in the clinic. Blood chemistry profile ruled out hepatic and renal dysfunction. After adequate preparation, the neoplastic mass was surgically excised, completely. The histopathological profile, characterized by clearly discernible infiltrative neoplastic proliferation of spindle shaped cells arranged in interwoven streams and bundles supported on a fibrovascular/ fibromyxomatous matrix, seven mitotic figures/ 10 dry high power (400x) fields, and anisocytosis and anisokaryosis of moderate intensity was interpreted as STS, Grade 2. Post-surgery weekly follow-up visits at the clinic showed that the dog patient was recovering well with no clinical signs of tumor recurrence. However, for added biosafety the case was referred to the veterinary clinical oncologist for post-surgery radiation therapy.

Keywords: Soft tissue sarcoma, Dog, Medical management

Comprising nearly 15% of all cutaneous and subcutaneous tumors, soft tissue sarcomas (STS) are a heterogeneous population of mesenchymal neoplasms, mostly affecting the middle aged and senescent dogs, without any breed/ gender predisposition (Cronin, 2006; Liptak and Forrest, 2007). Appearing as pseudo-encapsulated masses, these tumors have poorly defined histomorphological borders and may, therefore, infiltrate through or extend along the fascial planes. Locally invasive, there is a high risk of post-surgery recurrence in the primary site. The rate of metastasis *via* the peripheral blood circulation is nearly 20%, the lungs remaining the main target organ (Selting, 2010). The validated histopathological tumor grade (Kuntz *et al*., 1997) is predictive of the chances of metastasis, and the geometrical configuration of resected tumor margin reflects the possibility of local recurrence (Baker-Gabb *et al*., 2003). Radiation therapy (McChesney *et al*., 1989; Forrest *et al*., 2000; Mc Knight *et al*., 2000) or chemotherapy (Ogilvie *et al*., 1991; Rassnick *et al*., 2003; Elsmlie *et al*., 2008; Selting *et al*., 2010) is beneficial post-surgery. Prognosis for STS dog patients, treated promptly, is generally favourable.

The origin, primary sites, and risk of metastasis in soft tissue sarcoma cases vary widely (Table 1). Local tumor control is often the most challenging job for the pet clinician; recurrence rates following surgery alone, or surgery-cum-radiation combination therapy range from 7% to 32%. The poor prognostic factors include large tumor size, incomplete surgical margins, and high histopathological tumor grade (Withrow and Vail, 2007). The metastasis rate in dogs with STS (median time lag of 12 months) varies from 8% to 17%. The risk factors also include the frequency of mitotic figures in the HPF (400x), extent (%) of necrobiosis, and the susceptibility to local tumor recurrence. Thus, the metastasis rate for dogs with Grade 1 or 2 STS is below 15%, *cf*. 41% for Grade 3. Metastasis is 5 times more likely when the tumors exhibit more than 20 mitotic figures/ 10 HPFs. With closed surgical margins in the biopsy sample, recurrence is infrequent in STS Grade 1, of intermediate frequency in Grade 2, and is very likely in the Grade 3 category (Withrow and Vail, 2007).

Case Histoy and Treatment

Pre-surgery preparations: Bonzo, a 10 year old male Labrador retriever was first presented to the Angel Animal Hospital in mid-June, 2015 with a small lump in the right carpal area. Initially, the client was reluctant to permit surgical ablation. However, he brought the dog to the clinic again after about six months, since the growth had enlarged conspicuously (Fig. 1).

On receipt of client's written consent, blood chemistries report and the veterinary clinical oncologist's opinion on histopathological evaluation of the referred biopsy sample, surgical treatment was scheduled. The BUN and serum creatinine values (Table 1) pointed to normal renal function, and circulatory titres of ALT attested to the structural and functional patency of the hepatocytes. Myocardial integrity was evidenced by normal AST value in the peripheral blood circulation. The serum level of ALP, on the lower side of the physiological range, indicated normal functioning of the long bones.

Surgical excision of tumor: On the 18th day of December, 2015, the patient was admitted to the clinic, fasted overnight in the owner's premises. The normal blood chemistry profile permitted safe surgical intervention. Pre-anaesthetic atropine medication was given subcutaneously. Anaesthesia was induced with propofol and maintained with isoflurane gas. The surgical site was shaved and scrubbed with chlorhexidine soap, followed with chlorhexidine tincture. The patient was administered isoosmotic Normosol^{R} intravenously and kept under close clinical surveillance with Pulse Oximeter and ApAlert monitors during the entire surgical procedure in the OT. The large (6.8 cm x 6.2 cm) tennis ball sized mass (Fig. 2) from palmaro-medial aspect of right antebrachium was carefully excised, using freshly sterilized surgical pack and a pair of gloves. A representative piece of the tumor tissue was preserved in 10% buffered formaldehyde solution, and dispatched to the nearby regional centre, IDEXX Diagnostics (USA) for histopathology. The open surgical wound (Fig. 3) was gently flushed with normal saline and chlorhexidine solutions, using sterilized surgical pack and a fresh pair of gloves, and bandaged. The in-house thoracic survey radiographs (Fig. 4) revealed no metastatic lesions.

Histopathological profile

Multifocal necrobiosis in <50% of the excised tissue mass (Fig. 5) was evidenced by the eosinophilic amorphous debris, signs of capillary bleeding and karyorrhectic degeneration. Evaginated lymphocytes and eosinophils along with occasional plasma cells were observed. The neoplastic cells extended right up to the margins of the biopsy tissue sample. On the established criteria (Kuntz *et al.*, 1997), the oncologist's microscopic interpretation was STS Grade 2. The microscopic picture of the biopsy sample further revealed infiltrative neoplastic metaplasia in the form of spindle shaped cells, arranged in interwoven streams and bundles, supported on a fibro-vascular to fibro-myxomatous matrix without discrete borders. The typical neoplastic cell revealed oval to round nucleus

Table 1. Types of soft tissue sarcomas**

Tissue of origin	Benign tumor	Malignant tumor	Primary site(s)	Risk of metastasis	Target organ(s)
Adipose tissue	Lipoma	Liposarcoma	Limbs, abdominal or chest cavity	Low to moderate	Lungs, liver, spleen, bone
Fibrous tissue	Fibroma	Fibrosarcoma	Limbs, oral cavity	Low to moderate	Lungs
Histio-cytes	Histio-cytoma	Histiocytic-sarcoma	Limbs	Moderate to high	Lymph nodes, lungs, spleen, liver, kidneys
Lymph vessels	Lymph-angioma	Lymphangio-sarcoma	Limbs	Moderate	Lymph nodes
Blood vessels	Hem-angioma	Hemangio-sarcoma	Spleen, heart, liver, muscle, bone, kidneys	High	Lungs, liver, lymph nodes, distant dermal sites
Nervous tissue	-	Peripheral nerve sheath tumor	Limbs	Low to moderate	Lungs
Skeletal muscle	Rhabdo-myoma	Rhabdomyo-sarcoma	Tongue, larynx, heart, bladder	Low to moderate	Lungs, liver, spleen, kidneys
Synovial tissue	Synovioma	Synovial cell sarcoma	Joints	Moderate to high	Lymph nodes, lungs
Myxoma tissue	Myxoma	Myxosarcoma	Limbs, joints	Low to moderate	Lungs

** Adapted from Withrow and Vail (2007)

Table 2 Patient's pre-anesthesia blood chemistry profile

Parameter	Observed Av. value	Normal range
Blood urea nitrogen (BUN) (mg/dL)	13	7-27
Serum creatinine (mg/dL)	1.2	0.5 - 1.8
BUN: creatinine ratio	11	
Alanine aminotransferase (ALT) (U/L)	74	10 – 125
Aspartate aminotransferase (AST) (U/L)	42	0 - 50
Alkaline phosphatase (ALP) (U/L)	26	23 - 212

Fig. 1. Lump in the right carpal area of the patient

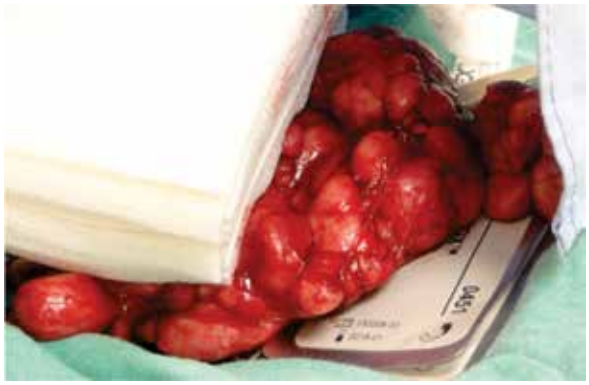

Fig. 2. The excised lump resembling a bunch of grapes

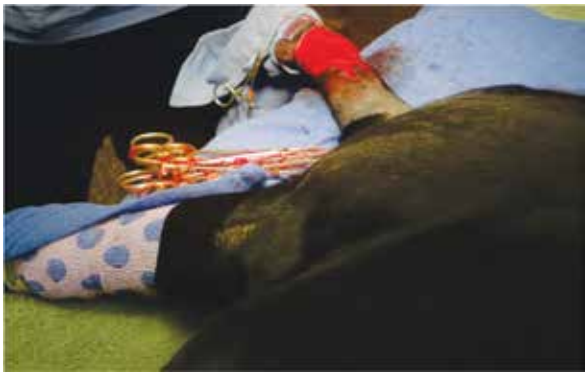

Fig. 3. The open surgical wound

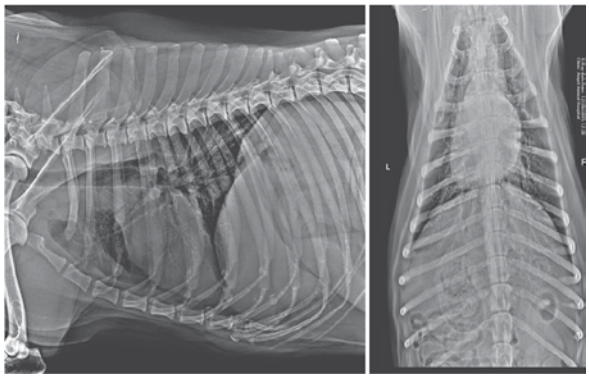

Fig. 4. Thoracic survey radiographs: right lateral/ ventro-dorsal view with no evidence of metastasis with no evidence of metastasis

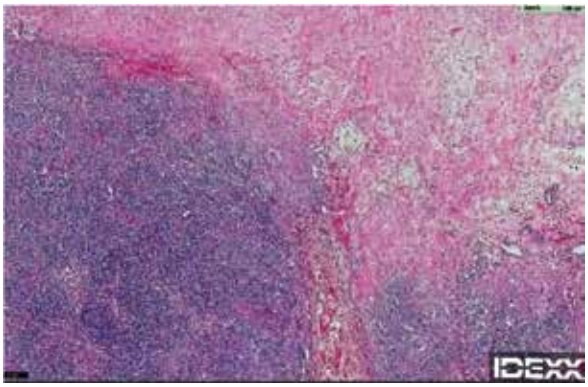

Fig. 5. Necrobiotic degeneration in excised tumor (HE, 40x)

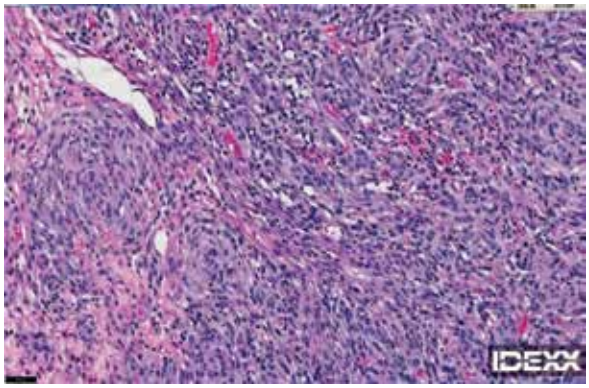

Fig. 6. Neoplastic cytomorphologic transformation (HE, 400X)

with 1 or 2 prominent nucleoli, eosinophilic cytoplasm and indistinct outlines. Total seven mitotic figures were visualized per 10 high power (400 x) fields. Anisocytosis and anisokaryosis of moderate intensity were also observed (Fig. 6).

Wound management and radiation therapy: The surgical wound was gently cleaned with chlorhexidine and betadine solutions, and proven beneficial honey bandage was applied on every alternate day for 3 weeks. For enhanced biosafety (Kuntz *et al*., 1997), the animal was referred to a veterinary clinical oncologist for serial radiation therapy. It is reiterated that mostly arising as solitary growths in the middle aged or senescent subjects soft tissue sarcomas (STS) are a heterogeneous population of mesenchymal tumors that constitute nearly 15% of all skin and subcutaneous tumors in dogs (Withrow and Vail, 2007). Recurrence is a common feature. Metastasis may occur haematogenously in up to 20% of cases. However, regional lymph node metastasis is unlikely, barring synovial cell sarcomas. In the instant case, based on the well-established criteria, laid down by Kuntz *et al*.(1997) and consistent with the report of Ettinger *et al*. (2006), the neoplastic growth was interpreted as soft tissue sarcoma (STS) Grade 2, possibly of peripheral nerve sheath origin. Bonzo, the dog patient showed no clinical signs of distress in the weekly post-operative follow-up visits, and responded well to the combination surgery-cum-radiation therapy. The wound healed completely, and the general health status also improved.

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Medical management of cutaneous mast cell tumor in a female Golden Retreiver

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Abstract

Cutaneous mast cell tumors (CMCTs), skin tumors with nearly 50% malignancy propensity are mostly located in the dermis, but may also incorporate the subdermis in some dog patients. Depending on the clinical presentation, the treatment options are complete surgical resection, fractionated radiation therapy, chemotherapy, or a judicious combination of these remedial strategies. In the client-owned 7 years old spayed female Golden Retriever dog, a small cantaloupe-sized mass, involving almost the entire left thoracic region, and also partly the opposite side was observed on presentation. The haemogram attested to the normalcy of the haemopoietic system and the circulating blood. The blood chemistry profile ruled out hepatic and renal dysfunction. Thus, in the instant case, surgical intervention was considered feasible. Accordingly, after adequate preparation, the large neoplastic mass was surgically excised with the veterinary oncologists' recommended minimum 3 cm lateral histologically tumor-free margins (HTFMs). The histopathological profile, characterized by the presence of mast cells arranged in cords and sheets within a scanty fibrous stroma, typical polygonal to ovoid transformed cellular entities exhibiting neoplastic transformation, notably high mitotic count (19/ ten HP, 400x microscopic fields), and anisocytosis and anisokaryosis of a moderate intensity, occasional binucleated cells, regional metastatic activity evidenced by neoplastic cells extending right up to the deep and lateral tissue margins, together with prominent hyperplastic peri-tumoral lymph node was interpreted by the pathologist as cutaneous mast cell tumor (CMCT), grade II (Patnaik system)/ High-grade (Kiupel system). In view of the high risk involved in surgical removal of an apparently massive neoplastic tissue mass, coupled with poor prognosis, euthanasia was advised, but the emotionally attached lady owner preferred to take a chance with surgical remedy. Accordingly, surgery was performed as per the standard protocol on 6th December, 2017, and the entire tumor mass (~4 kg) was completely removed uneventfully in about 2.5 hours time. The dog patient recovered from the ordeal remarkably fast. The post-operative clinical condition was closely monitored during the periodical follow-up visits to the clinic. Till date, the patient is doing well with no issues relating to eating, defaecation and urination.

Keywords: CMCT, Surgery, HTFMs, Biopsy, Histopathology, Prognosis, Dog

Mast cell tumors (MCTs), originating from the mast cells are one of the most common skin tumors in dogs. The cutaneous presentation represents the most prevalent form. However, MCTs can appear also in the subcutis, mucocutaneous structures, and the internal organs (Kiupel, 2017). The clinical presentation, gross appearance, pathogenesis, and pathogenicity of cutaneous mast cell tumors (CMCTs) vary markedly, and nearly 50% are malignant (O'Keefe, 1990). Thus, CMCTs may range from small low grade neoplastic growths to highly metastatic tumors that affect the pet's quality of life (Patnaik *et al*., 1988; Sledge *et al*., 2016; Kuipel, 2017). Euthanasia or mortality is the end result of local recurrence, regional and/or distant metasteses and associated disorders (Misdorp, 2004). Histopathological evaluation of a referred biopsy: the microscopic appearance of the neoplastic cells and grading of the pathogenic propensity by a veterinary oncologist provides important diagnostic and prognostic cues to the pet clinician pertaining to the predicted biological behaviour and the surgical margin assessment. Further insight into the potential bio-impact of CMCTs may be obtained through specialized testing: mast cell tumor prognostic panels (MCT-PPs), *viz*. mitotic count (MC), Ki67 (immuno-histochemical method that delineates the actively dividing neoplastic cells), *c-KIT* (PCR-based procedure to demonstrate the presence of *c-KIT* mutations in exon 8 and 11 of the oncogene) on the same biopsy sample.

Grading: The CMTs have been graded for pathogenicity in the traditional 3-tier Patnaik scale (grade I, II and III). However, the more recent 2-tier (low-grade/ high-grade) Kiupel system is aimed to provide increased inter-observer consistency and superior prognostic inputs. In the USA, the IDEXX pathologists currently employ both grading systems.

Mitotic count: MC (earlier named the mitotic index, MI), the number of mitotic figures per 10 HP (400x) microscopic fields is a simple, dependable clinical prognosis indicator.

Surgical margins: Veterinary oncologists recommend histo-morphologically tumor-free margins (HTFMs) of at least 3 cm lateral, and deep margin of one fascial plane during surgical ablation of neoplastic mass. However, Donnely *et al*. (2015) reported 36% localized recurrence rate for high-grade CMCTs, more likely to recur locally than low-grade tumors. No single criterion/ test is yet available that would unequivocally establish the prognosis of CMCTs, and the dog patients need to be evaluated on a case-by-case basis from histo-pathological profile, staging and PCR analysis for gene mutations (Sledge *et al*., 2016; Kiupel, 2017).

Case History and Management

The 7 year old spayed female Golden Retriever, named Sasha-Lingenfelter Rose (21.1 kg body wt) was presented at the Angel Animal Hospital, Farmington Hills, MI, USA on December 1st, 2017 with a prominent growth on the left shoulder. On anamnesis, the lady owner informed that the initially small bump in the affected skin area, noticed by her one week earlier, was assumed to be simply the result of bug bite. Further, to control the allergic reaction, she had also tried topical application of Benadryl ointment as a home remedy. However, since the bump continued to grow at an alarming rate, she decided to seek urgent veterinary medical care.

Physical examination: On palpation, the bump felt like a small cantaloupe-sized firm tissue growth involving virtually the entire left lateral thoracic region. The possibility of soft tissue tumor (STS) was uppermost among the clinical conditions. The client was advised to get the growth removed at the earliest open surgery date in the clinic, 6th December, 2017.

Pre-surgery preparations: On receipt of haematological and blood chemistry reports from the referral Diagnostic Lab, IDEXX, and the written consent of the client, surgery was scheduled. The haemogram (Table 1) pointed to normalcy of the haemopoietic system. The circulatory titres of alanine amino-transferase, ALT attested to the structural and functional patency of the hepatocytes. Normal functioning of the long bones was evidenced by serum titre of alkaline phosphatase, ALP on the lower side of the physiological range (Table 2). The blood urea nitrogen, BUN and serum creatinine values pointed to unimpaired renal function. However, the increased value, above the reference range, of symmetric dimethylarginine, SDMA (a more sensitive biomarker of both acute and chronic forms of renal dysfunction, *vs*. creatinine) mandated added safety precautions: appropriate i.v. fluid therapy during surgery and intensive post-operative patient management.

Surgical excision of the tumor: On 6th December,

Table 1. Pre-anesthetic haemogram of the patient.

Parameter	Observed value	Reference range
TEC (x 10^6/ µL)	7.2	5.4-8.7
Haematocrit value (%)	49.9	38.3-56.5
Haemoglobin (g/dL)	17.9	13.4-20.7
MCV (fL)	69.0	59.-76
MCH (µg)	24.9	21.9-26.1
MCHC (g/dL)	35.9	32.6-39.2
Reticulocytes (%)	0.6	
TLC (10^3/ µL)	43.0	10-110
Neutrophils (%)	69.4	
Lymphocytes (%)	25.1	
Monocytes (%)	4.5	
Eosinophils (%)	0.9	
Basophils (%)	0.1	
Thrombocytes (10^3/µL)	304.0	142-448

2017, the dog patient, with no other concurrent health issues, was admitted to the clinic, fasted overnight in the owner's premises. Final physical examination revealed that the growth in the proximity of spinal area had enlarged, covering the entire left lateral thoracic region, and also partly the opposite side. The anterior margins extended close to the jugular vein (Fig. 1). Pre-anaesthetic atropine was injected subcutaneously. Anaesthesia, induced with propofol, was maintained with isoflurane gas. The surgical site was shaved and scrubbed well with chlorhexidine soap, followed with chlorhexidine tincture. The neoplastic mass (~ 4 kg), in a virtual pool of blood (Fig. 2), was opened carefully for visual appraisal. Focal necrobiosis, adjoining the areas of heavy vascularization was clearly discernible (Fig. 3). In view of the involvement of some major blood vessels, muscles and nerves, total surgical excision was highly challenging with no guaranteed success, apart from the real risk of recurrence. The owner was accordingly advised to get the pet euthanized, but she opted for surgical ablation, regardless of the outcome In the OT, the patient was administered iso-osmotic Normosol solution intravenously, and strict surveillance throughout the surgical procedure was ensured with the ECG, Pulse Oximeter and Apalert gadgets. The clinical parameters were also closely monitored. Surgery was completed in 2.5 hr time. After total resection of the CMCT mass, the entire area was flushed with sterilized normal saline solution. The extensive surgical wound was closed patiently, using new surgical pack and a new pair of gloves, and a sterile drain tube was inserted. The line of suture was sanitized with lidocaine/ bupivacaine ointment. Baytril was also given. The patient was given Polyflex and Carpofen. Sterile bandage was applied around the wound site before discharge. Sasha recovered remarkably well after the prolonged surgical ordeal and actually walked off uneventfully, a pleasant surprise.

Biopsy: A representative piece of tumor tissue was preserved in 10% buffered formaldehyde solution, and dispatched to the nearby regional centre, IDEXX Diagnostics, USA for histo-pathological evaluation.

Post-operative clinical condition

On December 11, 2017, Sasha was brought to the clinic, covered with a T-shirt to prevent any damage to the sutures, for recheck and bandage change. The over-all clinical status and the behavioural profile had markedly improved. The owner informed that the pet was doing well, and her daily dietary intake had also increased gradually with no issues pertaining to defaecation and urination. On December 15, 2017 follow-up visit to the clinic, the drain tube was gently pulled out, and the patient's surgical wound site was re-bandaged. The incision site was healing uneventfully. On December 21, Sasha had another bandage change. The owner scrupulously follows the advisory on food and nutrition, and sends regular telephonic feedback on the pet's health status.

Histopathological profile: Infiltrative moderate to densely cellular tumor mass revealed the presence of mast cells, arranged in cords and sheets within a scanty fibrous stroma (Fig. 4).The typical polygonal to ovoid neoplastic cell with distinct borders revealed pale, amphophilic cytosol containing clearly discernible fine basophilic granules and a large, round to oval nucleus

Table 2. Pre-anaesthetic blood biochemical profile.of the patient.

Parameter	Observed value	Reference range
Blood glucose (g/dL)	78.0	63-114
SDMA (µg/dL)	15.0	0-14
Serum creatinine (mg/dL)	1.3	0.5-1.5
BUN (mg/dL)	13.0	9-31
BUN: creatinine ratio	10.0	
Serum total protein (g/dL)	5.7	5.5-7.5
Albumin (A) (g/dL)	3.2	2.7-3.9
Globulin (G) (g/dL)	2.5	2.4-4.0
A: G ratio	1.3	0.7-1.5
Serum ALT (U/L)	31.0	18-121
Serum ALP (U/L)	24.0	5-160

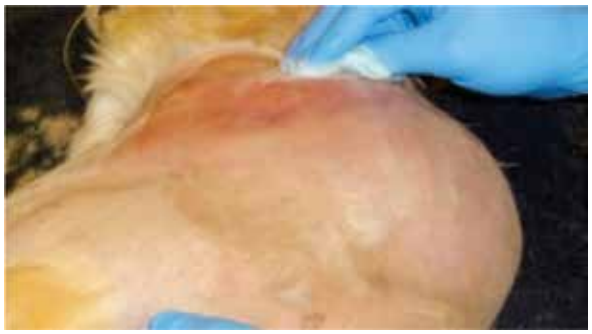

Fig. 1. Preparing for surgical excision of tumor.

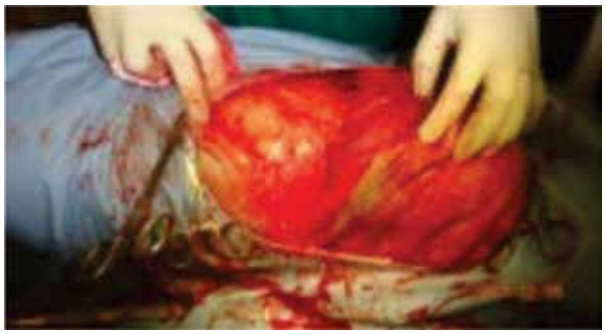

Fig. 2. Exploratory surgical incision of dermis.

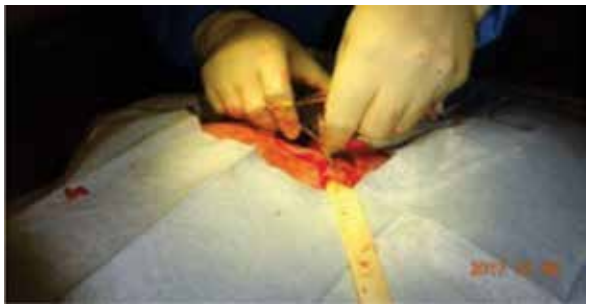

Fig. 3. Surgical wound closure with sutures.

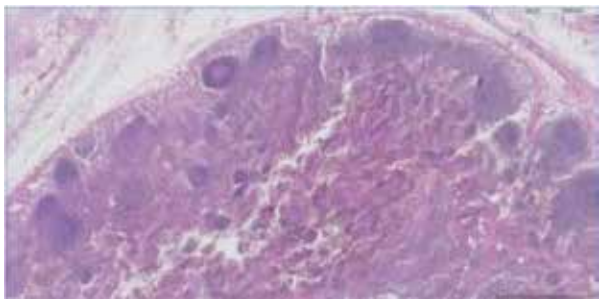

Fig. 4. Patchy necrobiosis in the exposed tumor.

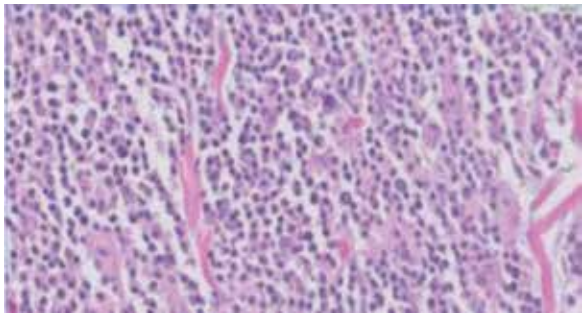

Fig. 5. Degenerative changes in the biopsy sample (HE, 400x).

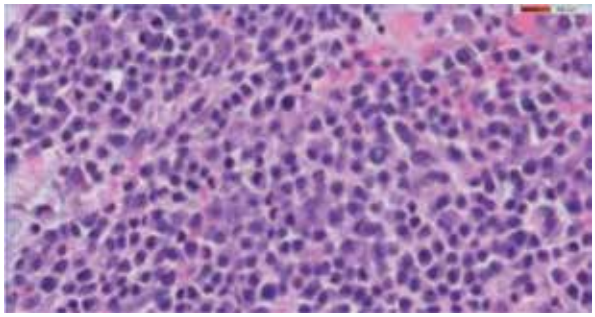

Fig. 6. Neoplastic cytomormophologic transformation (HE, 1000x)

with finely stippled chromatin, and an inconspicuous nucleolus. Anisocytosis and aniso-karyosis of moderate intensity and occasional binucleated cells were observed. The mitotic count was 19/ ten HP (400x) fields (Fig.5). Scattered areas of capillary bleeding and necrobiosis were observed. Evaginated eosinophils were observed, randomly scattered throughout the neoplastic mass (Fig. 6). Regional metastatic activity was evidenced by the Neoplastic cells, extending to the deep and lateral tissue margins, and conspicuous hyperplastic peri-tumoral lymph node. The IDEXX pathologist's microscopic interpretation was cutaneous mast cell tumor grade II (Patnaik system)/ high-grade (Kiupel system) with regional metastatic activity.

Comments: CMCTs in the dog are located in the dermis with or without extension into the underlying subdermis structure. Mitotic count remains a reliable clinical prognostic indicator in pet practice. Romanisk (2007) reported that CMCT dog patients with MC of 5 or less had a median survival rate of 70 months, *cf*. only 2 months with MC of 5, or more. In the instant case, need-based adjuvant fractionated radiotherapy and/ or chemotherapy will be recommended on confirmed post-surgery local recurrence.

Future perspectives: Surgical margins are stated to influence the final outcome; lateral margins of at least 3 cm, and deep margins of one fascial plane are recommended. But this rational approach *per se* may not effectively overcome the adverse bio-impact of tumor grade. Thus, Donnely *et al*. (2015) reported as high

as 36% recurrence rate of high-grade (Kieupel system) CMCTs despite histologically tumor-free margins (HTFMs) during total surgical excision of the neoplastic mass. PCR analysis for specific ITD mutations in the exon 11 of the cKIT gene, performed on the paraffin embedded biopsy sample, may facilitate advanced therapy with tyrosine inhibiting chemotherapeutic agents in the near future.

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A case report of pox in pigeons (*Columbia* spp.)

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Avian pox is a contagious, slow spreading viral disease of birds which occur all over the world (Tripathy and Cunningham, 1984). Pox infections in avian are the most common viral infectious diseases encountered both in domestic and in about 60 wild bird species including pigeons (*Columbia livia*) (Joshi *et al.,* 2012). Avian pox is a disease of economic importance particularly in commercial poultry and classically divided into 4 subgroups: fowl pox, turkey pox, canary pox, pigeon pox (Pandey and Mallick, 1974) and pigeon pox is a common virus disease of unvaccinated susceptible pigeons. It is the epitheliotropic DNA virus has a predilection for skin and mucous membranes with the formation of visible wart-like, "pock" lesions on the surface epithelium.

Case History and Observations

Among forty four pigeons of a poultry farm, five pigeon were presented to Medicine Clinic of TVCC, CVAS, Bikaner with the history of nodular growth on eye, legs and on beak also difficulty in feed and water intake. Physical examination revealed that pigeons have grayish white scattered multifocal firm nodules on the head and legs. The sizes of nodular lesions were varying from 1-6 millimeters. The lesions were seen around the eyes, on nasal septum, beak and between the toes (Fig. 1& 2). The pigeons were exhibiting symptoms of anorexia, depression and weakness.

Out of five pigeons two were died during course of treatment. The post-mortem of these pigeons revealed multiple yellowish nodules on mucosa of pharynx, larynx and oesophagus. Lungs revealed multifocal black spots adjacent to the borders and blackish multifocal granules in the alveolar walls.

Based on the clinical and post-mortem findings, the disease was diagnosed as pigeon pox. To combat the secondary bacterial infection tetracycline hydrochloride @ 1 g per liter of water. The birds were received Charmil® ointment for topical application for seven days and multivitamin Proviboost® 2 drops daily for ten days.

The regression of lesions were started after three days of treatment and completely recovered after ten days. The birds were started to take feed and water normally.

Discussion

Pigeon pox is a slow spreading disease which is responsible for morbidity and mortality in all age groups of pigeons. In natural infection, mortality is low but can be complicated with parasitism or poor condition of the flock (Singh *et al.,* 1990 and Tripathy, 1991). Pox virus is not fatal in all infected individuals, but it can reduce viability and predispose affected birds to predation, secondary infection, and accident (Reece, 1989). In this case, the bird might have died due to asphyxia and starvation because of the nodules in the pharynx and larynx.

Mechanical transmission of the virus is the result of wound created by mosquitoes and sucking insects such as mites or pigeon louse flies. These parasites serve purely as, which appears to localize on or in the proboscis of mosquitoes.

Upon gross examination, all the carcasses were found to be poor, emaciated and revealed small, focal, nodular greyish white lesions on the beak, around the eyes (Fig. 1), on the abdomen and on the limbs. Similar observations were reported earlier in pigeons (Mohan and Fernandez, 2008 and Hemanth *et al.,* 2014).

Supportive treatment can be attempted in captive birds; effective treatment of free-living birds under field conditions is not possible. To prevention and control of the disease only sensible hygiene and pox vaccine is effective in pigeons.

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*Corresponding Author

Fig. 1: Pox lesions on beak and head of pigeon

Fig. 2: Pox lesions on toes of pigeon

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Dated: 29 January, 2019 **Swaran Singh**

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