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Research Article

Serotyping of FMD virus from bovine tongue epithelium and virus visualization using Transmission Electron Microscopy

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Abstracts

For serotyping of FMD virus, 20 tongue epithelium samples were collected. Sandwich ELISA and RT-PCR were used for serotyping the FMD virus while Transmission Electron Microscope was used for study shape and size of virus. Overall typeability was 65.00% by sandwich ELISA and most prevalent serotype was O (69.23%) followed by serotype A (30.77%). Species-wise prevalence of serotype O and A were 66.67% and 33.33% respectively in cattle while 75.00% and 25.00% in buffaloes respectively. Although, overall typeability of FMD virus by RT-PCR was 80.00% and most prevalent serotype was O (68.75%) followed by serotype A (31.25%). Within species, serotypes O (72.73% and 60.00%) were most prevalent in both cattle and buffaloes respectively followed by serotype A (27.27% and 40.00%). Type C and Asia-1 could not recovered by both sandwich ELISA and RT-PCR. FMD virus seems to have spherical symmetry with size 40 nm to 45 nm in transmission electron microscope. However surface of virus seems to be rough with small projections.

Keywords: Buffalo, Cattle, FMD virus, RT-PCR, TEM, Sandwich ELISA and Tongue epithelium

Foot-and-mouth disease (FMD) is an acute, vesicular disease of cloven-hoofed ruminants, pigs and wild mammals with 100% morbidity and low case fatality rate (OIE, 2007). Cell culture and ELISA are routine methods for isolation and detection of FMD virus (Ferris and Dawson, 1988) but these are time consuming and labour intensive. Of late PCR-based molecular methods have been developed for detection of viral RNA from clinical samples using different primers (Callens and DeClercq, 1997; Reid *et al*., 1998). Presently, less time consuming real-time PCR assays is favoured (Moonen *et al*., 2003).

Transmission electron microscopy with negative staining of FMD suspected field samples with urinyl acetate is useful for initial identification of unknown viral agents in particular outbreaks and is recommended by regulatory agencies for investigations of the viral safety of biological products and/or the cells used to produce them (Philippe, 2008).

Materials and Methods

Total 20 tongue epithelium (8 cattle and 2 buffaloes from Udham Singh Nagar; 1 cattle and 3 buffaloes from Dehradun; 2 cattle and 1 buffaloes from Almora; 2 cattle and 1 buffaloes from Nanital districts) were collected and stored in 50% phosphate buffer glycerol (PBG) at 20°C till used. The samples were thawed at room temperature for 10 minutes, gently washed thrice with PBS (pH, 7.4) and triturated in sterile pestle and mortar to prepare a 10% (w/v) in PBS. Further equal volume of chloroform was added to the suspension, shaken vigorously and centrifuged at 3000 rpm for 10 minutes. The clarified supernatants were used for serotyping by sandwich ELISA and RNA isolation.

Sandwich ELISA was done at FMD Regional centre, Mathura. All the reagents were procured from Central FMD virus typing laboratory, IVRI, Mukteshwar. Type specific O, A, C and Asia-1 anti-146S rabbit sera were diluted in coating carbonate-bicarbonate buffer (pH 9.6) as 1:3000, 1:5000, 1:5000 and 1:8000 respectively. Similarly, type specific anti-146S guinea pig tracing sera against O, A, C and Asia-1 was diluted to 1:1000, 1:1000, 1:5000 and 1:3000 respectively, in blocking buffer. The conjugate was used at dilution of 1:3000. Test was carried out in flat-bottomed immunoassay plates as per method described by Bhattacharya *et al.* (1996).

For RT-PCR, RNA from FMD virus was isolated from tongue epithelium samples by GeNei™ TRIsol as per procedure mentioned in manufacturer instruction of GeNei™ TRIsol. Purity of RNA was judged on the basis of optical density ratio at 260:280 nm. The samples with acceptable purity (*i.e.* ratio 1.7-2.0) were quantified using the following formula and

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used for reverse transcription (Sambrook *et al.,* 1989; Manchester, 1996).

$$\text{Quantity of RNA } (\mu g/\mu l) = \frac{OD_{260} \text{ x dilution factor x } 40}{1000}$$

RNA was amplified using RT-PCR by GeNei™ One Step M-MuLV RT-PCR Kit. Reaction Mix I contains; RNasin (1µl), GeNei™ 2X RT PCR Reaction Mix (25 µl), GeNei™ RT-PCR Enzyme Mix (2 µl), Primers; NK-61, ARS-4, A-$1C_{562}$ and As1-$1C_{505}$ (25 picomole/reaction each for forward and backward primers) and nuclease free water (upto 40 µl). Master Mix II was setup in separate 0.2 ml PCR tubes. It contains template RNA (5 µl/50 µl reaction) and was denatured at 65°C for 5 minutes, subsequently chilled on ice. Master Mix II (10 µl) were added to the Master Mix I (40 µl) into each PCR tube. It was subjected to following thermal cyclic conditions: one cycle at 95°C for 5 min, 30 cycles each at; 94°C 1 min, 45°C 1 min, 72°C 2 min for serotype O; 94°C 1 min, 55°C 1 min, 72°C 1.5 min for serotype A; 94°C 1 min, 55°C 1 min, 72°C 1.5 min for serotype Asia-1, followed by one cycle at 72^0 C for 5 min. The size of PCR product was confirmed by electrophoresis (100 volts for 30 min) in 1.5% agarose containing ethidium bromide (0.5 µg/ml).

Negative staining using 2% urinyl acetate was performed as per method of Brenner and Horne (1959) in electron microscopy laboratory, GBPUA&T Pantnagar. Specimens (Grid) was mounted in a dedicated holder and analyzed using a TEM (JEM 1011) with a BioTwin lens configuration with a Lab6-filament operating at an acceleration voltage of 80 kV.

Results

Of 20 tongue epithelium sample, 13 showed positive results by sandwich ELISA indicating overall typeability as 65.00% (Table I). Serotype O (69.23%) was the most predominant followed by serotype A (30.77%). Serotypes C and Asia-1 could not be recovered. Among cattle, 9 (69.23%) were positive of 6 (66.67%) and 3 (33.33%) were positive for serotype O and A, respectively. In buffaloes, 4 samples (57.14%) were positive of which 3 (75.00%) and 1 (25.00) revealed serotype O and A, respectively (Table 2).

By RT-PCR, 16 samples gave amplicons 865bp (Figure I) and 1301bp (Figure II) respectively, for serotype O and A, whereas amplicons of Asia-1 and C could not be amplified. Among cattle and buffaloes, typeability was 84.61 and 71.43%, respectively (Table I). Most prevalent serotype was O (72.73%) followed by serotype A (27.27%) in cattle as well as buffaloes (Serotype O - 60.00% and A - 40.00%). In sandwich ELISA positive sample, the virus particles were visualized by TEM as small spherical structure (Figure III) of sizes 40 - 45 nm. The small projections from outer surface of virus particle have also been recorded that is surface look like rough.

Discussion

The typeability of virus by sandwich ELISA was 65.00%. Non-typing of virus in remaining samples may be due to the fact that either samples were not

Table I: Prevalence of FMDV by sandwich ELISA in bovines.

Sl. No.	Species	Samples tested	Virus recovered	Types of Virus			
				O	A	C	Asia-1
	Sandwich ELISA						
1	Cattle	13	9 (69.23)	6 (66.67)	3 (33.33)	-	-
2	Buffaloes	7	4 (57.14)	3 (75.00)	1 (25.00)	-	-
	Total		20 (65.00)	13 (69.23)	9 (30.77)	4	- -
	RT-PCR						
1	Cattle	13	11 (84.61)	8 (72.73)	3 (27.27)	-	-
2	Buffaloes	7	5 (71.43)	3 (60.00)	2 (40.00)	-	-
	Total		20 (80.00)	16 (68.75)	11 (31.25)	5	- -

* Figure in parenthesis are respective per centage

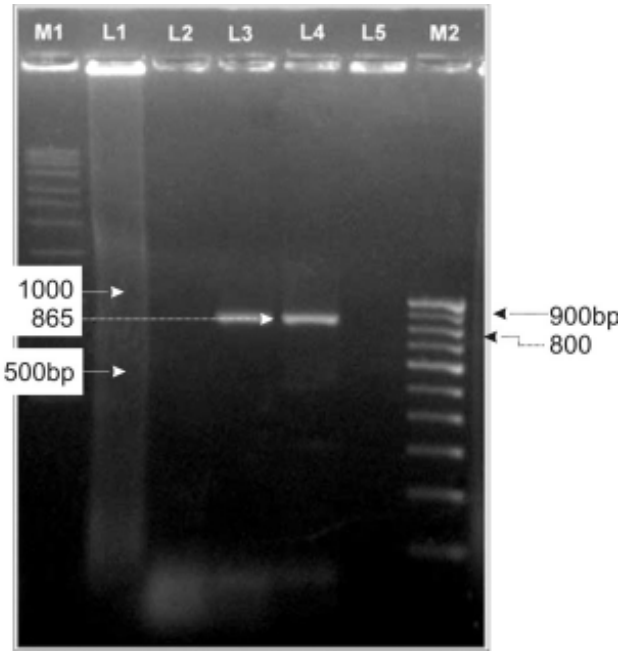

Fig I: Agarose gel electrophoresis of 865 bp PCR products amplified from RNA isolated from the tongue epithelium samples of bovine suspected for FMD.

M 1 : DNA marker 1 kb
L 1 : Negative sample
L 2 : Negative sample
L 3 : Positive sample with primer dimer
L 4 : Positive sample with primer dimer
L 5 : Negative control
M 2 : DNA marker 100 bp

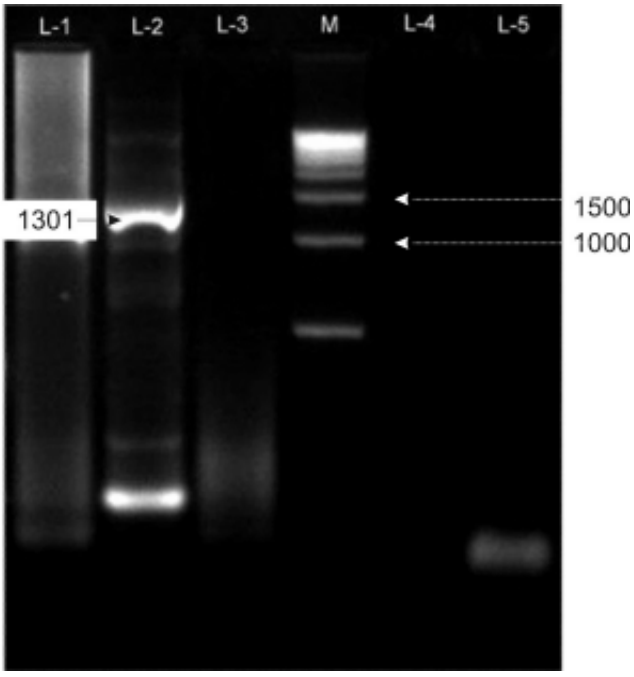

Fig. II: Agarose gel electrophoresis of 1301 bp PCR products amplified from RNA isolated from the tongue epithelium samples of bovine suspected for FMD

M : DNA marker 1 kb
L 1 : Positive sample
L 2 : Positive sample with non specific amplifications
L 3 : Negative sample
L 4 : Negative sample
L 5 : Negative control with primer dimer

collected at proper time of clinical manifestation of disease or were not suitably preserved (Prasad *et al.,* 1992). Among them more samples were characterized as serotype O. Serotype C has not been reported from any of the outbreaks in recent years (Kumar *et al.,* 1994) from India.

The highest prevalence of serotype O (69.23%) in present investigation is in concordance with the findings of Mann *et al.* (1998) who reported the dominance of type O in country. The molecular epidemiological studies have established that the Pan-Asia strain was the major cause of outbreak of FMD involving serotype O in India (Bandyopadhyay, 2004).

In cattle and buffaloes serotype O were dominated. Gurhan *et al.* (1993) identified that second most prevalent serotype was type A. However Rana *et al.* (1991) noticed type A as the most dominant type in West Bangal. Occurrence of the disease due to type A was 12.5% during 1971-1977 in the country and during 1995-1997; it was recorded in only 3.50% of total outbreaks (Adlakha, 2003).

In the present study, the typing results revealed the predominance of type O followed by A in both cattle and buffalo irrespective of sex, breed or age group. Therefore, it appears that the occurrence of different serotype has no relation with species, sex, breed and age group of the animal. Sudhan *et al.* (1990) observed outbreaks in pigs due to type A_{22} and Kumar *et al.* (1994) identified type Asia-1 as the cause of outbreaks in Gaurs.

By RT-PCR, 80.00% samples were found positive suggesting that RT-PCR was more sensitive than sandwich ELISA as reported by Clavijo *et al.* (2003) and King *et al.* (2006) also. The higher sensitivity of RT-PCR may be because of its ability to detect very small number of virus as well as detection of RNA of non-viable FMD virus (Mohapatra *et al.,* 2007).

Actual size of FMD virus has been reported as 27 nm by X-ray crystallography though it varies from 25 to 30 nm. The large size of FMD virus recorded by

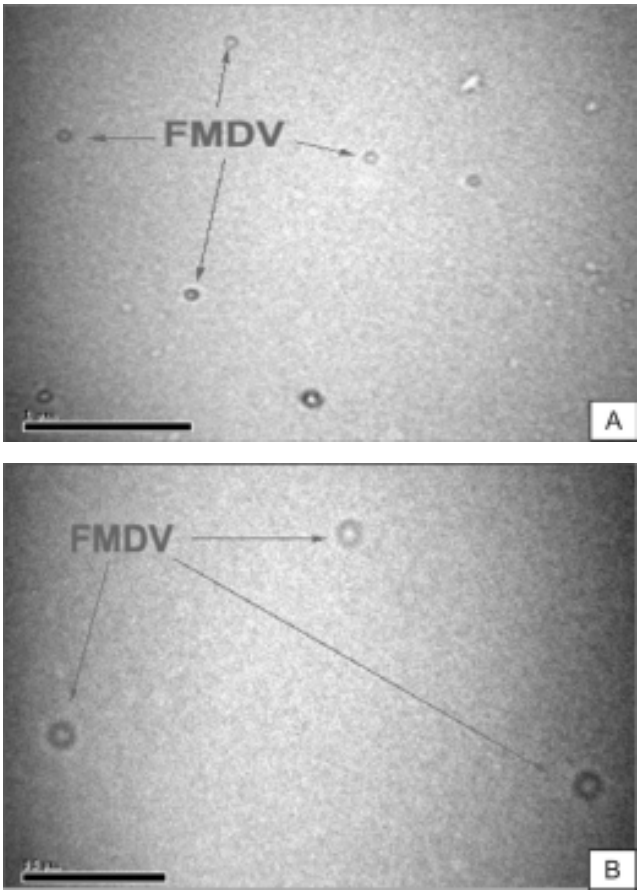

Fig. III: Negative staining of FMDV isolated directly from clinical sample (Tongue epithelium) visualized in Transmission Electron Microscope at resolution of 672×520 pixels and accelerating voltage 80 KV.
(A) FMDV at magnification X30000 and exposure time 1.48273s.
(B) FMDV at magnification X80000 and exposure time 3.94521s.

transmission electron microscope may be due to attached secretory antibody to the FMD virus. These attached antibodies (IgA) gives rough morphology, same region attributed to large size FMD virus. The full-sized Ab has estimated molecular dimensions of 15 x 7 x 3.5 nm (Jung *et al.,* 2008). So the actual virus size will be approximately 30 nm. Le *et al*. (1994) also noticed similar results and they treated the FMD virus culture with virus binding portion (*Fab*) of antibody and hence size of virus becomes approximately 45 nm. In this case *Fab* contributes 16 nm to the virus diameter. FMD virus having comparatively smooth surface in comparison to other picornavirus, which was evident by X-ray crystallography but Fab part attached in the clinical samples gives it rough spherical appearance in transmission electron microscope.

Conclusion

Serotype O was most prevalent in different districts of Uttarakhand in both cattle and buffaloes followed by serotype A, while serotype Asia-1 and C could not be recovered from the samples. RT-PCR might be more specific and sensitive test than Sandwich ELISA for antigen detection on tongue epithelium samples. FMDV can be visualized in clinical samples with negative staining and may be used as a semi quantitative test for diagnosis of FMD outbreaks.

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Haemato-biochemical and therapeutic studies on Dilated Cardiomyopathy in dogs

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Abstract

Eight dogs presented with clinical signs suggestive of cardiac problems and later confirmed for DCM were utilised for the detailed treatment studies. Signalment, history, electrocardiography, radiography, echocardiography, haematology, serum biochemistry and response to treatment of enalapril @ 0.5 mg/kg bid, valsartan @ 2 mg/kg bid and lasilactone @ 2mg/kg bid orally were studied. All the ECG measurements were within the normal range except for a slight increase in the P wave duration indicating left atrial enlargement. Echocardiographic four chamber view revealed left ventricular dilatation all cases. Pericardial effusion was present in 12.5% of the cases. M–mode measurements showed reduced myocardial contractility in all cases. Mean values of RBC and haemoglobin on day 30 showed a significant reduction indicating development of anemia due to direct effect of angiotensin on erythropoesis. Clinical improvement was present in 62.5% of the cases.

Keywords: Dilated cardiomyopathy, Valsartan, Dog.

Dilated cardiomyopathy (DCM) could be defined as a cardiovascular disease in which the degree of myocardial dysfunction was not explained by the abnormal loading conditions or the extent of ischaemic damage (Ristic,2004). Diagnosis of DCM requires active exclusion of other causes of dilated and hypokinetic heart. Stringent diagnosis of DCM requires all the following, left ventricular dilation, depressed systolic function and altered geometry of the left ventricle (McEwan *et al*., 2003).

Judicious inhibition of renin – angiotensin system is the widely accepted way of treatment of DCM in dogs. Recently other pathways of angiotensin II production were identified. Blocking angiotensin at the receptor level by using angiotensin II receptor antagonist may be a better way of treatment, to improve the quality and survival rate of affected dogs. Hence the study has been undertaken with the objective to evaluate the efficacy of angiotensin II receptor antagonist for the clinical management of DCM.

Materials and Methods

Twenty five dogs brought to the Veterinary College Hospital, Mannuthy and University Veterinary Hospital, Kokkalai with signs of cardiac problems *viz.* lethargy, weakness, dyspnoea, cough, syncope and exercise intolerance were used for the study. They were screened for cardiac problem by detailed clinical examination. Based on electrocardiographic, radiographic and echocardiographic abnormalities eight confirmed cases of dilated cardiomyopathy were selected and utilised for further treatment studies with enalapril @ 0.5 mg/kg body weight, furosemide and spironolactone @ 2 mg/ kg body weight and valsartan @ 2 mg/ kg body weight orally twice daily for a period of one month. Echocardiographic observations were performed on the day of admission and on day 30 of treatment and radiographic observations on the day of admission only.

Detailed history and results of clinical examination including temperature, pulse and respiration of eight selected dogs were recorded paying special attention to the cardiovascular system.

Electrocardiogram were recorded as per the standard procedures using BPL – CARDIART _ 6108® ECG machine on the day of admission, 15th and 30th day of treatment. Three standard bipolar limb leads (I, II, III) and three augmented unipolar limb leads (aVR, aVF, aVL) were used for the study.

Lateral plane radiographs of thorax of diseased animals were taken for cardiac evaluation. Animals were subjected to both two - dimensional and M – mode echocardiography in left and right parasternal view using L&T SYMPHONY® 4.0 scanner with 7.5 MHz transducer. Left ventricular dimensions were measured in the right parasternal short axis view. Values for each parameter were determined by the average of three to five cardiac cycles.

Blood samples (approximately 5 ml) were collected from diseased dogs on the day of admission, 15th and 30th day of treatment for haematobiochemical analysis viz. total RBC, haemoglobin, volume of packed

red cells, total leukocyte count, differential count, serum creatine kinase, creatinine, potassium and sodium were estimated. Serum creatine kinase and creatinine were estimated by spectrophotometry in Merck 200 spectrophotometer using commercially available kits (AGAPPE Diagnostics). Serum sodium and potassium were estimated using SYSTRONICS 128® flame photometer.

Treatment response was monitored based on clinical response, electrocardiography, echo-cardiography, haematology and serum biochemistry. Data collected on various parameters were statistically analyzed by paired samples T test as described by Snedecor and Cochran (1994) at 1% level of significance.

Results and Discussion

Dilated cardiomyopathy was more commonly observed in the middle aged dogs. Age of affected dogs were ranged from 2 to 13 years with a mean of 6 ± 1.25 years. Sexwise prevalence of DCM in dogs revealed that both males and females were equally affected. The breed wise distribution of DCM indicated that Labrador Retriever was more prone to dilated cardiomyopathy (37.5%) followed by Boxer (25%), German Shepherd (12.5%), Spitz (12.5%) and Non-descript (12.5%) dogs.

Major clinical signs included cough (37.50%), ascites (75%), anorexia (75%), polydypsia (50%), syncope (25%) and oedema of hind limbs (25%). Exercise intolerance and dyspnoea were present in all cases.

Clinical data were within the normal range. Irregular pulse was present in 37.5% of the cases on the day of admission. Femoral pulse was weak in 75% of the cases. Pale mucous membrane was seen in 25% of the cases. Thoracic auscultation revealed tachycardia (25%) and pulmonary crackles (75%). Pulse deficit was present in 50% of the cases. Ascites was confirmed by tactile percussion and later by ultrasonography in 75% of the cases. Positive hepato-jugular reflex and

Table 1: Echocardiographic M - mode measurements of dilated cardiomyopathic dogs on day 1 and 30.

Observation period	Left ventricular end diastolic diameter (cm)	Left ventricular end systolic diameter (cm)	Fractional shortening(%)	Ejection fraction (%)
Day 1	6.02 ± 0.50	4.84 ± 0.43	19.80 ± 1.71	43.43 ± 4.16
Day 30	5.29 ± 0.75	4.32 ± 0.78	19.93 ± 0.03	40.25 ± 0.06

Table 2: Haematological values of dilated cardiomyopathic dogs on day 1, 15 and 30 of treatment.

Parameters		Diseased dogs		
		Day 1	Day 15	Day 30
RBC (millions/cu.mm)		6.5 ± 0.93^{a*}	4.66 ± 0.67^{b*}	4.64 ± 1.70 ^{*b}
Hb (g%)		11.67 ±1.59 ^{a*}	9.45 ± 1.63 ^{b**}	9.45 ± 1.60 ^{b*}
VPRC (%)		32.61 ± 4.24 *	28.45 ± 4.08	28.82 ± 4.27
TLC (per cu.mm)		15191.67± 3346.70	11983.33 ± 2351.80	11300 ± 1865.30
DLC	N	73.33 ± 9.59	76.33 ± 3.32	75.17 ± 2.54
	L	23.50 ± 9.77	20 ± 2.49	21.5 ±2.23
	M	1.0 ± 0.82	0.83 ± 0.54	0.67 ± 0.49
	E	2.17 ± 0.60	2.83 ± 0.95	2.67 ± 0.88

Table 3: Serum creatinine, CPK, sodium and potassium of dilated cardiomyopathic dogs on day 1, 15 and 30 of treatment.

Parameters	Diseased dogs		
	Day 1	Day 15	Day 30
Creatinine (mg/dl)	1.08 ± 0.16	1.45 ± 0.27 ^{a}	2.19 ± 0.31^{*b}
CPK (U/L)	230.83 ± 52.46 **	267.50 ± 64.34 ^{a}	142.50 ± 55.14^{*b}
Sodium (mEq/L)	142.83 ± 2.57	148.33 ± 2.97^{a}	142.67 ± 2.50^{*b}
Potassium (mEq/L)	4.22 ± 0.23	4.73 ± 0.23	4.75 ± 0.24

*Represents a significant difference ($p<0.05$) compared to day 1

** Represents a highly significant difference ($p<0.01$) compared to day 1

Means within the same row of the same parameters with different superscript differ.

A, B compared with control a, b compared with day 1 values.

venous distension were present in 75% of the cases which were features of right sided heart failure.

Sinus tachycardia (25%), atrial fibrillation (12.5%), Ventricular Premature Complexes (25%) and ventricular pre – excitation (12.5%) were the most common abnormalities encountered in ECG. Mean heart rates were increased on day 15 and decreased on day 30. Atrial fibrillation and ventricular premature complexes were absent on day 15 and day 30. Atrial tachycardia was present in 12.5% of the cases on day 15 and day 30.

Radiographic examination revealed generalised cardiomegaly (75%), tracheal elevation (100%), pulmonary congestion (75%) and pericardial effusion (12.5%).

Echocardiography revealed cardiac dilatation in all cases. Left ventricular dilatation was evident in all cases in cardiac four chamber view. Pericardial effusion was present in 12.5% of the cases. Results of M – mode measurements are presented in table. There was no significant difference in the M – mode measurements on day 30 when compared to day 1 which indicated the treatment caused no significant alterations on the structure of heart.

Haemato-biochemical results are presented in the table (2 and 3). Normal to slightly reduced haaemocrit values were obtained in DCM and this might be due to fluid retension secondary to heart failure caused by activation of renin angiotensin mechanism causing haemodilution and reduced haemocrit. Slightly increased total leukocyte count along with increased neutrophil count was suggestive of a stress leukogram which was similar to the finding of Abbott (1998), Guglielmini and Civitella (2004) and Ristic (2004).

A statistically significant reduction in the mean values of RBC and haemoglobin on day 15 ($p < 0.05$) and day 30 ($p < 0.01$ and $p < 0.05$ respectively) might be suggestive of the role of angiotensin in erythropoesis which was supported by the finding of Cole *et al.* (2000) who reported 12 to 20% reduction in haematocrit value in angiotensin converting enzyme deficient mice. Mechanism of development of anemia was uncertain and the authors ruled out hemolysis, bonemarrow suppression and renal failure. There was no significant difference in the hemodynamic characteristics of the groups receiving enalapril and valsartan alone (Kasama *et al.*, 2003) which showed angiotensin could have direct effect on erythropoesis.

No significant difference could be obtained in total and differential leukocyte count on day 15 and 30 when compared to day 1 values which was according to the finding of Cole *et al.* (2000).

Mild elevation of creatinine level was observed on day 1. Creatine phosphokinase activities were elevated in cardiomyopathies due to reduced coronary perfusion. Sodium was within the normal range but towards the lower limit which was in accordance with the finding of Deicas *et al.* (1995). Patients with moderate to severe CHF experienced a significantly reduced renal blood flow and enhanced tubular reabsorption of sodium and free water. Consequent activation of renin – angiotensin – aldosterone mechanism led to mild hyponatrimia. Potassium was within the normal range. No significant difference could be detected in day 15 values when compared to day 1 values.

Persistant elevation of CPK might be due to reduced coronary perfusion and active myocardial necrosis which was a consistent feature of DCM. Significant increase for creatinine value was observed on day 30 when compared to day 1 value. According to Eric *et al.* (1995) older age, diuretic therapy, and diabetes were associated with decreased renal function in enalapril therapy. This might be a cause for significant increase in creatinine concentration on day 30.

Significant reduction ($p<0.05$) in the CPK value might be due to the action of enalapril that improved transmural myocardial perfusion at rest and after chronotropic stress and restored impaired subendocardial coronary flow and vasodilator reserve in DCM. The effects of enalapril were bradykinin mediated and nitric oxide dependent and were not recapitulated by angiotensin receptor blockers (Nikolaidis *et al.*, 2002). These data suggested the beneficial effects of ACE inhibitors on the coronary circulation in DCM that are not shared by AT_1 receptor antagonists. Following diuretic therapy there was loss of sodium ions which might be responsible for the significant reduction ($p<0.05$) in sodium level on day 30 when compared to day 15 value (Kittleson, 2002).

One case (case No. 3) developed cough

following the treatment. Skin rashes developed following treatment in two cases (case No.3 and case No.6). Anemia which was evidenced by reduction in mean values of RBC and haemoglobin developed following treatment. The side effects of ACE inhibitors were first-dose hypotension, dry cough, acute renal failure, angioneurotic edema, hyperkalemia, skin rashes, fetopathic potential (oligohydramnios, fetal growth retardation, and fetal death may be due in part to fetal hypotension), proteinuria, dysgeusia, neutropenia, glycosuria, and hepatotoxicity. Angiotensin receptor blockers had less side effects and production of dry cough been reduced (Kumar *et al.*, 2000).

Conclusion

Based on the above studies, it could be concluded that diagnosis of DCM requires a combination of clinical examination, electrocardiography, echocardiography, radiography and haematobiochemical studies. Fractional shortening is more significant indicator of cardiac function than ejection fraction in dogs. A combination of enalapril, valsartan and lasilactone could be used for treating DCM and the treatments improve clinical signs and prolong the survival in dogs. Along with the above treatment haematinics also should be added so that anaemia can be counteracted.

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Evaluation of urea mollases multi-nutrient blocks enriched with area specific mineral mixture in crossbred Cattle

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Abstract

To assess the effects of supplementary feeding of urea-molasses multi-nutrient block (UMMB) enriched with area specific mineral mixture on productive and reproductive traits 20 crossbred cattle were allowed to lick a UMMB @ 400-600g daily for 30 days. Blood samples were analyzed for haemato-biochemical parameters, macro and trace elements and hormonal status (T_3, T_4 and progesterone) at the beginning and after completion of trial. Significant increase in total plasma protein, albumin and globulin levels was observed. An average increase of 18.18% in fodder intake, 12.76% in milk yield and 6.31% in milk fat following UMMB supplementation was noticed. The anoestrus animals came in heat during the study period.

Keywords: Biochemical changes, Cattle, Haematology, UMMB

Livestock reared in hills are generally fed local grasses, tree leaves, wheat, rice straw and maize stalk which are low in fermentable nitrogen, mineral, and readily available carbohydrate resulting in poor animal growth rate, poor reproduction, long calving interval, and unthrifty condition. Inadequate nutrition is one of the factors that frequently limit the full utilization of the productive and reproductive potential of livestock in hills. Developing alternate feeding strategies for ruminant production based on agro-industrial wastes is, therefore, of prime importance. A UMMB prepared from locally available agro-industrial by-products has been adoptable feed supplement which improves nutritional status of animals (Kang *et al*., 2007). Successful treatment and control of mineral deficiencies lies in effective and practical methods of supplementation. Feeding of merely the deficient minerals may not improve the general health status, production and reproduction of animals in these areas. The present study was undertaken to evaluate the effect of UMMB containing area specific mineral mixture as supplementary feeding on the general health condition, milk yield and reproductive performance of crossbred cattle.

Materials and Methods

Total 20 crossbred cattle of 2-8 yr age from a village in irrigated belt near the international border of Jammu in subtropical zone reared under conventional small farming system and fed through grazing and variable quantity of barseem, sorghum, local grasses and wheat straw were selected for the present study conducted during April and May months. Out of 20 selected crossbred cattle, 10 animals showed anoestrus and 14 animals were in lactation. The UMMB were prepared by mixing mollases (35%), urea (10%), deoiled rice bran (10%), oiled rice bran (10%), groundnut meal (10%), area specific mineral mixture (14% containing DCP- 70%, $MgSo_4$-29%, Potassium Iodate-0.09%, $CuSo_4$-0.5% and $MnSo_4$-0.5%), common salt (1%) and cement (10%) as binding material. All the animals were allowed to lick a 2 kg block of UMMB @ 400-600 g/ day for 4-6 hr daily for 30 days. Close observations were made on selected animals on changes, if any, in feed intake, milk yield, milk fat production and oestrus activity.

Blood samples were collected on day 0 and 30th and Hb and PCV were analysed by standard methods. Glucose, total proteins, albumin, alkaline phosphatase, calcium and urea nitrogen were estimated using Autopak kits supplied by Bayer Diagnostics India Ltd. Levels of triiodothyronine (T_3) and thyroxine (T_4) were assayed by radioimmunoassay technique using RIA kits procured from BARC (Radiopharmaceuticals Operations Board of Radiation and Isotope Technology, BARC's Vashi Complex, Navi Mumbai, India). Progesterone estimation was done by liquid phase radioimmunoassay (RIA) procedure (Kamboj and Prakash 1993). Cu, Fe, Zn, Mn and Co were measured by Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI). Mg (diagnostic kit of AGAPPE Diagnostics), inorganic phosphorus (Taussky and Shorr,

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1953) and plasma inorganic iodine (Aumont and Tressol, 1987) were also estimated. Statistical analysis of data was done as per Snedecor and Cochran (1989).

Results and Discussion

All the selected crossbred cattle showed emaciation with poor body condition. Anoestrus was the major problem as 50 per cent of the selected cattle had not shown estrus for the last over 6 months. The health condition of the selected cattle suggested that malnutrition might have been the cause of anoestrus. However, after 30 days of supplementation marked improvement in the general health condition was observed. Observations on all closely monitored crossbred cattle revealed an average increase of 18.18% in fodder intake, 12.76% in milk yield and 6.31% in milk fat following UMMB supplementation. At initiation of UMMB supplementation the daily fodder intake was 22 kg (10 kg dry and 12 kg green), daily milk yield 4.70± 2.02 kg and milk fat per centage was 3.80± 0.12%, which, increased to 26 kg (10 kg dry and 16 kg green), 5.30± 1.87 kg and 4.04± 0.18%, respectively, at the end of trial. The increased milk production and fat per centage may be attributed to higher supply of crude protein, energy and minerals to animals and increased digestibility of the ration (Rafiq *et al*., 2000). Earlier study carried by Tripathi *et al.* (2006) reported an average increase of 14.13 per cent in DM intake in cattle following UMMB feeding reflected through improved milk yield by 24%. Better intake and utilization of dietary nutrients following UMMB supplementation in crossbred cattle has been reported by Toppo *et al.* (1997). Vu *et al.* (1999) observed 10.3-11.9% increase in milk production and 3-5% increase in milk fat following UMMB supplementation in cattle. The mean values of Hb and PCV were within the normal range quoted by Radostits *et al*. (2000). Based on Hb and PCV, anaemia was observed in 50 and 60 per cent animals having levels below 8 g/dl and 24%, respectively (Table 1). Significant (P<0.05) increase in the average value of total plasma protein after 30 days of trial i.e., 7.41± 0.24 g/dl was observed (Table 1), which could be attributed to significant (P<0.05) increase in the albumin and globulin levels. Considering the critical level of 5.7 g/dl hypoproteinemia was observed in 80 per cent animals at the beginning of trial with none of the animal having hypoalbuminemia (level <2.1 g/dl). However, after 30 days of supplementation not even a single animal was having hypoproteinemia. No-significant (P<0.05) effect of UMMB supplementation on total plasma protein and albumin level in anoestrus buffaloes after 4 weeks was observed by Brar and Nanda (2008). Earlier study carried by Qreshi *et al.* (2002) have reported that excessive levels of crude protein in the diet elevated BUN levels, altered uterine pH and reduced fertility in buffaloes. However, in present study no-significant (P<0.05) effect on urea nitrogen following UMMB supplementation was observed and finding corroborates with study of Brar and Nanda (2008) in buffaloes. The glucose level of crossbred cattle increased significantly (P<0.05) from 49.87mg/dl to 64.24 mg/dl after 30th day of supplementation of UMMB. The alkaline phosphatase values observed were within the normal range of 35-350 u/l quoted by Radostits *et al.* (2000). Thus, the UMMB supplementation was not having any harmful effect on the liver and bones.

No-significant variation in the levels of plasma T_3 and T_4 was observed (Table 1). The average values of T_4 observed was lower than the average value of 82.4 nmol/l (normal range of 54-110.7 nmol/l) quoted by Kaneko *et al.* (1999). The average progesterone level

Table 1. Effect of UMMB supplementation on Haemato-biochemical, hormonal and mineral status in crossbred cattle. (Mean ± SE)

Parameter	Day of trial	
	0	30th
Hb (g/dl)	8.20±0.56	7.47±1.08
PCV (%)	25.40 ±2.04	27.55±2.55
TPP (g/dl)	5.30 ± 0.09 [a]	7.41± 0.24 [a]
Albumin (g/dl)	2.70± 0.05 [a]	3.21 ± 0.19 [a]
Globulin (g/dl)	2.59± 0.12 [a]	4.19± 0.37 [a]
A:G ratio	1.06 ± 0.06	0.84 ± 0.12
Glucose (mg/dl)	49.87± 2.37	64.24 ±6.75
ALP (I.U.l-l)	113.06± 28.89	113.60 ± 26.95
BUN (mg/dl)	12.97± 1.98	12.55 ± 0.90
T_3 (nmol/l)	0.63± 0.11	0.53 ± 0.08
T_4 (nmol/l)	48.10± 0.11	45.44± 6.82
Progestrone (ng/ml)	0.773± 0.189	0.817 ± 0.162
Ca (mmol/l)	2.17± 0.10	2.27 ± 0.07
Pi (mmol/l)	2.00± 0.10	2.34± 0.12
Mg (mmol/l)	0.92 ± 0.04	1.09± 0.16
Cu (µmol/l)	7.07± 0.95	7.68± 1.42
Fe (µmol/l)	51.33± 6.97	69.1 ± 7.90
Zn (µmol/l)	237.90± 25.09	204.45± 43.41
PII (ng/ml)	61.11± 6.70	64.14± 5.92
Mn (µmol/l)	0.75± 0.19	0.82± 0.38

[*] Means marked with similar superscript "a" differ significantly (P<0.05) in a row.

of plasma samples from crossbred cattle showing anoestrus was 0.58 ± 0.17 ng/ml (range values 0.33 to 1.20) at the beginning of trial which showed non-significant (P<0.05) increase to 0.92± 0.23 ng/ml. Interestingly, all anoestrus animals came in heat during the study period. Vu *et al.* (1999) reported significantly shorter intervals from calving to onset of ovarian activity (91–94 days), to estrus (110–114 days), to conception (121–122 days) and the calving interval (13.4–13.6 months) in the UMMB supplemented cattle than those in the control group (112, 135, 152 days and 14.4 months, respectively).

The average value of Ca, Pi and Mg showed non-significant (P<0.05) increase at the end of trial as shown in Table 1. Tiwari *et al.* (1990) reported increased (P<0.01) balances of calcium and phosphorous in buffalo calves supplemented UMMB and fish meal for 130 days. Considering the critical level of 2.1 mmol/l, 30% crossbred cattle were having hypocalcaemia at the beginning however, after 30 days of supplementation 22.22% showed hypocalcaemia. The average values were within the normal range quoted by Radostits *et al.* (2000). The average value of copper in plasma samples of crossbred cattle showed non-significant (P<0.05) increase on 30th day of supplementation. The average values of copper in both the pre- and post-supplementation groups were lower than the normal range of 9.5 - 23.6 ìmol/l as reported by McDowell (1992). The overall prevalence of hypocupraemia was 88.88 per cent before the start of trial, of which 37.50% animals were marginally deficient (plasma level 7.9-9.59 µmol/l) and 62.50% per cent were having level <7.9 µmol/l. However, at the end of supplementation trial, 66.66% animals were copper deficient of which 16.66% animals were marginally deficient and 83.33% per cent were having level <7.9 µmol/l. Likewise, the iron level of plasma samples increased from 51.33 ± 6.97 µmol/l (range values 23.27 – 83.26 µmol/l) at beginning of trial to 69.13 ± 7.90 µmol/l (range values 48.34 – 128.02 µmol/l) on 30th day of supplementation. The average values observed were higher than the normal range of 17.9 – 35.8 µmol/l quoted by Radostits *et al.* (2000). Zinc level of the plasma samples from crossbred cattle decreased non-significantly. The overall mean values of PII and Mn in crossbred cattle showed non-significant (P<0.05) increase. Before the supplementation of UMMB, sub-clinical deficiency was observed to be prevalent in all the crossbred cattle with marginal deficiency (plasma level 50-104.90 ng/l) in 60% animals and 40% animals were having low level of iodine i.e., <50ng/l. After 30 days of UMMB supplementation, PII deficiency was prevalent in all animals with marginal deficiency in 77.77 per cent animals and 22.22 per cent animals having low level of iodine. Thus, it can be concluded that UMMB enriched with area specific mineral mixture enhanced milk yield, milk fat, dry matter intake, general health status and reproductive performance of the crossbred cattle.

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Comparative efficacy of some alkalinizing fluids for treatment of acidosis in induced colibacillotic diarrhoeic calves

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Abstract

Twenty five newborn male calves were subjected to induction of diarrhoea by oral administration of pathogenic enterotoxigenic *E.coli*. Diarrhoea of moderate to severe degree was produced with significant dehydration, depression, hyponatraemia, hyperkalaemia, and metabolic acidosis. Five calves, which were not given oral *E.coli* infection but maintained under same feeding and management conditions, served as healthy control. After induction of diarrhoea these calves were divided into five groups of five calves each for therapeutic evaluation of five intravenous fluids with different alkalinizing agent viz. bicarbonate, acetate, gluconate, l-lactate and ringer lactate. Cotrimazine was given @15mg/kg b.wt.twice daily in all the groups as specific antibacterial therapy. Based on findings of present study parenteral sodium bicarbonate was found fastest alkalinizing agent to correct metabolic acidosis of severe diarrhoea. Parenteral sodium l-lactate and sodium acetate solution were found to be the next most effective to counteract metabolic acidosis of severe diarrhoea. Ringer lactate solution was effective in acidosis associated with mild diarrhoea while gluconate was found least effective among all the fluids.

Key words- Acidosis, Alkalinizing fluids, Calf, Diarrhea, *E. coli*

Diarrhoea in calves is one of the major causes of morbidity and mortality worldwide (Cambier *et al.,* 2005). The treatment of infectious calf diarrhoea with specific therapy is apt to give disappointing results if supportive therapy for correction of fluid, electrolyte and acid base imbalances is not supplemented. As regards the use of intravenous (i/v) fluids in severely diarrhoeic calves, the conventional treatment uses Ringer Lactate (RL) solution for correction of dehydration, electrolyte imbalances and acidosis. The RL contains racemic mixture of D and L isomers of lactate and D isomer is not effectively metabolized in diarrhoeic calf to combat acidosis (Radostits *et al*., 2007). The objective of this study was to compare the alkalinizing potential of RL, sodium l-lactate, sodium bicarbonate, sodium acetate and sodium gluconate in treating acidosis of diarrhoeic calves.

Materials and Methods

The study was conducted on 30 newborn calves weighing 24.66±0.60 kg under the protocol as approved by CPCSEA. Calves were procured from an organized dairy herd (LPR C&B, IVRI, Izatnagar) immediately after calving. The newborn calves were given colostrum @1.5L at the age of 10 hour to provide inadequate immunity to facilitate the induction of diarrhoea. On 4th day of postnatal life, diarrhea was induced in 25 of such calves by oral administration of 10g sodium bicarbonate followed within 2-3 minutes by 10ml of *E.coli* suspension containing approximately 10^{10} organism of a pathogenic enterotoxigenic *E.coli* (serotype O120) isolated from the faeces of clinical cases of diarrhoea(Groutides and Michell, 1990). The calves were considered diarrhoeic when they had passed profuse watery faeces at least for three times. These calves were maintained on whole milk diet and *ad lib* drinking water until completion of experiment.

Strain of *E.coli* was grown in 5 ml of brain heart infusion (BHI) broth overnight at 37^{o}C. The broth culture was centrifuged at 3000rpm for 8 minutes till a soft pellet formed. The cell pellet was washed twice in a phosphate buffer saline (PBS). After washing, cell pellet was suspended in PBS and cell concentration was adjusted to 10^{10} micro organism/ml by matching with Brown's tube opacity no.10.

The *E.coli* culture from nutrient agar slant was inoculated in 5ml of BHI broth tubes. These tubes were incubated at 37^{o}C for 18 hours. A single loopful of broth was streaked on EMB agar plates and incubated for 24 hour. Single round convex dark centered colonies with metallic sheen were picked up and further tested for *E. coli* organism by gram's staining, biochemical tests and serotyping. These cultures were stored on nutrient agar slant at 4^{o}C for a maximum of one month and revived systematically as mentioned above.

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The four-i/v fluids were prepared in the laboratory with composition (mmol/L) as Na-150, K-4, Cl-115. Bicarbonate, acetate, L-lactate and gluconate were taken as 40 mmol/L in different I/V fluids. Ingredients of each solution were dissolved separately in one litre of triple distilled water and sterilized at 15psi for 15 minutes. Ringer lactate solution was available commercially.

On development of diarrhoea, the calves were assigned into five groups of 5 calves each for trial of i/v fluids. Cotrimazine was given in diarrheic calves @ 15mg/kg b.wt. p.o. bid as specific antibacterial therapy based on cultural sensitivity test of isolated *E. coli*. The i/v fluids were given as 3L per calf for first day and 2L per calf per day for next two days. The fluid deficit was corrected on the first day and for the next two days; the fluid was given to meet continuing loss and maintenance requirement. The therapeutic response was judged based on clinical and biochemical recovery of diarrhoeic calves. Five calves, which were not given oral *E. coli* suspension but maintained under same feeding and management conditions, served as healthy control.

The clinical symptoms like body temperature, appetite, color of faeces etc. of the diarrhoeic calves were noted. Approximately 0.5 ml of whole blood was collected in heparinized syringe anaerobically and kept in ice for blood gas analysis. Blood gas analysis was done within half an hour of collection of samples. Five ml of blood was collected in glass test tubes and processed for separation of serum for biochemical analysis. Blood was collected on day 0(before treatment) and subsequent to start of treatment on day 3 and day 7. Packed cell volume (PCV) was determined as per method suggested by Coles,1980. Sodium and potassium were estimated by flame photometery. Samples for blood pH, HCO_3 and TCO_2 were analyzed by use of a blood gas analyzer (Stat Profile-M).

Statistical analysis

The data were statistically analyzed using ANOVA to find the significance of difference between the mean values of different groups (Snedecor and Cochran, 1994).

Results and Discussion

In the present study, diarrhoea of moderate to severe degree was successfully induced in the calves after 3 to 5 days of oral *E.coli* infection as evidenced by profuse watery faeces of yellowish white to greenish color with significant dehydration and depression.

The administration of sodium bicarbonate before *E.coli* administration was done to overcome abomasal acidity, which might have adversely affected the survival of *E. coli* organism interfering with the induction of diarrhoea in calves (Groutides and Michell, 1990). The diarrhoeic calves were found to be inappetant, lethargic, dull, dehydrated and with watery offensive faeces of yellow white to green color. The biochemical alterations observed were haemoconcentration, hyponatraemia, hyperkalaemia and metabolic acidosis.

Regarding the concentration of electrolytes incorporated in the parenteral fluids used, Na, K and Cl were taken in the concentration similar to extra cellular fluid since fluid lost in the diarrhoea through intestinal lumen is mainly extracellular. Similarly alkalinizing agents (bicarbonate, L-lactate, acetate, gluconate) were taken as per the base requirement of a severely diarrhoeic calf of the present study of 25 kg body weight (Radostits *et al.,* 2007).

Hematobiochemical profile of experimental calves is given in table1. All the fluids yielded comparable response on day 3 and 7-post therapy in respect of PCV. As regards improvement in hyponatraemia of diarrhoeic calves significantly ($p<0.05$) better results were obtained with use of bicarbonate, acetate, gluconate and l-lactate solution than RL by day 3 post therapy. However by 7^{th} day of treatment, all the five fluids showed similar improvement in serum sodium concentration. In respect of improvement in the altered values of serum potassium, blood pH, HCo^3 and TCo_2, greater improvement was observed with the use of bicarbonate solution on day 3 and 7 post therapy followed by l-lactate, acetate, RL and gluconate solution in decreasing order of efficacy.

In the present study intravenous bicarbonate solution was found to be the best alkalinizing agent in combating acidosis of severely diarrhoeic calves. This effect was probably due to the fact that bicarbonate does not require cellular metabolism to exert an alkalinizing effect (Kasari and Naylor, 1985). The bicarbonate containing fluid also performed better in terms of improvement in depression and lowering of increased

Table 1. Haemato-biochemical profile of experimental calves.

Parameter	Group	Days of therapy		
		0	3	7
PCV (%)	Gr. I	35.40±1.86^{Aa}	34.40±2.16 ^{Aa}	35.20±1.62^{Aa}
Mean±S.E.	Gr. II	44.40±1.03^{Ab}	35.20±1.06^{Ba}	33.20±1.28^{Ba}
	Gr. III	45.40±1.03 ^{Ab}	32.60±0.92 ^{Ba}	31.40±0.74 ^{Ba}
	Gr. IV	45.60±1.07 ^{Ab}	36.20±0.80 ^{Ba}	34.40±0.92 ^{Ba}
	Gr. V	46.20±1.65 ^{Ab}	34.20±1.35 ^{Ba}	34.40±1.50 ^{Ba}
	Gr.VI	46.60±1.63 ^{Ab}	35.60±1.28 ^{Ba}	34.80±1.46 ^{Ba}
Sodium (mmol/L)	Gr. I	132.00±0.20^{Aa}	131.33±1.56 ^{Aa}	132.83±1.48^{Aa}
Mean±S.E.	Gr. II	115.40±0.81^{Ab}	126.20±1.24^{Bb}	133.20±1.02^{Ca}
	Gr. III	113.40±092 ^{Ab}	132.20±1.15 ^{Ba}	134.40±0.92^{Ba}
	Gr. IV	115.60±1.07 ^{Ab}	130.80±0.96 ^{Ba}	135.20±0.80^{Ca}
	Gr. V	113.60±1.08 ^{Ab}	131.40±1.36 ^{Ba}	135.60±1.07^{Ca}
	Gr.VI	114.40±1.20 ^{Ab}	130.40±1.07 ^{Ba}	134.00±0.89^{Ca}
Potassium (mmol/L)	Gr. I	4.48±0.16^{Aa}	4.46±0.06^{Aa}	4.54±0.10^{Aab}
Mean±S.E.	Gr. II	5.82±0.08^{Ab}	4.92±0.07^{Bb}	4.74±0.10^{Bbc}
	Gr. III	5.66±0.16 ^{Ab}	4.52±0.06^{Ba}	4.44±0.05^{Ba}
	Gr. IV	5.86±0.22 ^{Ab}	4.96±0.13^{Bb}	4.50±0.05^{Bab}
	Gr. V	5.72±0.13 ^{Ab}	5.39±0.08^{Bc}	4.88±0.09^{Cc}
	Gr.VI	5.72±0.20 ^{Ab}	4.56±0.09^{Ba}	4.44±0.10^{Ca}
PH	Gr. I	7.45±0.03^{Aa}	7.41±0.03^{Aa}	7.42±0.02^{Aa}
Mean±S.E.	Gr. II	7.15±0.02^{Ab}	7.26±0.02^{Bb}	7.36±0.01^{Cb}
	Gr. III	7.17±0.02 ^{Ab}	7.45±0.10 ^{Ba}	7.50±0.01 ^{Cc}
	Gr. IV	7.18±0.01 ^{Ab}	7.34±0.01 ^{Bc}	7.41±0.01 ^{Ca}
	Gr. V	7.15±0.01 ^{Ab}	7.20±0.01 ^{Bd}	7.31±0.01 ^{Cd}
	Gr.VI	7.16±0.01 ^{Ab}	7.35±0.01 ^{Bc}	7.43±0.01 ^{Ca}
HCO3 (mmol/L)	Gr. I	27.80±1.40^{Aa}	26.66±1.06^{Aa}	27.38±1.22^{Aa}
Mean±S.E.	Gr. II	15.40±1.01^{Ab}	23.64±0.57^{Bb}	25.88±0.60^{Cab}
	Gr. III	15.36±0.77^{Ab}	29.26±0.59^{Bc}	30.28±0.75^{Bc}
	Gr. IV	16.16±1.12 ^{Ab}	25.84±0.77^{Ba}	27.12±0.96^{Ba}
	Gr. V	16.58±0.82 ^{Ab}	20.12±0.28^{Bd}	24.14±0.46^{Cb}
	Gr.VI	14.78±0.77 ^{Ab}	25.64±0.87^{Ba}	27.56±0.89^{Ba}
TCO_2	Gr. I	21.58±0.61^{Aa}	22.22±0.98^{Aab}	21.28±0.82^{Aa}
(mmol/ L)	Gr. II	12.48±0.83^{Ab}	18.60±0.46^{Bc}	21.94±0.60^{Ca}
Mean±S.E.	Gr. III	12.78±0.27 ^{Ab}	23.16±0.70^{Bb}	24.80±0.38^{Bb}
	Gr. IV	14.44±0.68 ^{Ab}	21.06±0.49^{Ba}	22.12±0.58^{Ba}
	Gr. V	13.68±0.75 ^{Ab}	16.16±0.33^{Bd}	18.48±0.23^{Cc}
	Gr.VI	13.74±0.72 ^{Ab}	20.52±0.50^{Ba}	22.60±0.41^{Ca}

The observation with different small letters in a column and different capital letters in a row as superscript differs significantly ($p<0.05$), Gr.I- Healthy control, Gr.II- Treated with oral cotrimazine + Ringers lactate solution I/V, Gr.III- Treated with oral cotrimazine + Bicarbonate solution I/V, Gr.IV- Treated with oral cotrimazine + Acetate solution I/V, Gr.V- Treated with oral cotrimazine + Gluconate solution I/V, Gr.VI- Treated with oral cotrimazine + L-lactate solution I/V

value of serum potassium than the fluids containing l-lactate, acetate, RL and gluconate. The restoration of K^+ value may be attributed to better alkalinizing potential of bicarbonate containing fluid since maintenance of plasma K^+ is dependent on transmembrane gradient between cells and ECF, which is dependent on acid base balance(Michell *et al.,* 1989). Similar findings with bicarbonate therapy have been reported earlier also (Cambier *et al.,* 2005, Coskun *et al*., 2010). In spite of aforesaid merits of sodium bicarbonate as an alkalinizing agent, its indiscriminate administration may have deleterious effects due to excess production of Co_2, which accumulates in the body (Naylor and Forsyth, 1986, Moon *et al*., 1997). However in present study no such type of complication was observed in any of the diarrhoeic calf because of the fact that all the calves were having good pulmonary ventilation and the fluid was administered slowly.

In this study sodium l-lactate was also found to be an effective blood alkalinizing agent in correcting the altered values of blood pH, HCO_3, TCO_2 and potassium in the diarrhoeic calves. From comparative analysis of data it was observed that l-lactate had slower alkalinizing action than bicarbonate. It may be attributed to the fact that l-lactate depends on metabolism by the body for its alkalinizing effect. Lactate is metabolized by the liver and peripheral tissues (Naylor and Forsyth, 1986, Groutides and Michell, 1990). Findings of study are in accordance with previous reports (Groutides and Michell, 1990, Nakagawa *et al*., 2009).

In this study acetate infusion gave significantly ($p<0.05$) better improvement in blood pH, HCO_3 and TCO_2 in comparison to RL and gluconate while it was found comparable to l-lactate in its efficacy. Sodium acetate also depends on metabolism by the body for its alkalinizing effect. Acetate is converted into acetylCoA which, enter the tricarboxylic acid (TCA) cycle and is oxidized to CO_2 and H_2O resulting in alkalinization (Kasari and Naylor, 1985). However it has been reported that acetate induced significantly better alkalinization than l-lactate in early phase (within four hour) of i/v fluid administration (Kasari and Naylor, 1985). In the present study the alkalinizing effect of different i/v fluids in early hours has not been estimated. Findings of present study are in accordance with earlier reports of Groutides and Michell, 1990 and Nakagawa *et al*., 2009. Acetate oxidation is not a liver dependent process. It is metabolized in muscles and other tissues thus hepatic diseases or acidosis that may inhibit hepatic metabolism does not affect the onset of alkalinization induced by acetate.

Ringer lactate was found less effective as supportive therapy in comparison to bicarbonate, L-lactate and acetate solutions in respect to correction of altered values of blood pH, HCO_3, and TCO_2. Improvement in serum sodium was also found significantly($p<0.05$) less in RL treated animals than with the use of other fluids. The probable reason for lower alkalinizing ability of Ringer lactate could be that the lactate as a bicarbonate precursor is present at lower concentration of 28 mEq/L as a racemic mixture of D and L isomers of lactate. Enzymes that metabolize the L-isomer are abundantly present in mammalian tissues but the D-isomer is not effectively metabolized (Radostits *et al*., 2007). Moreover, higher level of D-lactate has been observed in serum of calves that suffered from metabolic acidosis and neonatal diarrhea (Lorenz, 2009). The significantly ($p<0.05$) less improvement in serum sodium value of RL treated animals might be due to its lower sodium content (130meq/L) as compared to 150meq/L of other fluids. The alkalinizing effect of RL has also been reported earlier (Kumar and Mandial, 2002, Nakagawa *et al*.,2009).

As per the findings of present study gluconate was found to have minimal alkalinization effect as compared to other fluids. The lesser alkalinization effect might be due to the fact that calves metabolize gluconate very slowly (Groutides and Michell, 1990, Radostits *et al*., 2007). Groutides and Michell (1990) also found that gluconate solution slightly improved acidosis in calves.

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Autologous cell-based therapy in chronic ulcerative wound at carpal region in a bullock

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Abstract

A 6 years old bullock was affected with chronic non-healing ulcerative wound on the dorso-medial aspect of carpal region of left foreleg. The animal was earlier treated with different types of topical application along with parenteral antibiotics and steroids preparations, but there was no recovery. After clinical examination and taking detail history bone marrow derived mesenchymal stem like cells (BM-MSCs) implantation was performed at the wound bed. Proper wound healing was observed at 25 days of treatment and there was no undesired reaction on the animal.

Keywords: BM-MSCs, Bovine, Chronic ulcerative wound.

Skin is a highly complex organ and is consisted of epidermis, dermis and associated adnexa (Fossum *et al.,* 1997). The thickness of skin varies from species to species and also with breed, sex, age, and also differ as per different parts within the animal body (Sisson and Grossman, 1953). The wound is a break in the continuity of skin, but ulcer is an inflammatory lesion of skin and mucous membrane with loss of epithelium due to molecular destruction of cells (O'Connor, 2005) which fails to undergo the natural process of healing (Frank, 2002). Therefore, one clinical trial was made targeting chronic, non-healing and full thickness cutaneous ulcerative wound at dorso-medial aspect of left carpal region of a Haryana bullock of 6 years old.

Materials and Methods

The owner of the animal was fully informed about the clinical trial, risk and benefits of the proposed cell based therapy. The wound was detected at dorso-medial side of carpal region extending both up and downwardly. Due to constant irritation, the animal was licking and making fresh wound irrespective of different types of parenteral and topical treatments for last 9 months. The wound measured 3.5 inches in length and 3.5 inches by breadth (Fig. 1 and Fig. 2). Swab was collected from wound bed for bacteriological culture and sensitive test and biopsy collected for histo-pathological and histochemical study. These examinations were performed under sedation with inj Xylazine @ 0.06 mg/kg body weight intramuscularly after the animal was fasted for 24 hours. The wound was cleaned and dressed with fly repellent spray. Under peronial nerve block and local infiltration with 2% lidocaine hydrochloride, the proposed site i.e antero-medial aspect of tibia was prepared aseptically for bone marrow collection. A 0.5 cm long incision was made at the antero-medial aspect of tibia and bone drilling was done by electrically operated orthopaedic drill with 3 mm drill bit (Fig.3). Then the drill bit was withdrawn and immediately one siphoned needle of 10 cm long (16 gauze) was inserted into the medullary cavity through the hole made. Bone marrow (20 ml) was aspirated into a sterile syringe primed with EDTA (Himedia) @ 1mg/ml (Fig.4). The aspiration of the BM was made as per the method of Crow and Walshaw (1997) with some modification. The bone marrow derived nucleated cells (BM-NCs) were collected from aspirate by volume reduction protocol as per method of Kasten *et al.* (2007) with some modification. The EDTA mixed bone marrow was despatched inside ice packed thermo cool to the stem cell laboratory of CIFA, Kausalyaganga, BBSR within 30 minutes for culture and growth.

For culture, conditioned media was prepared with commercially available basic ingredients as per Table 1. The collected bone marrow layered over Histopaque (Sigma, Aldrich, used for isolation of mononuclear cell) and was centrifuged at 2000 rpm for 20 minutes. The monocytes in buffy layer were seeded on to a 0 · 1% gelatin-coated six well plate (Tarson) and 100 µl of cells were poured into 6 well plate containing 2 ml of composed media at 37^0 C and 5% CO_2 in the CO_2 incubator (Contherm Scientific Ltd). The medium was changed on every third day and cell morphology was examined under a Nikon phase contrast microscope (Fig. 5 and Fig. 6). After complete colony formation, passaging was done at 5–7 day intervals by

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Table 1: Constituents of the composed media.

Constituents of media	Amount
FBS (Fetal Bovine Serum) 10% , Lonza	5ml
Sodium Pyruvate 0.1% Himedia	0.5ml
NEA (Non essential amino acids) 0.1%, Himedia	0.5 ml
DMEM (Dulbecco-modified Eagle medium) (MP pharmaceuticals)	18.5 ml
Streptomycin (Sigma, Aldrich)	0.5 ml
L 15 (Livosys 15) washing media	25 ml
Total	50 ml

Table 2: Biochemical parameters measured before and after implantation.

Parameters	Before start of therapy	On day 25 post therapy
Glucose (mg/dL)	62.4	74.4
Serum urea (mmol/L)	8.13	8.52
Serum Creatinine (mg/dL)	1.43	1.37
Serum Cholesterol (mg/dL)	65.21	73.41
Triglyceride (mg/dL)	8.5	12.06
Serum AST (IU/L)	88.06	96.08
Serum ALT (IU/L)	33.21	29.73
Serum ALP (IU/L)	115.17	103.62
Phosphorous (mg/dL)	6.23	5.89
Calcium (mg/dL)	10.55	10.98

trypsinization.

The bullock was sedated with xylazine @ 0.03 mg/kg body wt for BM-MSCs application. The ulcerative wound site was prepared aseptically. The prepared BM-MSCs was diluted with NSS at 2: 1 ratio and injected intradermally at the wound margin and then at different points on wound bed (Fig.7). It was kept as such for 20 min for proper adherence of the implanted MSCs at the site and bandaged with paraffin wet bandage. Outwardly fly repellent spray was sprinkled.

The physiological parameters like rectal

Table 3: Measurement of tensile stress and tensile strain of nonhealing ulcerative wound after application of BM-MSCs and normal skin as control.

Sample label	Sample width (mm)	Sample thickness (mm)	Sample Area (mm^2)	Sample Length (mm)	Maximum Load (N)	Tensile stress at maximum load (MPa)	Extension at break (mm)	Tensile strain at break (%)
Bullock (normal skin as control)	12	2.51	30.12	24	643.28	21.35	15.87	66.12
Bullock (healed tissue after cell based therapy)	11	2.67	29.37	20	489.86	16.68	11.51	57.55

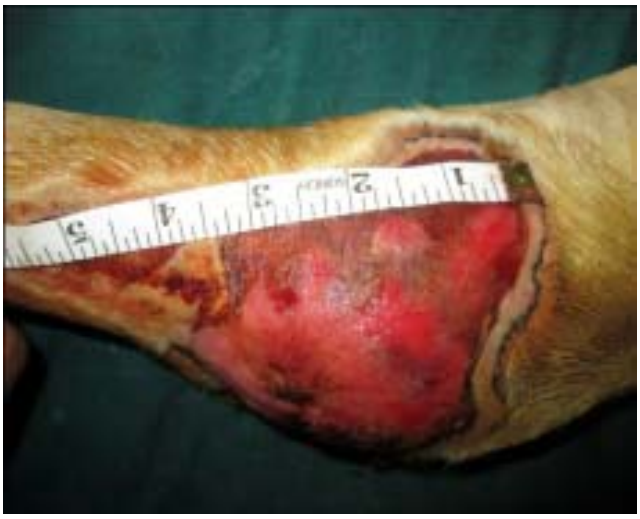

Fig 1. Measurement of the wound length on the day of presentation (3.5 inches).

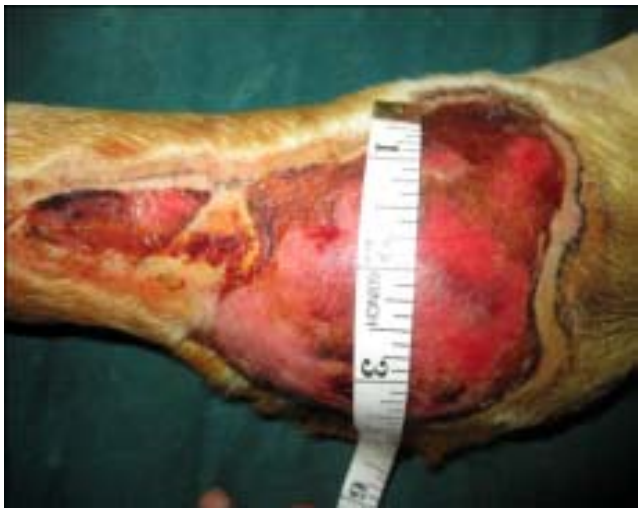

Fig 2. Measurement of the wound breadth (3.5 inches).

Fig 3. Drilling at antero-medial aspect of tibia for BM collection.

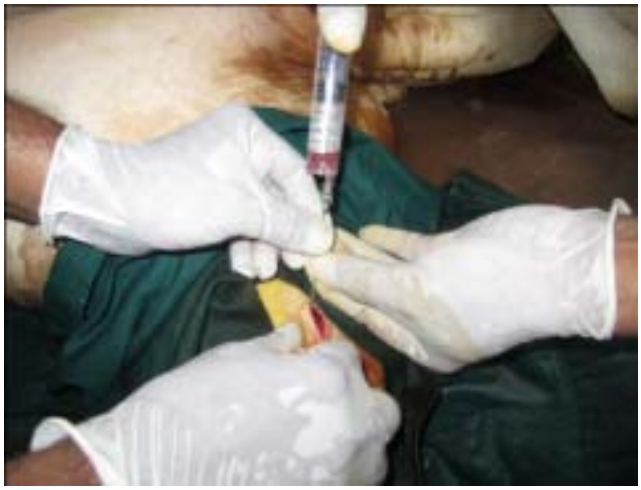

Fig 4. Collection of BM.

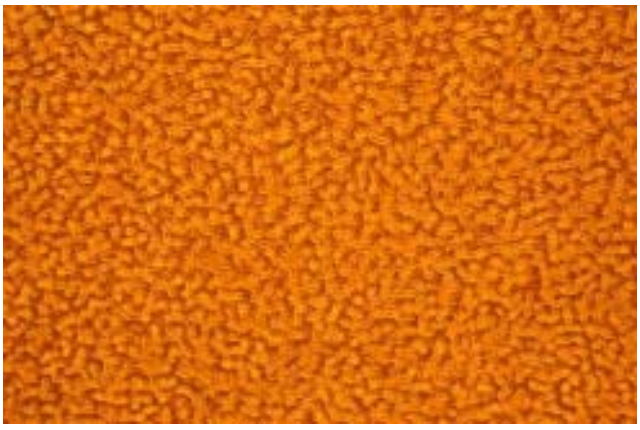

Fig 5. Bone marrow-derived nucleated Cells on 1st day (10X phase contrast view).

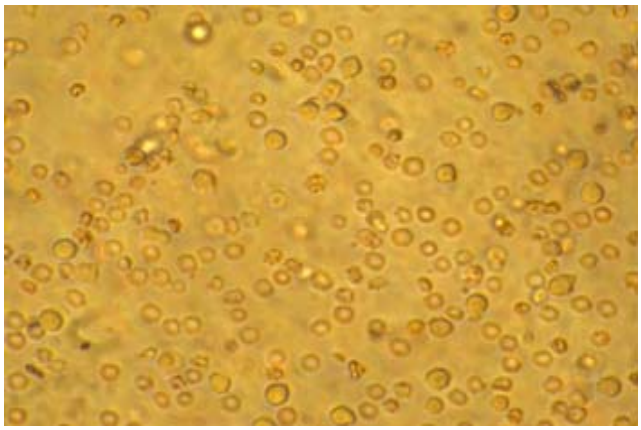

Fig 6. Confluency of BM-MSCs after 5 days of culture at CIFA Kausalyaganga (4x view).

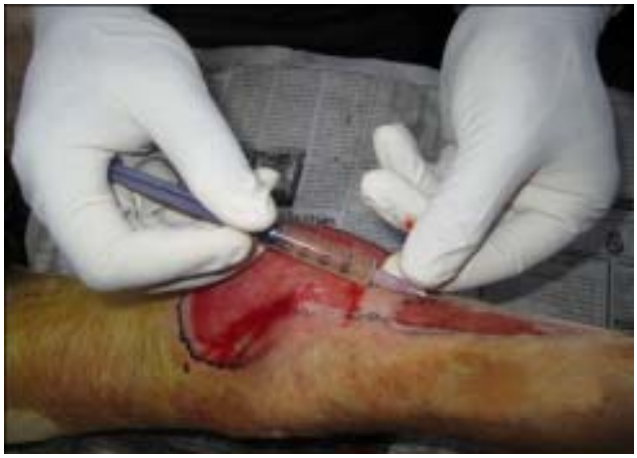

Fig 7. Implantation of BM-MSCs on wound bed.

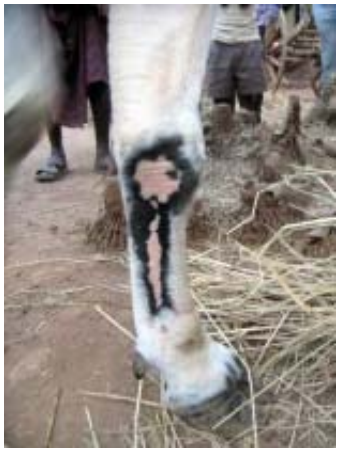

Fig 8. Photograph showing healing of wound after 25 days of stem cell therapy.

temperature (^{0}C), heart rate (beats/min), respiration rate (breaths/min) and status of visible mucous membrane were recorded on the day of presentation, BM collection, BM-MSCs implantation and 25 days after healing. The size of the ulcerative wound, types of exudates, extend of exudates, swelling and wound contraction were studied on 0-3 scale basis in different stages and also after healing along with study of pain free walking

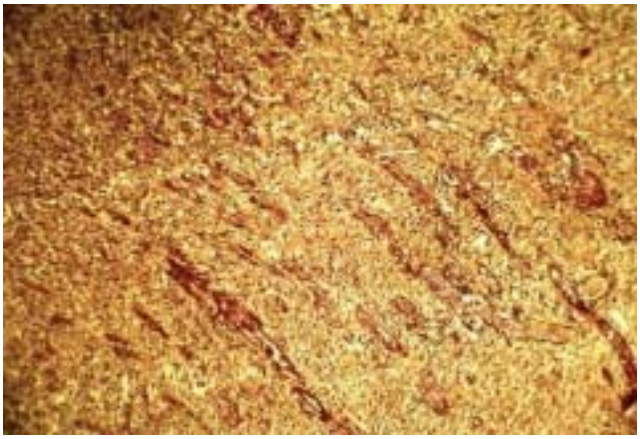

Fig 9. H & E stain showing formation of new capillary bed.

Fig.10. Tensile strength measurement by universal testing machine (Instron-3382).

distance. Biochemical parameters on day of presentation and after 25 days of healing were estimated (Table. 2). Histopathology with H and E stain and histochemical study with Masson-Trichrome stain for study of collagen content were carried out. Estimation of collagen content of tissue was also done with Sircol™ method by help of spectrophotometer at 555 nm wave length. The study of tensile stress and strain of tissue were carried out after 60 days of healing and compared with normal skin of the adjacent site with the help of Universal testing machine (Instron 3382) with running rate 50 mm/minute at 27^0 C temperature and 65% humidity. As per culture and sensitivity test of the wound bed the antibiotic inj. Ciprofloxacin was administrated @ 5 mg/kg body weight was administered parentrally for 5 days before stem cell implantation.

Results and Discussion

The bone marrow sample was aspirated from the bullock without any adverse effect. The success rate for isolating the BM-derived Mononuclear Cells (BM-MNCs) in the culture was more than 80%. On attaining 90% confluence the total number of cells harvested was counted using a haemocytometer and the population doubling time was also calculated. The BM-MNCs cultured in Dulbeco-Modified Eagle Medium (DMEM)-low glucose medium maintained typical morphology. On day 2 the rounded cells showed attachment and attained confluence on day 3, but on day 5 the elongated morphology turned to spindle-shaped cells and on day 7 it was attained the optimal stage for implantation.

As per culture and sensitivity test of wound bed the *Bacillus* and *Staphylococcus* organisms were isolated and antibiotics like Ciprofloxacin and Levofloxacin were found to be most sensitive. So inj. Ciprofloxacin was administrated @ 5 mg/kg body weight to the animal before application of BM-MSCs, in order to keep the wound bed in sterile condition for stem cell therapy as per protocol. The physiological parameters like rectal temperature, pulse rate and respiration rate were studied before therapy (103.2^0F, 74/min. and 24/min. respectively) and after 25 days of therapy (102^0 F, 72/min. and 20/min. respectively) though varies, but remained within the normal physiological range. The haematological parameters like Hb (gm%), TEC (10^6/cu mm), TLC (10^3/ cu mm), PCV (%) and DC (%) values before therapy and after healing showed no significant difference. The safety laboratory data of glucose (mg/dL), urea (mmol/L), creatinine (mg/dL), cholesterol (mg/dL), triglyceride (mg/dL), AST (IU/L), ALT (IU/L), ALP (IU/L), phosphorous (mg/dL) and calcium (mg/dL) were measured before implantation and after 25 days of post implantation and found no significant difference. It is indicating the normal functioning of body physiology, suggesting that the chronic non-healing wound (on body of animal) was unable to alter the parameters beyond normal range. This might be due to the small sized wound in comparison with the total body surface area. In this clinical research the estimated values correlates with the works of Gopinathan *et al*. (2007) in horses and Borena *et al.* (2009) in dogs which shows no significant difference in their research works. The estimated data regarding gross observation of wound viz. extent of exudates, types of exudates, swelling and wound contraction were found to be highly significant difference ($P<0.05$) while comparing with the values before and

after therapy.

There was rapid formation of granulation tissue after cell based therapy and the wound was healed after 25 days (Fig. 8). Eruption of hairs on skin occurred afterwards. This might be due to the accelerated wound healing process produced by BM-MSCs application. This was supported by other workers that the BM-MSCs accelerate the wound healing process in cutaneous wound in case of Sprague-Dawley rats (Mc Farlin *et al*., 2006). Correlating this Vojtassak *et al*. (2006) also reported healing within 4 weeks following BM-MSCs application of a 25 year open wound in human patient who was affected with diabetes since long.

Histopathology with H & E stain showed affluent granulation and neovascularisation with increased vascular bed along with marked proliferation of fibroblastic cells at the site (Fig. 9). The fibroblasts are the major effectors of wound repair and regenerating the wound healing process. There were development of hair follicles and sebaceous glands in the dermis and optimal regeneration of epidermis was found after MSCs application. The histochemical study of tissue i.e. Masson-Trichrome stain done for study of collagen content showed there was formation of more collagen content after cell therapy. The biochemical study for quantitative estimation of collagen content (µg/mg of tissue) revealed that on zero day it was 12.56 µg/mg which was increased to 27.11 µg/mg on 14th day and 28.97 µg/mg after healing, which is suggesting to be increased due to MSCs application. This increased quantity indicating progression in wound healing as supported by the works of Pascoe (1985), Curtis (1993), Singh and Singh (1993). Since the collagen is a key regulator of tissue tensile strength, so these collagen fibrils must be cross linked for proper alignment and function (Olaso *et al.,* 2011).

The closed wounds undergo different phases of attainment of wound strength, such as early wound strength and late wound strength. During wound healing process the adhesive strength is attributed to epithelialization as does in growth of capillary into ground substance (Singh and Singh, 1993). According to the work of Toporcer *et al.* (2006) the measurement of maximal breaking strength (MBS) is the preferable method for wound healing evaluation (Moelleken and Mathes, 1995; Pickett *et al.,* 1996). In their experiment they suggested that a cubic relationship exist in between healing time and tensile strength. So the wounds closed under tension exceeds that of tensionless wounds as early as day 7 following surgery.

The tensile stress and strain estimated by Universal testing machine (Fig. 10) were given in table no. 3. While comparing between normal skin and healed skin after BM-MSCs therapy, the normal skin showed higher value for breaking strength, but on the other hand the healed tissue was advancing towards proper stretchability and gaining towards normal tensile strength. The required tensile strength and elongation at break was achieved due to the formation of collagen during the healing period and their structural disposition in the tissue. This indicates that the regeneration capacity of the healed tissue was due to the effect of stem cell therapy. There was remarkable increase in pain free walking distance from 109.54 m. to 825.43 m., which was measured by subjective evaluation method by 3 surgeons. The 8 fold increase in the pain free walking distance is suggestive of wound healing in the said animal.

From the above study, it is said that the autologous implantation of BM derived MSCs in bovine is safe, effective, quicker and simple procedure for therapeutic management of chronic non-healing ulcerative wound.

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Comparative efficacy of some oral supportive therapies in calf diarrhea

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Abstract

Twenty four crossbred calves suffering from E.Coli diarrhoea were randomly divided into four groups I, II, III and IV each consisting of 6 calves for trial of four oral supportive therapies. Animals of group I were given oral electrolyte solution with high energy and glutamine (OESHEG). Group II animals received combination of Bel fruit powder and Shisham leaves powder. Group III received Glucose along with Bel and Shisham while calves of group IV were given combination of Bel, Shisham, Glucose and Glutamine. OESHEG containing Na-150, K-20, Cl-140, HCo3-30, Dextrose-300 and Glutamine-30 mmol/lit was used @ 1lit/calf twice daily. In the above combinations Bel and Shisham were used @ 2.4g/10kg b wt,10g/10kg b wt twice daily respectively. Glucose and glutamine were given@300mmol and 30mmol twice daily respectively in these combinations. All the supportive therapies were used along with cotrimazine @15mg/kg b wt as specific antibacterial therapy. The treatment was continued for three days. The efficacy of oral formulation containing bel + shisham + Glucose + Glutamine was found very close to OESHEG followed by combinations of bel +shisham + Glucose and bel + shisham in decreasing order of efficacy.

Keywords: *Bel, Calves,* Diarrhea*, E. coli, Shisham*

Diarrhea in calves is one of the most important conditions for the dairy industry. As regards various causes of diarrhea in calves *Escherichia coli* (*E. coli*) is the most common cause of diarrhea in calves aged up to 4 month. There are number of indigenous preparations to treat the cases of calf diarrhea, which are in use in rural India since long with encouraging results. *Shisham* leaf powder and *bel* fruit powder separately has been found effective in mild to moderate cases of calf diarrhea (Sena *et al.,* 2005). This study was taken to evaluate the therapeutic efficacy of *bel* and *shisham* in combination and with supplemental energy (glucose) and nutrient support (glutamine).

Materials and Methods

Twenty four (24) crossbred calves of either sex aged up to three months maintained at livestock production and research (cattle &buffalo) unit of the institute and suffering from *E. coli* diarrhea were selected for this study. They were randomly divided into four groups I, II, III and IV each consisting of 6 calves.

Treatment: Animals of group I received oral electrolyte solution with high energy and glutamine (OESHEG) @1lit/calf bid. This was constituted in the laboratory with composition per litre Na-150, K-20, Cl-140, Hco_3-30, Dextrose-300 and glutamine-30mmol. Animals of group II were given combination of *bel* fruit powder and *shisham* leaf powder. Group III animals received glucose along with *bel* and shisham while group IV animals were supplemented with the combination of *bel, shisham*, glucose and glutamine.

The unripe *bel* fruit was cut into slices and dried under the shade. This was made to powder for trial @24g/10kg b wt bid. *Shisham* leaves were freshly plucked, dried under the shade and made to powder for trial@10g/10kg b wt bid. Glucose and glutamine were given @ 300mmol and 30 mmol bid respectively in these combinations. All the four combinations were evaluated for their therapeutic efficacies along with cotrimazine (Sulphamethoxazole + Trimethoprim)@15mg/kg b wt bid. Cotrimazine was selected on the basis of cultural sensitivity test of faecal isolates. The therapy was continued for three days.

Clinical profile: The clinical symptoms like body temperature, appetite, color of faeces etc. of the diarrheic calves were noted. The scores (0-3) for faecal consistency, dehydration and depression of each calf were recorded on day 0(before therapy) and day 3 and day 7(after therapy) as per Walker *et al*., 1998.

Sample collection and hematobiochemical profile: Approximately 0.5 ml of whole blood was collected in heparinized syringe anaerobically and kept in ice for blood gas analysis. Five ml of blood was collected in glass test tubes and processed for separation of serum for biochemical analysis. Blood was collected

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on day 0(before treatment) and subsequent to start of treatment on day 3 and day 7. Packed cell volume (PCV), glucose were determined as per method suggested by Coles, 1980. Sodium and potassium were estimated by flame photometry. Samples for blood pH, HCo_3 were analyzed by use of a blood gas analyzer (Stat Profile-M).

The data were statistically analized using ANOVA to find the significance of difference between the mean values of different groups (Snedecor and Cochran, 1994).

Results and Discussion

The calves suffered with mild to moderate diarrhea with presence of semisolid to watery offensive faeces. The calves were found dull and depressed. Most of the calves suffered with reduced appetite. The color of the diarrheic faeces was yellowish white to green. The diarrheic calves were found free from parasitic infection based on laboratory examination of faecal samples. This may be due to routine preventive deworming schedule being practiced at the farm. Cultural examination of all the diarrheic calves revealed the presence of *E.coli* as evident from metallic sheen on eosine methylene blue media, which was subsequently confirmed by biochemical tests.

The clinical profile of diarrheic calves has been given in table1. The rectal temperature of calves of all the groups remained in the normal range (100.86 ± 0.183^0F to 102.08 ± 0.382^0F) throughout observation period. The comparative evaluation of results obtained in respect to scores of faecal consistency, similar response was observed on day 3 post therapy in all the four groups with the value becoming normal. As regards improvement in the dehydration and depression score of the diarrheic calves, highest improvement was observed in group I on day 3 post therapy followed by group IV, III and II animals. However on 7th day after therapy calves of all the groups showed full clinical recovery.

In respect of PCV, better improvement towards normal was observed in group I followed by group IV, III and II on day 3 post therapy. As regards changes in serum glucose similar improvement was observed in group I and IV, which was the maximum, followed by group III and II on day 3 after therapy. However, on 7th day, the performance of group I, III and IV was found comparable to each other and significantly ($p<0.05$) less improvement was observed in group II animals. In respect of changes in value of serum sodium and potassium, significantly ($p<0.05$) better improvement was observed in group I followed by group IV, III and II on day 3 of therapy. The value of serum sodium and potassium became similar in all the groups, 7th day post therapy. As regards changes in blood pH and HCo_3 values, group I calves revealed better recovery towards

Table 1: Clinical profile of experimental calves

Parameter	Group	Days of therapy		
		0	3	7
Faecal consistency Score (0-3)	Gr. I	2.33 ± 0.21^{Aa}	0 ± 0^{B}	0 ± 0^{B}
	Gr. II	2.66 ± 0.21^{Aa}	0 ± 0^{B}	0 ± 0^{B}
	Gr. III	2.50 ± 0.22^{Aa}	0 ± 0^{B}	0 ± 0^{B}
	Gr. IV	2.16 ± 0.22^{Aa}	0 ± 0^{B}	0 ± 0^{B}
Dehydration Score (0-3)	Gr. I	1.83 ± 0.16^{Aa}	$0\pm0B^{a}$	0 ± 0^{B}
	Gr. II	2.00 ± 0.25^{Aa}	0.83 ± 0.16^{Bb}	0 ± 0^{C}
	Gr. III	2.16 ± 0.16^{Aa}	0.67 ± 0.21^{Bb}	0 ± 0^{C}
	Gr. IV	2.33 ± 0.21^{Aa}	0.16 ± 0.16^{Ba}	0 ± 0^{B}
Depression Score (0-3)	Gr. I	1.67 ± 0.21^{Aa}	$0+0^{Ba}$	0 ± 0^{B}
	Gr. II	1.83 ± 0.30^{Aa}	0.83 ± 0.21^{Bb}	0 ± 0^{C}
	Gr. III	2.00 ± 0.36^{Aa}	0.33 ± 0.21^{Ba}	0 ± 0^{B}
	Gr. IV	2.00 ± 0.25^{Aa}	0.16 ± 0.16^{Ba}	0 ± 0^{B}

The observation with different small letters in a column and different capital letters in a row as superscript differ significantly ($P<0.05$)

Gr. I - Treated with oral cotrimazine + oral electrolyte solution with high energy and glutamine
Gr. II - Treated with oral cotrimazine + beI + shisham
Gr. III - Treated with oral cotrimazine + bel + shisham + glucose
Gr. IV - Treated with oral cotrimazine + beI + shisham + glucose + glutamine

Table 2: Haemato-biochemi[Ca]l profile of diarrhoeic [Ca]lves

Parameter	Group	Days of therapy		
		0	3	7
PCV (%)	Gr. I	43.16±1.07^{Aa}	35.83±0.87^{Ba}	33.33±1.20^{Ba}
	Gr. II	44.33±0.76 ^{Aa}	33.16±0.79^{Bb}	35.16±1.10^{Ca}
	Gr. III	42.33±0.42 ^{Aa}	38.16±1.19^{Bb}	34.33±0.66^{Ca}
	Gr. IV	44.33±0.66^{Aa}	35.50±0.71^{Ba}	33.16±0.70^{Ca}
Glucose (mg/dl)	Gr. I	44.89±1.10^{Aa}	60.22±0.41^{Ba}	62.47±0.80^{Ba}
	Gr. II	44.61±0.98^{Aa}	51.89±0.98^{Bb}	53.54±1.01^{Bb}
	Gr. III	46.59±0.97^{Aa}	56.95±0.95^{Bc}	60.44±1.09^{Ca}
	Gr. IV	43.46±0.64^{Aa}	59.63±0.78^{Ba}	61.09±1.88^{Ba}
Sodium (mmol/L	Gr. I	115.67±1.08^{Aa}	129.33+0.88^{Ba}	130.33±1.38^{Ba}
	Gr. II	117.66±1.05^{Aa}	122.83±0.47^{Bb}	128.16±1.32^{Ca}
	Gr. III	118.66±1.08^{Aa}	125.83±0.91^{Bb}	128.83±0.47^{Ca}
	Gr. IV	117.50±0.80^{Aa}	126.50±1.20^{Bc}	129.66±1.20^{Ba}
Potassium (mmol/L)	Gr. I	5.26±0.16^{Aa}	4.23±0.07^{Ba}	4.20±0.03^{Bab}
	Gr. II	5.15±0.11^{Aa}	4.85±0.12A^{Bb}	4.55±0.11^{Bb}
	Gr. III	5.10±0.08^{Aa}	4.73±0.11A^{Bc}	4.21±0.18^{Bab}
	Gr. IV	4.86±0.17^{Aa}	4.48±0.11A^{Bac}	4.10±0.10^{Ba}
pH	Gr. I	7.230±0.018^{Aa}	7.380±0.005^{Ba}	7.400±0.003^{Ba}
	Gr. II	7.270±0.011^{Ab}	7.300±0.005^{Bb}	7.340±0.008^{Cb}
	Gr. III	7.240±0.007^{Aab}	7.320±0.012^{Bb}	7.350±0.003^{Cb}
	Gr. IV	7.250±0.008^{Aab}	7.300±0.010^{Bb}	7.380±0.008^{Ca}
HCO3 (mmol/L)	Gr. I	17.93±0.38^{Aa}	24.80±0.74^{Ba}	27.43±0.40^{Ca}
	Gr. II	18.18±0.33^{Aa}	20.98±0.19^{Bb}	24.86±0.21^{Cb}
	Gr. III	18.36±0.47^{Aa}	21.12±0.14^{Bb}	24.86±0.43^{Cb}
	Gr. IV	17.78±0.37^{Aa}	22.00±0.39^{Bb}	27.060±0.80^{Ca}

The observation with different small letters in a column and different Capital letters in a row as superscript differ significantly (P<0.05)

Gr. I - Treated with oral cotrimazine + oral electrolyte solution with high energy and glutamine
Gr. II - Treated with oral cotrimazine + beI + shisham
Gr. III - Treated with oral cotrimazine + bel + shisham + glucose
Gr. IV - Treated with oral cotrimazine + beI + shisham + glucose + glutamine

normal in these parameters than group II, III and IV on day 3 of therapy while on day 7 of therapy improvement in these parameters were similar in group I and IV that was maximum followed by group III and II which registered similar increase (Table 2).

In this study, the use of OESHEG yielded comparatively faster clinical and biochemical recovery because it contains the necessary element i.e. sodium, potassium, chloride, bicarbonate, dextrose and glutamine to correct the secondary complications of dehydration, electrolyte and acid base imbalances associated with diarrhea. The fluid loss in diarrhea is principally extra cellular and initially the ions lost through the intestinal lumen are sodium and chloride. The hyperkalaemia seen in association with diarrhea is mainly attributable to the shift of K^+ from intracellular to extra cellular compartment under the influence of metabolic acidosis (Michell *et al.,* 1989). This shows that in case of diarrhea the patient suffers from hypokalaemia in terms of body's total potassium content. Therefore replacement of all these ions as quickly as possible is required. The OESHEG had the potential to correct electrolyte and acid base imbalances associated with diarrhea.

In the present study, incorporation of 300mmol/L glucose in group I, III and IV found better in terms of improvement in depression by providing more energy to ailing calves in comparison to group II calves on 3rd day of therapy. Findings of the study are in accordance with earlier reports of Brooks *et al*. (1997) and Sena *et al.* (2005). In this study the addition of glutamine @30mmol/L in group I (OESHEG) and IV (*bel*+*shisham*+glucose+glutamine) was found beneficial because the therapeutic efficacy of group I and IV drug regimen were statistically similar in terms of improvement in many parameters like dehydration, PCV,

glucose on day 3 after therapy while comparatively less improvement in these parameters were observed in group III and II calves which were not given glutamine. Glutamine is the main nucleotide precursor for intestinal cells. It promotes intestinal absorption of sodium, hepatic uptake of glucose and helps in sustaining villous form and function (Vanderhulst *et al.,* 1993).

The combination therapy of *bel+shisham* (group II) also yielded good results. The efficacy of *bel+shisham* was found comparable to other groups on 7th day of therapy however on day 3 it was found to be less than other oral therapies. The effectiveness of *shisham* leaves in diarrhea could be due to its nonspecific spasmolytic activity (Kumar *et al*., 2000). Sarg *et al.* (1999) indicated that the alcoholic extract of green branches of aerial parts (leaf) of *shisham* had a dose dependent inhibitory effect on the motility of isolated rabbit duodenum. Sena *et al.* (2005) observed that powder of *shisham* leaves is effective in checking diarrhea @ 105g/100kg b wt bid for 3 days. This marked astringent property could be due to high tannin content of leaves.

The unripe fruit of *bel* is known to be used for antidiarrhoeal effect (Dukes *et al.,* 2000; Nadkarni, 2000; Sena *et al*., 2005). Dukes *et al.* (2000) observed that unripe *bel* fruit possessed antibacterial, antispasmodic, demulcent, antiviral activities and used in diarrhea, dysentery and *E.coli* infection in human beings. The fruit pulp of *bel* contains reducing sugar, tannin, furocoumarin (marmelosin) as the active ingredient (Nadkarni, 2000). Sena *et al*. (2005) observed that *bel* fruit powder @24g/100kg b wt bid for three days was effective as primary therapeutic agent in nonspecific diarrhea.

Findings of present study conclude that oral electrolyte solution with high energy and glutamine is the most effective supportive therapy in mild to moderate calf diarrhea. The efficacy of oral formulation containing *bel* + *shisham* + Glucose + Glutamine was found very close to OESHEG followed by combinations of *bel* +*shisham* + Glucose and *bel* + *shisham* in decreasing order of efficacy. These herbal preparations are easily available and cost effective and therefore, can serve as suitable alternative for OESHEG. Further research aiming at isolation and characterization of ingredient with antidiarrheal action in these herbs is needed for their commercial utilization at large scale.

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Short Communication

Effect of environmental determinants on prevalance of Amphistome infection in cattle in and around Pantnagar

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Abstract

In the present study overall incidence of amphistome in cattle were recorded as 22.83%. The infection rate was highest (30.33%) in year 2008 followed 21.99% infection in 2006 and lowest (16.66%) in 2007. Month wise incidence showed highest (39.19%) in month of July followed by June having 37.66%. The least infection (6.52%) was observed in month of December. The data revealed that relative humidity and total rainfall were on higher side in the months of higher intensity of infection. Seasonal clubbing of the data was found to be further conformity of these observations as the amphistome infection was found to be more prevalent (31.05%) in rainy season followed by 27.68% and least i.e. 9.75% in winter.

Keywords: Amphistome, Cattle, Incidence

Helminthic infestation of gastro-intestinal tract, particularly *Amphistome* is one of the major causes of wastage and decreased productivity through loss of blood and plasma proteins in G.I.T. Effective parasite control has not been possible due to lacuna of information on parasite epidemiology. However, the prevalence rate may vary and clinical disease may or may not appear due to number of factors: Environment factors are most important determinant among them. Some literature is already available that a deal with climatic responses on variety of parasites (Kates, 1965), but constant vigil is needed on this aspect. Environment has profound effect on parasitism. Several environmental factors influence the development of eggs to larvae, survival of larvae and their availability to hosts. Such studies help in taking decision on strategic and tactical schedule of deworming in livestock farming, with this view, the present work was taken to study the effect of environmental determinants on the severity of amphistome infestation in cattle under semi-arid zone.

Materials and Methods

The present study was conducted in and around Pantnagar at Animal Disease Diagnostic Center of the University from January, 2006 to December, 2008. Monthly incidence rate was estimated on the basis of qualitative faecal examination (MAFF, 1971). Meteorological Data was plotted in the form of climatograph, as suggested by Gardon (1948). Statistical analysis was done as described by Snedecor and Cochran (1968).

Results and Discussion

In the present study over all incidence of amphistome in cattle were recorded as 22.83%. Barua *et al.,* 2009 reported 18.26% incidence of amphistome infestation in year 2007 in Uttarakhand. These variations may be attributed to differences in managemental practices viz type and composition of pasture, grazing pattern, type of soil and climatological variations in the respective year under study (Radostits *et al.* 1994).

It was observed that the infection rate was highest (30.33%) in year 2008 (Temperature Fluctuation, 12.18 °C, Relative humidity, 85.04%, Rainy days, 0.833) followed 21.99% infection in 2006 (Temperature Fluctuation 12.09 °C, Relative humidity 85.13%, Rainy days 0.67) and lowest (16.66%) in 2007 (Temperature Fluctuation 11.96 °C, Relative humidity 88.00%, Rainy days 0.81) . Temperature fluctuation recorded in year 2008 was higher than 2006 and 2007. It suggests that the temperature fluctuation is one of the stress factors, which increased susceptibility of host for parasitic infection in host. Any form of increased stress on the host may lead to increase in rates of infection (Esch *et al*., 1975).

In general, it was observed that amphistome infestation was highest (39.19%) in month of July (Max Temperature 31.46° C, Min Temperature 25.41° C Relative humidity 89.6%, Total rainfall 104.72 mm)

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followed by June having 37.66% (Max Temperature 33.27 °C, Min Temperature 25.16 °C Relative humidity 80.25%, Total rainfall 68.48 mm). The least infection (6.52%) was observed in month of December (Max Temperature 22.42 °C, Min Temperature 8.24 °C Relative humidity 71.75%, Total rainfall 0.80 mm).

The data revealed that relative humidity and total rainfall were on higher side in the months of the years of higher intensity of infection. Pandey (1994) also concluded that rainfall and intensity of infection was directly related to each other. Our results indicated that these months are favorable for development of infection in the host in this semi arid zone. Moisture content to parasite is dependent on temperature and rainfall. It is well-established fact that higher relative humidity had direct impact on incidence on parasite infection (Hawkins, 1945).

Seasonal clubbing of the data was found to be further conformity of these observations as the amphistome infection was found to be more prevalent (31.05%) in rainy season (Max Temperature 31.26 °C, Min Temperature 22.84° C, Relative humidity 87.96%, Total rainfall 70.26 mm) followed by 27.68% in summer (Max Temperature 33.36° C, Min Temperature 18.89 °C, Relative humidity 75.56%, Total rainfall 24.56 mm) and least i.e. 9.75% in winter(Max Temperature 23.50 °C, Min Temperature 8.84 °C, Relative humidity 70.57%, Total rainfall 4.16 mm). Pandey *et al*., (1994) also reported the peak incidence during rainy season. Shekher and Haque (2009) also reported similar seasonal incidence and direct effect of relative humidity temperature and rainfall on incicence of infection. Thus amphistomosis that occur in rainy season can be attributed to high rain fall. It might have increase the snail population that in turn resulted in development of cercariae in snails in the coming months. Incidence of parasite may found to be directly proportional to mean temperature and relative humidity (Hawkins, 1945).

The mean maximum temperature is low in winter season thus not conducive for larval growth. The mean minimum temperature with high relative humidity during winter season might be responsible for arrested development of larvae as cold stimuli encourage hypobiosis (Hutchinson *et al.,* 1972). Further short photoperiod during winter season contribute to reduction in faecal out put (Chapman, 1982), as reduced day length reduces the grazing period of animals, which in turn lower the chances of infection and finally resulting in decrease output of faecal eggs.

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Evaluation of micro mineral profile in the growing male and female Vrindavani cattle

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Abstract

Present study was conducted to record the micro minerals i.e. copper (Cu), iron (Fe) and zinc (Zn) status, in growing Vrindavani cattle with respect to sex of the animal in different age groups. The animals were divided in four different age groups containing 12 animals each (six male and six female). Micro minerals concentration was determined by atomic absorption spectrophotometery. The results were compared between and within groups. Within groups, male and female animals showed no significant variation ($p \leq 0.05$) in Cu, Fe and Zn concentration except for group IV which varied significantly ($p \leq 0.05$) in Cu concentration, whereas between groups male and female animals of group I and IV varied significantly ($p \leq 0.05$) from those of group II for Fe concentrations. Similarly, male and female animals of group I, III and IV varied significantly ($p \leq 0.05$) from those of group II for Zn concentrations.

Keywords: *Vrindavani cattle, micro minerals, gender.*

The economic importance of any organized or unorganized cattle farm relies heavily on the health status and reproductive performance of its young stock especially heifers which are the future cows for the farm. Hidiroglou (1979) reported that various minerals affect reproductive performance in ruminants either singly or in combination. As micro minerals have essential role in the wide spread physiological and metabolic processes. So the proper monitoring of the serum/blood concentration of these micro minerals, their variations with different factors (like age, breed, sex etc) and optimum supplementation of their required amounts can improve performance of the animals.

The concentrations of trace elements in blood are frequently analyzed for the evaluation of deficiency/ excess as they have significant correlation with nutritional status of some of the trace minerals in the animals (Levander, 1986).

The issue of evaluation of individual micro mineral deficits in the youngest calf categories has been discussed only superficially(Pavlata *et al*., 2004) and evaluation is often made using the same values as for adult animals or knowledge of metabolism of a particular micro mineral is applied to other micro mineral (Pavlata *et al*., 2004). Also variation of the mineral profile of cattle with age, breed, lactation etc is widely reported but reports about the variation with sex of animal are meagre. The present study was carried to study the variation of micro mineral status in young growing cattle with respect to sex of animals.

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Materials and Methods

The study was carried out at Cattle and Buffalo (C&B) farm of LPM section, IVRI, Izatnagar during the month of April to May 2010. Selection of animals was made randomly from the farm herd of Vrindavani growing cattle kept and maintained on similar feeding and management practices. The animals were divided into four groups (Group I, II, III and IV) based on age (0-6, 6-12, 12-18 and 18-24 months) containing 12 animals each (six male and six female animals).

Blood samples (10 ml) were collected using sterile disposable syringe during early morning hours from jugular vein in a sterilized acid washed test tubes containing EDTA as anticoagulant and immediately transported to laboratory on ice pack.

Blood concentration of Cu, Fe and Zn were determined by subjecting the samples to nitric acid/ perchloric acid digestion as described by Kolmer *et al.* (1951) and analyzed by atomic absorption spectrophotometry (AAS 4141, EICL, Hyderabad, India).

Differences in blood concentration of Cu, Fe and Zn among different groups were compared using ANOVA and Duncan's Multiple Range Test (Snedecor and Cochran, 1994).

Results and Discussions

Mean Values of blood concentration of Cu, Fe

Table 1: Concentration of micro minerals in blood(µg/ml) samples of growing Vrindavani cattle (Mean ± SE).

Group	Sex	Cu	Fe	Zn
I	Male (n=6)	1.10 ± 0.03^{bc}	1.54 ± 0.10^{c}	1.17 ± 0.007^{b}
(0-6m)	Female (n=6)	1.11 ± 0.04^{bc}	1.46 ± 0.13^{bc}	1.17 ± 0.007^{b}
II	Male (n=6)	1.17 ± 0.007^{c}	0.90 ± 0.02^{a}	0.99 ± 0.02^{a}
(6-12m)	Female (n=6)	1.11 ± 0.006^{bc}	0.90 ± 0.02^{a}	0.99 ± 0.02^{a}
III	Male (n=6)	1.17 ± 0.007^{c}	1.15 ± 0.06^{ab}	1.16 ± 0.007^{b}
(12-18m)	Female (n=6)	1.14 ± 0.01^{c}	1.16 ± 0.06^{ab}	1.09 ± 0.01^{ab}
IV	Male (n=6)	0.75 ± 0.05^{a}	1.40 ± 0.05^{bc}	1.16 ± 0.05^{b}
(18-24m)	Female (n=6)	1.00 ± 0.02^{b}	1.36 ± 0.06^{bc}	1.18 ± 0.06^{b}

Values with different superscripts vary significantly ($pd \leq 0.05$) between and within groups.

and Zn of growing male and female Vrindavani cattle are depicted in Table 1.

Copper concentration in the male and female animals of group I, II and III shows no significant variation but that of group IV shows significant ($p \leq 0.05$) variation implying different requirements by the male and female animals for the Cu during the maturity of animal. Bremner (1980) reported that Cu absorption may be regulated by oestrogen. Cu plays an important role in the reproductive development of female especially hormone secretion, oestrus and ovulation (Radostits *et al*., 2000). Cu deficiency affects hypothalmo-hypophyseal axis as well as hypophyseal ovarian axis (Du Plessies *et al*., 1999). Impaired steroidogenesis and hormonal pattern due to Cu deficiency in rabbit was reported by Tohamy *et al. (*1997). Impaired progesterone secretion due to Cu deficiency was reported by Van Niekerk and Van Niekerk (1989). Cu concentration in the female animals of group III and IV and in the male animals of the group I and IV; III and IV shows significant ($p \leq 0.05$) variation. This may be due to the increase in micro mineral requirement with increasing age for growth and developmental processes and/or reduced absorption from available sources. Kay *et al.* (1973) reported that Cu absorption declines significantly from intestine after weaning.

Iron concentration in the male and female animals of group I, II, III and IV showed no significant ($p \leq 0.05$) variation. Hidiroglou (1979) reported no difference in circulatory Fe levels in infertile/fertile animals. Fe concentration in the male animals of group I and IV and in the female animals of group I and IV vary significantly ($p \leq 0.05$) from those of the group II. Serum Fe level of young animals decline initially with age as milk is poor source of iron but this level attains normal value as animals start taking feeds and fodders (Radostits*et al*., 2000).

Zinc concentration in the male and female animals of group I, II, III and IV shows no significant ($p \leq 0.05$) variation. Male animals of group I, III and IV and the female animals of same groups vary significantly ($p \leq 0.05$) from those of the group II. This may be due to the fact that Zn has diverse role in the synthesis (Singh *et al*., 1998), secretion (Sugino *et al*., 1999) and action (Tourtellotte *et al*., 2000) of hormones (oestrogen and progesterone). In male animals, Zn is involved in testicular development and spermatogenesis (Radostits *et al*., 2000). Positive correlation was observed between serum progesterone level and serum Cu and Zn levels in cows and heifers throughout the oestrous cycle (Yildiz and Akar, 2001).

Generally requirements for micro minerals increase as animal grows but the availability being low (as milk is poor source of micro minerals especially Cu and Fe) so initially serum micro mineral levels fall. These levels are brought to normal concentrations by the intake of other micro mineral sources like feeds and fodders. DeMaria (1978) reported that there is significant fall in Cu level in milk 48 hours after weaning. Pavlata *et al.* (2004) reported lower Cu concentration in calves than their dams.

Thus the present study showed significant variation of Cu concentration in male and female animals during maturity and non-significant variation in Fe and Zn concentration.

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Haematobiochemical alterations in chicks challenged with chicken infectious anaemia virus

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Abstract

The present investigation was undertaken to assess the haematobiochemical alterations induced by experimental inoculation of Chicken infectious anaemia virus (CIAV) in SPF chicks. All the CIAV challenged birds showed a significant alterations in haemograms, leukogram and biochemical parameters as compared to control group.

Keywords: Chicken Infectious Anaemia, Haematobiochemical, Chicks

Chicken infectious anaemia (CIA) is a highly contagious disease with a high prevalence in countries with intensive poultry production. Anaemic signs are observed in chicks on the featherless parts of the body at 14-16 days post infection (dpi) and also evident as low haematocrit value (Goodwin *et al.,* 1992; Dhama *et al.,* 2003; Praveen, 2005). Panmyelopathy and transient generalized lymphoid atrophy are observed at the peak of the disease (Taniguchi *et al*., 1982, 1983; Goryo *et al*., 1989a; Chettle *et al*., 1989; Pope, 1991). Dysfunction of haemopoiesis drastically affects the production of mature red blood cells (erythropoiesis) and myelopoiesis leading to transient destruction of erythroblastoid and granuloblastoid cell lineages in bone marrow resulting in hypoplasia, anaemia and panleukopenia. The present study was carried out to study the haematobiochemical alterations in chicks challenged with chicken infectious anaemia virus (CIAV).

Materials and Methods

A total of 40 day old specific pathogen free (SPF) chicks were maintained in standard managemental conditions and were given antibiotic (Enrofloxacin, 10% @ 1ml/lit of drinking water) for six days. The experimental chicks were vaccinated with primary doses of Newcastle disease (NCD) and infectious bursal disease (IBD) on first and twelfth day, respectively. CIAV propagated in MDCC-MSB1 cells maintained in the Avian disease section, Division of Pathology, IVRI, Izatnagar, UP (India) was used as challenge virus. A 0.5 ml (10%) suspension of the virus was inoculated intramuscularly twice daily on day 15th and 16th of the study. The chicks were randomly divided into 2 equal groups. Group I chicks of this group were kept on normal basal ration and provided with plain drinking water and served as negative control. Group II chicks were given normal basal ration and plain drinking water and were challenged by CIAV by intra muscular route. No medication was given before and after the virus challenge. About 5ml of pooled blood samples were collected from 5 birds taken at random from each group on day 14th, 21st and 28th.

Packed cell volume (PCV) and haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leucocyte count were determined as per standard procedure. Aspartate amino tranferase (AST) and alanine amino transferase (ALT) were estimated as per the mehod of Tietz (1998). Creatinine kinase (CK) and uric acid (UA) were estimated using reagent kits. The results were analyzed by standard method (Snedecor and Cochran, 1994).

Results and Discussion

In the present study, a significant ($P<0.05$) decline in the haemoglobin, PCV and total erythrocyte count was observed in all the CIAV challenged groups as compared to the control group. In leukocytic lineages also, a significant decline in total leukocyte count, per cent lymphocyte count and per cent basophil and eosinophil count was observed (Table I). This significant decline in erythrocytic and lymphocytic series cells can be attributed to depression of haemopoietic precursor cells due to CIAV (Dhama *et al.* 2008).

The plasma levels of AST, ALT, CK and UA in group I showed a significant ($P<0.05$) increase as compared to control group on day 21st and 28th of the study. CIAV has a wide spread distribution in the body resulting in damage and focal necrosis of the liver, kidney

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Table I : Haematobiochemical alterations in CIAV inoculated chicks.

Parameters	Groups	Days of observation		
		14	21	28
Haemoglobin (g/l)	I	119.24±1.00	121.96±2.20^{B}	123.58±2.01^{B}
	II	119.14±1.67	101.30±1.10^{Ab}	67.66±1.72^{Aa}
PCV (l/l)	I	0.33±0.01	0.34±0.01^{B}	0.34±0.00^{B}
	II	0.32±0.01^{c}	0.28±0.00^{Ab}	0.20±0.01^{Aa}
TEC (x10^{12}/l)	I	3.36±0.05	3.43±0.06^{B}	3.43±0.09^{C}
	II	3.37±0.06^{c}	2.67±0.05^{Ab}	1.99±0.011^{Aa}
TLC (x10^{9}/l)	I	22.17±0.76	23.16±0.31^{C}	22.68±0.60^{B}
	II	22.33±1.04^{c}	18.90±0.28^{Ab}	15.88±0.15^{Aa}
PLC (%)	I	60.17±0.76	60.76±1.55^{B}	59.71±0.67^{B}
	II	60.83±1.61^{c}	52.66±0.69^{Ab}	48.50±1.36^{Aa}
PHC (%)	I	29.13±1.31	30.08±1.22^{A}	30.83±0.45^{A}
	II	29.90±1.15^{a}	39.88±2.26^{Bb}	45.22±1.10^{Bc}
PMC (%)	I	7.80±0.98	6.02±0.54	6.51±1.07
	II	6.02±1.66	5.39±1.90	4.98±1.20
P(B+E)(%)	I	2.90±0.10	3.07±0.16^{B}	3.19±0.24^{B}
	II	2.92±0.18^{b}	2.41±0.41^{Ab}	1.38±0.15 ^{Aa}
AST (U/l)	I	115.08±2.50	115.35±2.81^{A}	114.48±2.80 ^{A}
	II	115.83±1.26^{a}	168.83±3.46 ^{Bb}	182.71±6.69 ^{Bc}
ALT (U/l)	I	33.17±1.26	33.81±0.70 ^{A}	33.61±0.54 ^{A}
	II	34.67±1.61^{a}	51.45±2.79^{Bb}	60.26±0.70 ^{Bc}
CK (IU/l)	I	54.52±1.03	56.10±0.77 ^{A}	56.33±0.53 ^{A}
	II	53.83±1.89 ^{a}	76.21±0.95 ^{Bb}	79.26±0.93 ^{Bc}
UA (mg/dl)	I	5.68±0.35	5.79±0.48 ^{A}	6.18±0.32 ^{A}
	II	5.57±0.31^{a}	7.41±0.49 ^{Bb}	8.09±0.13 ^{Bc}

The values (Mean±SD) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly (P<0.05) for a parameter.

and spleen. Erosions in the gizzard, skin necrosis on the wings and consolidation of lungs have also been reported. Haemorrhages in the proventricular mucosa and subcutaneous and muscular haemorrhages within the wing tips are sometimes associated with severe anaemia (Yuasa and Imai, 1986; Engstrom *et al*., 1988). In our study, an increase in the activities of enzymes ALT, AST and CK and uric acid can be associated with damage to the organs like liver, muscles, skin and kidneys of CIAV inoculated chicks.

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Sera status of PPR in nomadic Sheep and Goats of Jammu region

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Abstract

Present investigation of Peste des petits ruminant (PPR) was done in nomadic sheep and goats of Jammu and Kashmir State of India. A total 432 serum samples comprising of 216 samples (108 sheep and 108 goats) of migratory and 216 samples (108 sheep and108 goats) of non-migratory flock of sheep and goat were screened for PPR antibodies by using competitive-ELISA. Overall seroprevalence of PPR in migratory flock (33.79%) was significantly (P<0.05) higher as compared to non migratory flock (24.07%). The seroprevalence of PPR in sheep (29.16%) was non-significantly (p<0.05) higher than that of goat (28.70%). Age wise seroprevalence was significantly (p<0.05) higher in >12 months age group (39.58%), followed by 8-12 months (26.38%) and 4-8 months (20.83%) age group. The finding of the present study may be correlated with variation of PPR with respect to species, age, sex, and husbandry practices in the state.

Keywords: cELISA, Nomadic, Peste des petits ruminant (PPR) and Seroprevalence.

Peste-des petits ruminant (PPR) an acute, viral disease of small ruminants, was first reported in India in 1987 from Tamil Nadu (Shaila *et al.,* 1989) and now being pronounced as the major threats to the sheep and goat population of this country with a combined susceptible population of about 200 million (Dhar *et al.,* 2002). The disease is caused by a Morbillivirus belonging to the family Paramyxoviridae (Gibbs *et al.,* 1979). The close relationship between PPR outbreaks and migration of sheep and goats flocks had been reported by Shankaran *et al.* (1998) and Dhand *et al.* (2002). Singh *et al.* (2004) discussed the relation of migration of animals with that of maintenance and transmission of PPRV in nature. Although there are several documentations of research finding related to various aspects of PPR throughout India. But still it is a poorly recognized disease, particularly with regard to epidemiological features such as transmission and propagation dynamics under different production systems (Diallo, 2007).

Materials and Methods

The present study was conducted during the year 2009-10. A total of 432 samples (216 each from migratory and non-migratory flocks), comprising of three different age groups viz 4-8 months, 8-12 months and more than 12 months were collected. Samples from migratory flocks were collected from 6 different migratory routes (Kathua-Bhaderwah-Paddar route, Samba-Dachan-Marwah route, Jammu-Pahalgam-Warwan route, Reasi-Margam-Krishnalla-Nunkun route, Rajouri-Kalakote-Kangan-Tlail route and Poonch-kralpthri route). Whereas from non-migratory flocks samples were collected from 6 different districts (Udhampur, Kathua, Samba, Reasi, Rajouri and Poonch) of Jammu region.

The PPR specific antibodies were detected by using competitive ELISA (cELISA) kit developed by National Rinderpest Laboratory, Division of Virology, IVRI, Mukteswar. As per protocol given in user manual the sera samples showing more than 40 per cent inhibition (PI) of mean OD values of the Cm (Monoclonal antibody control) wells were taken as positive for PPR antibodies provided other controls fell within the range. The plate reading was rejected if the PI in the control panel did not fall within the expected range: conjugate control: 91 to 105%; strong positive serum (C^{++}): 81 to 100%; weak positive serum (C^{+}): 51 to 80%; negative control serum (C^{-}): -25 to +25%.

The statistical difference of seroprevalence with regard to species (sheep and goats), age, migration and non-migration was analyzed through Chi-square test by using on line programme (http://statpages.org/ctab2x2.html).

Results and Discussion

The overall seroprevalence of PPR antibodies was 28.93%. The seroprevelence was significantly higher (p<0.05) in migratory (33.79%) than non migratory (24.07%) flocks. The seasonal movement of these migratory animals may increase the chance of transmission of disease. Moreover, in migratory flocks, there is transportation stress, underfeeding and

Table I. Antibody based sero-prevalence of PPR in sheep and goats of migratory and non-migratory flocks.

Age groups	Migratory						Non-migratory					
	Sheep		Goat		Total		Sheep		Goat		Total	
	ST	SP	ST	SP	ST	SP	ST	SP	ST	SP	ST	SP
4-8 months	36	11(30.55)	36	5(13.88)	72	16(22.23)	36	8(22.23)	36	6(16.67)	72	14(19.44)
8-12 months	36	11(30.55)	36	10(27.78)	72	21(29.16)	36	8(22.23)	36	9(25.00)	72	17(23.61)
>12 months	36	16(44.45)	36	20(55.56)	72	36(50.00)	36	9(25.00)	36	12(33.34)	72	21(29.17)
Total	**108**	**38(35.18)**	**108**	**35(32.40)**	**216**	**73(33.79)**	**108**	**25(23.15)**	**108**	**27(25.00)**	**216**	**52(24.07)**

ST – Samples Tested, SP- Samples Positive, Figures in the parenthesis indicates per cent positive

Table II Antibody based route wise seroprevalence of PPR in sheep and goats of migratory flocks

Migratory routes	Sheep		Goat		Total	
	ST	SP	ST	SP	ST	SP
Kathua route	18	3 (16.66)	18	8(44.45)	36	11(30.55)
Samba route	18	8 (44.45)	18	7 (38.88)	36	15 (41.66)
Jammu route	18	7 (38.88)	18	5 (27.78)	36	12 (33.34)
Reasi route	18	9 (50.00)	18	7 (38.88)	36	16 (44.45)
Rajouri route	18	6 (33.33)	18	4 (22.23)	36	10 (27.78)
Poonch route	18	5 (27.78)	18	4 (22.23)	36	09 (25.00)
Total	**108**	**38 (35.18)**	**108**	**35 (32.40)**	**216**	**73 (33.79)**

ST – Samples Tested, SP- Samples Positive, Figures in the parenthesis indicates per cent positive

Table III. Antibody based district wise seroprevalence of PPR in sheep and goats of non-migratory flocks

District	Sheep		Goat		Total	
	ST	SP	ST	SP	ST	SP
Udhampur	18	08 (44.45)	18	02 (11.12)	36	10 (27.78)
Samba	18	05 (27.78)	18	03 (16.67)	36	08 (22.23)
Kathua	18	04 (22.23)	18	07 (38.88)	36	11(30.55)
Reasi	18	03 (16.67)	18	05 (27.78)	36	08 (22.23)
Rajouri	18	02 (11.12)	18	04 (22.23)	36	06 (16.67)
Poonch	18	03 (16.67)	18	06 (33.33)	36	09 (25.00)
Total	**108**	**25 (23.14)**	**108**	**27 (25.00)**	**216**	**52 (24.07)**

ST – Samples Tested, SP- Samples Positive, Figures in the parenthesis indicates per cent positive

underlying parasitic infection which may decrease the immunity, thereby increasing the susceptibility of animals to disease. Similarly Khan *et al.* (2007) reported higher seroprevalence of PPR in southern and western parts of Punjab in Pakistan where nomadic rearing of sheep and goats was common.

Species wise overall seroprevalence was non–significantly higher in sheep (29.16%) than goats (28.70%) and could be due to the fact that the disease is more severe in goat as compared to sheep (Radostits *et al.,* 2000). Hence, more number of infected animals survives the infection leading to increase in the seroprevalence. The result follows the similar pattern as observed by Singh *et al.* (2004), from India.

Age wise the overall seroprevalence was significantly ($p<0.05$) higher in >12 months age group (39.58%) followed by 8-12 months (26.38%) and 4-8 months (20.83%) age group respectively (Table I). The lower prevalence of PPR in young stocks may be due to the passive immunity provided by colostrum. Ata *et al.* (1989) reported a lower prevalence of PPR in kids of dam with previous history of PPR up to 3-5 months of age.

Analysis of route wise data from various migratory routes showed a higher seroprevalence of PPR in animals of Reasi route and lowest in Poonch (Table II). Detailed study of these routes revealed that the animals of Reasi route had to travel approximately 536 km (to and fro) during their course of migration, for approximately 90-120 days from an altitude of 2000 ft to 9500 ft, whereas the animals of Poonch route had to travel only 196 km (to and fro) during their course of

migration in approximately 30 days. These environmental, geographical and meterological factors may contributes to reduction in immunity of these animals. These findings are in concurrent with Abubakar *et al.* (2009) who found that species and geographic location were the main factors affecting the prevalence of the PPR.

District wise analysis of data of non-migratory flocks revealed higher seroprevelence in the animals of Kathua (33.55%) and lowest in the animals of Rajouri (16.67) district (Table III). The possible reason for higher seroprevalence in animals of Kathua district may be due to fact that these non migratory flocks share their pasture land more often with the migratory flock of nomadic community. The results of present study are also supported by the various workers in India and abroad, viz; Danad *et al.* (2002) in Punjab of India and Abubakar *et al.* (2009), Khan *et al.* (2008) and Khan *et al.* (2007) in their respective studies in Pakistan found that the migratory flock act as primary source of PPR infection and spread the infections to native non migratory flocks.

In a nut shell it can be concluded that PPR is fairly prevalent in nomadic sheep and goats of J&K and should be considered as one of the priority animal diseases whose control should be considered important for poverty alleviation in the state where about 14% of the population are dependent on sheep and goats rearing for their livelihood.

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Chronic diarrhea caused by combined infection of *Strongyle* worms and *Proteus* spp in a mare

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Abstract

A Kathiawari mare was investigated for the chronic moderate diarrhea, reduced appetite and progressive weight loss. The previous treatment with fluid, antimicrobials and anthelmentics failed to respond. Faeceal examination revealed combined infection of *Strongyle* worms and *Proteus* spp. Neomycin injections and a single shot of oral ivermectin was given, which cured the animal. The present findings suggested that chronic diarrhea in horses may have multiple etiology. Enteric bacteria, like *Proteus* spp may be involved and hence use of antibiotics is warranted specially in chronic refractory cases.

Keywords: Diarrhoea, Equine, Neomycin, *Proteus* spp, Strongyle-worms

Diarrhoea is a potentially serious disease condition in horses. It may be caused by change in feed or fodder, stress, bacterial, viral or fungal infections or may be idiopathic in nature (Radostits *et al*., 2000). Under natural conditions two or more etiological factors may be involved simultaneously complicating the disease process. The present paper reports the successful clinical management of chronic diarrhea caused by combined infection of *Proteus* spp and *Strongyle* worms in a Kathiawari mare.

A three years old Kathiawari mare was presented with 4 months old history of moderate diarrhea, progressive weight loss, reduced appetite and general apathy. The mare was treated by local veterinarians with fluid, antibiotics and anthelmintics for long period of time with no clinical response. At the time of presentation the mare had poor body condition, dry and rough hair coat. Animal appeared dull and depressed, passing increased volume of liquid faeces without formation of normal faecal balls. All vital signs were within the normal range. Blood sample was collected for haematological examination. Faecal sample was examined for parasitic eggs/ ova/ oocyst. Rectal swab was collected aseptically for bacteriological isolation and identification.

Haematological examination revealed low haemoglobin concentration (10 g%), normal total leukocytes (7400/ cubic mm), neutorphils (50%) and lymphocytes (50%) counts with moderate increase in platelet count and presence of few degenerated neutrophils. Faecal sample was positive for *Strongyle* eggs. *Proteus* spp was isolated from the faecal sample. The animal was treated with injection Unimycin (Neomycin, Unichem Laboratories Limited) @ 15 ml, intramuscular, twice daily for five days. The very next day, the animal showed improvement in faecal consistency and appetite. The owner was advised to continue the same treatment. The animal was given one bolus of Hitek (Ivermectin 80 mg) orally once. One week later, complete clinical cure was reported by the owner and there was no relapse of condition even three months after treatment.

Intestinal parasites are common causes of diarrhea in equines. *Strongyle* infection in equine is rampant throughout the world (Radostits *et al*., 2000). In small numbers they may not cause any clinical disease. In present case, only few eggs were evident in faecal samples suggesting low parasitic burden, it was decided to give first antibiotic and then anthelmintic drug. Moreover, anthelmintic resistance is common in *Strongyles* (Kaplam, 2009) which may be a reason behind poor response to earlier therapy. Scientists across the world are now recommending judicious use of anthelmintics in equines in view of growing anthelmintic resistance.

The common infectious causes of equine diarrhoea include Rotavirus and *Cryptosporidium* in foals and *Salmonella* spp., *Clostridium* spp. and *Aeromonas* spp. in all age groups. Multi-resistant *Proteus* spp are commonly present in gastrointestinal tracts of equine (Singh, 2009). However, there was no report indicating their role in cases of equine diarrhea. In present case, quick response of neomycin administration suggested possible role of *Proteus* spp

in chronic diarrhoea in horses. Following intramuscular administration, neomycin is rapidly absorbed in equines where therapeutic concentration against variety of bacterial pathogens is maintained for long time (Baggot *et al*., 1981). However, care should be taken since it may cause nephrotoxicity when used for prolong time (Edwards *et al*., 1981). *Proteus* spp isolated from animals are reported to be resistant against tetracyclines, sulfonamides and chloramphenicol (Grobbel *et al*., 2007).

From the present report, it is concluded that chronic diarrhea in equines may have multiple etiology. Thorough clinical and laboratory examinations are warranted if therapy against one etiological factor is not responding. Administration of antibiotics may also be required, but their judicious use is always warranted.

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Effect of Ashwagandha extract on infectious bursal disease virus replication

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Abstract

In present study, hydro alcoholic extract of Ashwagandha roots was evaluated for its inhibitory action on Infectious Bursal Disease Virus Replication (IBDV). The extract showed inhibitory action at its maximum nontoxic concentration i.e. 25µg/ml which was confirmed by May Grunwald and Giemsa staining method and Quantitative Real Time PCR results. Decrease in VP2 gene expression proved the inhibitory action of extract on Infectious Bursal Disease Virus Replication.

Keywords: Infectious Bursal Disease Virus, Ashwagandha, Hydroalcoholic extract, Real Time PCR, Antiviral

Infectious Bursal disease is a highly contagious disease of young chickens characterized by marked immunosuppression and mortality in affected flocks. The causative agent is Infectious Bursal Disease Virus (IBDV), a non-enveloped ds RNA virus of genus Avibirnavirus and family Birnaviridae. As both medication and vaccination failed in controlling the infection, recent success in using medicinal/herbal plants extract as phyto- antiviral agents has raised some optimism (Jassim and Naji, 2003). Recent studies have shown that Ashwagandha (*Withania somnifera*) has inhibitory activity against HIV virus (Usha *et al.*, 2003), Herpes simplex virus type-1 (Kambizi *et al*., 2007) and IBD Virus (Ganguly, 2009).

Materials and Methods

The roots of Ashwagandha were cut, dried and ground into a fine powder. The powder was allowed to be continuously agitated in 50% ethanol (v/v) for 48 hours at 40ºC in a shaking incubator. The mixture was filtered through muslin cloth and filter paper. The final hydro-alcoholic extract was obtained by drying the filtrate under hot circulating air at 40ºC.

Phyto-chemical screening tests were done for alkaloids, tannins, flavonoids and steroids (Trease and Evans, 1983). Different passages of UABz-2 strain of IBD virus maintained at Veterinary Biochemistry lab of G.B.P.U.A. & T., Pantnagar were used and Chicken Embryo Fibroblast (CEF) Culture was used for the propagation of virus. Primer set (23 mer) (Bangalore Genei, India) was used for amplification of 248 bp fragment of hypervariable region of VP2 gene of IBDV (Ikuta *et al*., 2001).

Forward primer 5' GTA ACA ATC ACA CTG TTC TCA GC 3' 804-826

Reverse primer 5' GAT GTG ATT GGC TGG GTT ATC TC 3' 1029-1051

Similarly β-actin primer set (20 mer) was used for 275 bp fragment amplification (Kumar, 2004).

Forward primer 5' GAG AAG CTG TGC TAC GTC GC 3'

Reverse primer 5' CCA GAC AGC ACT GTG TTG GC 3'

Primary CEF cell cultures were prepared using 9-11 days old chicken embryos (Cunningham, 1973). In order to standardize the dose and eliminate the cytotoxic effect of Ashwagandha (*Withania somnifera)*, MTT Dye Reduction assay was done. In this method CEF monolayer were grown in 96- well plate for 24 hours and after washing with EMEM different concentrations of Ashwagandha extract were added and the plate was kept at 37°C in an atmosphere of 5% CO_2 for 24 h. After removing extract solution 50-ml of MTT solution (4mg/ml in EMEM) was added to each well and plate was incubated at 37°C for 4 h. Afterwards MTT solution was removed and 200 ml of Di Methyl

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Sulfoxide (DMSO)solution was added to each well. Then 25 µl of glycine buffer was added and absorbance was recorded at 570 nm. The rate of growth inhibition was calculated as formula in which per cent cytotoxicity is equal to the 100 – [OD extract treated/ OD of Control]) in per centage. Culture flasks with 80-90% confluency were taken and inoculated with 1ml of previously CEF adapted virus. These flasks were then incubated with regular tilting in every 6-8 min for 45 min at 37°C for the adsorption of the virus. Then the inocula was decanted and the fresh maintenance medium (EMEM with 2% FBS) was added and flasks were kept in incubator at 37°C and 5% CO_2 for development of cytopathic effects characterized by small round refractile cells for 3-4 days.

The infected cultures (with and without treatment of WS extract) were stained using May-Grunwald and Geimsa stains at 48 h intervals post infection (hpi) (Merchant *et al*., 1960). In order to confirm the inhibitory action of *Withania somnifera* total RNA was isolated using Trizol reagent (Invitrogen life technologies). RNA pellet was solubilized in 1% DEPC (Diethyl pyrocarbonate) treated water. The c-DNA from total RNA was prepared and denatured by keeping the mixture at 95°C for 3 minutes. Then this c-DNA is used for Real Time Quantitative Polymerase Chain Reaction.

The cDNA from Ashwagandha extract treated and untreated infected CEF (48 hpi) with IBDV was used**.** The 248 bp fragment of VP2 gene and 275 bp fragment of β-actin gene were amplified.

Results and Discussion

In Phytochemical screening the extract showed positive results for alkaloids, tannins, flavonoids and steroids. In May-Grunwald and Giemsa (MGG) staining less expression of CPE (Cytopathic effects) was found with Ashwagandha extract treatment. In Real Time Quantitative PCR the expression of β-actin gene was considered as constant as it is a housekeeping gene whereas decrease in expression of VP2 gene indicated the inhibition of IBD viral replication by Ashwagandha extract.

Table1: Variation in the expression of VP2 gene in untreated and Ashwagandha extract treated samples showing the decrease expression of VP2 gene which proves the inhibitory action of Ashwagandha extract on IBD Virus replication.

S.No.	Sample	Expression of VP2 gene in per centage
1	Untreated	100
2	Treated	44.5

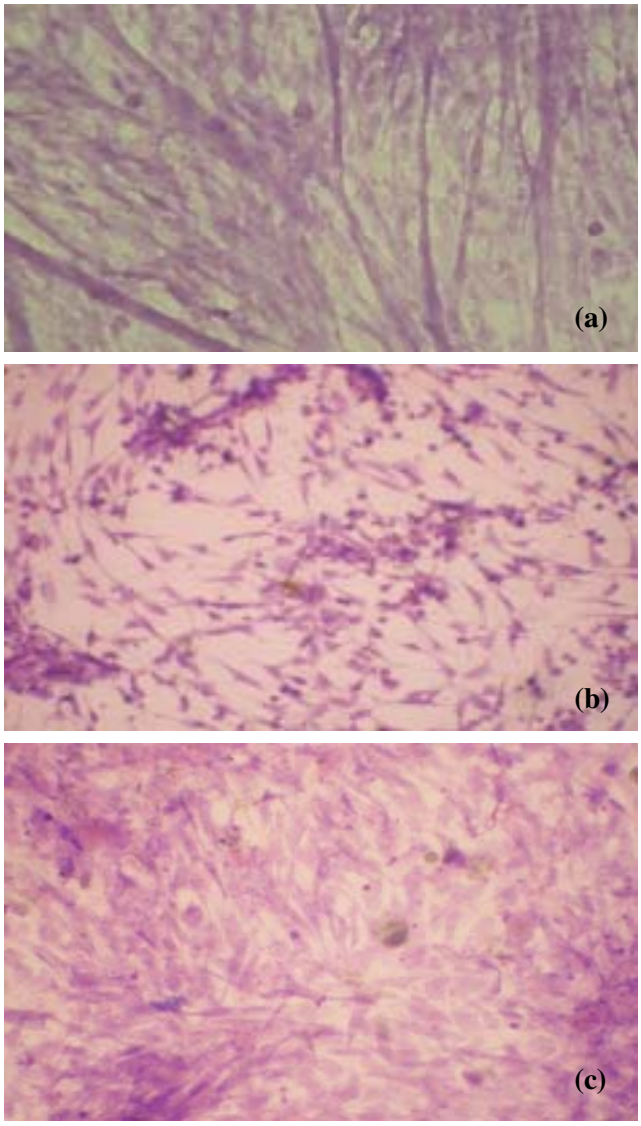

Fig. 1: Comparision of Ashwagandha(Withania somnifera) extract treated and untreated IBD virus infected CEF cells: (a)Control CEF culture (b) CEF culture infected with virus showing CPE (c) Effect of Withania somnifera extract extract on Virus infected CEF culture showing less CPE.

There are many mechanisms for the inhibitory effect of Ashwagandha (*Withania somnifera)*. Ashwagandha is known to increase inducible nitric oxide synthetase expression (Iuvone *et al*., 2003). Nitric oxide synthases (NOSs) synthesizes NO from the amino acid

l-arginine. NO secretion has been reported in monocytes, macrophages, smooth muscle cells, microvascular endothelial cells, fibroblasts, cardiomyocytes, hepatocytes, and megakaryocytes after appropriate infection (Mollace *et al*., 2005). Malik *et al*. (2007) also found *Withania somnifera* extract to cause enhanced secretion of nitrite *ex vivo*. Nitric oxide has been shown to be inhibitory to IBDV (Ashraf, 2005). Jena (2008) also found nitric oxide to exert an inhibitory effect on IBDV. Another mechanism is the induction of apoptosis. WS is a well-known inducer of apoptosis causing upregulation of *Bax*, down-regulation of Akt phosphorylation and inhibition of NFêB (Oh *et al*., 2008; Prasanna *et al.*, 2008). It activates both intrinsic and extrinsic signaling pathways leading to apoptosis (Malik *et al*., 2009). By these mechanism Ashwagandha extract exerts inhibitory effect on IBD Virus replication.

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Clinical and therapeutic aspects of Canine Monocytic Ehrlichiosis

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Abstract

Seven dogs of various breed and sex presenting common signs viz., oculonasal discharges, pale mucosae, lymphadenitis and respiratory distress along with hemorrhagic gastro-enteritis, epistaxis, enlargement of popliteal lymph nodes and blanched conjunctival mucosa in few were diagnosed for *E. canis*. Low levels of TEC, Hb and PCV with leukopenia, eosinopenia and thrombocytopenia along with increased ALT and decreased serum proteins and albumins but increased globulins were the significant hemato-biochemical findings. Treatment with doxycycline showed clinical improvement from day 2-4 and complete clinical recovery by day 15.

Keywords: Canine monocytic ehrlichiosis, Doxycycline, Treatment.

Ehrlichiosis, also termed as canine monocytic ehrlichiosis or tropical canine pancytopenia is commonly transmitted by ticks and is many a times undiagnosed cause for non-responsive / recurrent pyrexia in dogs. Canine ehrlichiosis, caused by the rickettsial organism, *Ehrlichia canis* is a serious febrile and potentially fatal disease of dogs is usually diagnosed by clinical signs, although no specific and pathognomic signs occur consistently (Woody and Hoskins, 1991). The present communication deals with specific clinical signs and constraints in therapeutic management of various stages of naturally occurring canine monocytic ehrlichiosis.

Seven dogs of various breed, sex and age presented to Veterinary Hospital Bhoiguda, TVCC of College of Veterinary Science, Hyderabad formed the material for this study. All these were being treated for pyrexia of unknown origin at various hospitals, but of no response. The common signalment were going down condition, non-response to recurrent episodes of fever, off food and epistaxis (GSD-2 and Lab-1). Moderate to severe tick infestation was also noticed in all the dogs. Blood was collected from peripheral vein for hematology and biochemical analysis and smears were made from ear vein. All the affected dogs were treated with two doses of parenteral ivermectin* @300 mcg/kg, sc, at weekly interval, oxytetracycline** @20mg/kg, iv, for 3 days followed by oral doxycycline*** @10 mg/kg wt for 21 days and oral hematonics**** @5ml, bid, 15 days.

*NEOMEC Inj. (Ivermectin 1mg/ml), M/S Intas Pharmaceuticals, Ahmedabad, **TERRAMYCIN Inj. (Tetracycline 50 mg/ml), M/S Pfizer, Mumbai, ***DOXT (Doxycycline 100 mg), M/S Dr. Reddy labs Ltd., Hyderabad, ****IMFERON Liq (Elemental Iron (as Carboral Iron) 25mg, Folic acid I.P 500mcg, Cyanacobalamine I.P. 6mcg, Elemental Zinc 11mg), M/S Shreya life sciences pvt. Ltd., Mumbai.

Serous oculonasal discharges, pale mucosae, peripheral lymphadenitis, dyspnoea and erythema of the skin of the abdomen and inguinal region were the significant manifestations. Associated signs like hemorrhagic gastro-enteritis, epistaxis, enlargement of popliteal lymph nodes and blanched conjunctival mucosa was also noticed in 2(GSD) and 1(Lab) dogs. Nervous signs such as incordination, seizures, droopy ears and edema of face were also recorded in one German shepherd dog of 6.8 yr. Complete blood picture revealed significantly low ($P<0.05$) levels of Total Erythrocyte Count ($5.1 \pm 0.28 \times 10^6$/ml), Hemoglobin (6.8 ± 0.58 gm/dl) and Packed Cell Volume ($39.8 \pm 0.46\%$) with leukopenia ($5.98 \pm 1.24 \times 10^3$/ml), eosinopenia ($0.84 \pm 0.36\%$) and thrombocytopenia ($98.2 \pm 2.22 \times 10^3$/µl). Biochemically there was increased enzymatic activity of Alanine amino transferase (316 ± 1.52 u/l) with low levels of serum proteins (4.6 ± 0.08 mg/dl) and serum albumins (1.62 ± 0.54 mg/dl) but increased globulins ($3.98 + 0.02$ mg/dl). Marked improvement was noticed in 4 dogs from day 2 onwards and 2 dogs from day 4 and complete clinical recovery with absence of organisms in peripheral blood smear by day 15. However, the owners were advised to continue doxycycline orally for one more week. Unfortunately, inspite of every effort we could not save one German shepherd dog of 6.8 yr beyond day 2, which was showing severe nervous signs and profuse epistaxis.

Ehrlichia canis causes a potentially fatal disease in dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favourable prognosis. The condition is characterized by three stages, viz., acute (first) stage manifested by fever, depression, dyspnoea, anorexia, and slight weight loss

with thrombocytopenia, leucopoenia, mild anaemia, and hyper gammaglobulinemia. The second phase is subclinical in which dogs can remain persistently infected for years without clinical signs but with mild thrombocytopenia. The ultimate stage is chronic, characterized by haemorrhages, epitasxis and edema which may often be complicated by superinfections by other micro organisms (Iqbal *et al.*, 1994 and Troy and Forester, 1990).

The signs and laboratory findings of the present study were in accordance with Ueno et al. (2009) and Scotarczak (2003), who opined that diarrhea, apathy, and anorexia were the major clinical signs and the most frequent laboratory abnormalities are non-regenerative anaemia, thrombocytopenia, lymphopenia, and eosinopenia. Animals with ehrlichiosis can also exhibit ocular and CNS signs, due to CNS vasculitis or hemorrhages. Dagnone et al. (2003) reported that dogs infested with brown dog tick, were diagnosed for ehrlichosis with thrombocytopenia and anemia. The authors further opined that the organism can remain alive in the developing tick for upto 5 months.

Drugs with known efficacy include tetracycline hydrochloride, oxytetracycline, (Amyx et al., 1971); minocycline and chloramphenicol (Woody and Hoskins, 1991). Doxycycline @ 10mg/kg, once daily for 3 weeks in conjunction with imidocarb dipropionate @ 5mg/kg, weekly once, is considered the treatment of choice, however, doxycycline alone can also be used (Harrus et al., 1997). It was also reported that, doxycycline can be effective if given at 5 mg/kg, for 3-4 weeks (Egenvall et al., 1997). Improvement in symptoms is usually very quick, but several weeks of treatment is usually needed to ensure a full recovery. In severe cases where blood cell counts are very low, blood transfusions may be needed. Doxycycline generally alleviates clinical monocytic ehrlichiosis, but its efficacy in the control of monocytotropic ehrlichial pathogens requires further investigation (Troy and Forrester, 1990). *Ehrlichia canis* was detected in dogs treated with doxycycline for 14 days and in ticks fed on these dogs, suggesting that treated dogs can remain reservoirs for *E. canis*. Dogs infected with *E. canis* remain infected for their entire lives, even if they received antibiotic treatment with doxycycline (Schaefer et al., 2007).

Hence, it may be concluded that canine monocytic ehrlichiosis, a highly fatal disease is one of the most important cause for pyrexia of unknown origin in dogs. The condition may vary from acute to chronic forms, later being worse with grave prognosis and increased case fatality rate. Though doxycycline is effective in alleviating clinical signs, its efficacy against control of *Ehrlichia* pathogen requires further investigation.

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Management of acute renal failure by using conventional therapy in a dog

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Abstract

A 7 year old Labrador female dog was presented with complaint of inappetance, vomiting, anuria and melena. On physical examination pale conjunctival mucous membrane, normal rectal temperature (102^0F) and dullness were noticed. Haemato-biochemical analysis showed decrease in Hb, TEC and increases in BUN and creatinine. Animal showed an uneventful recovery after day 10 of treatment.

Keywords: dog, ARF, diuresis, anaemia

Acute renal failure (ARF) is a serious disease with guarded prognosis, and results from sudden loss in renal function. It is characterized by a rapid onset of renal insufficiency/failure, reduction in glomerular filtration rate and renal plasma flow, and the clinical and biochemical aftermath of the excretory failure leads to accumulation of waste products within the blood stream. Causes of ARF include pre-existing renal disease, ischemia, exposure to toxins, and other events that decreases renal perfusion (Graner, 2007). Immune-mediated disease, pyelonephritis, hypercalcemia, urinary tract obstruction, and infectious diseases (e.g., leptospirosis) can also result in ARF (Cowgill and Elliot, 2000). Mortality associated with all causes of ARF in dog's ranges from 56% to 80% (Stokes *et al.,* 2006). Acute renal failure (ARF) is potentially reversible under intensive care. Failure to rapidly recognize ARF and initiate aggressive therapy may result in irreversible renal damage and patient death. The present therapy was directed toward preventing further complications of renal failure, restoring kidney function and preventing additional renal injury.

A 7 year old Labrador female dog of 25 kgs body weight was presented with complaint of inappetance, vomiting, anuria and melena for 4 days. Physical examination showed pale conjunctival mucous membrane, normal rectal temperature (102^0F) and dullness. Haemato-biochemical analysis revealed Hb (7.2gm%), PCV (23%), total erythrocyte count (2.45 $x10^6$/µl), total leukocyte count (10.65 $x10^3$/µl) neutrophils (75%), lymphocyte (16%), monocyte (6), eosinophil (3%), BUN (169.6 mg/dl), creatinine (4.4 mg/dl), ALT (27 IU/L), AST (45 IU/L), SAP (53 IU/L) and GGT (16 IU/L). Blood smear examination and bacterial culture showed negative result for haemo-protozoan and leptospirosis, respectively. Ultrasonography of liver and kidneys demonstrated normal architecture and echogenicity. Based on BUN and creatinine value along with other findings, the present case diagnosed as an acute renal failure. Treatment was instituted with ringers lactate @ 500ml bid iv, ceftriaxone and tazobactum (Intacef-Tazo) 562.5 mg od iv, furosemide @ 2 mg/kg iv, metoclopramide @ 0.2 mg/kg q 8-12 hr im, ranitidine @ 2 mg/kg 8-12 hr im, B-complex @ 2ml iv od for 7 days apart from dextrose 25% 100 ml iv od for three days and iron dextran 2 ml iv at 3 days of interval (3 times). Owner of dog advised to restrict salt in diet and prescribed *Boerhavia diffusa* one cap bid orally for 15 days.

Animal showed recovery after day 10 of treatment. BUN and Creatinine values goes down on day 5 at 27.2 mg/dl, 2.34 mg/dl, respectively and on day 10 at 2.2 mg/dl, 1.3 mg/dl, respectively after treatment. High values of serum creatinine and BUN in ARF is due to decrease in GFR, which leads to accumulation of non-protein nitrogenous compound in the blood. The serum creatinine is not metabolized and is excreted by kidneys almost entirely by glomerular filtration whereas low protein diets, anabolic steroids, severe hepatic insufficiency or post systemic shunting (DiBortola *et al.,* 2005) can decrease BUN concentration. Low urine output or complete absence of urine production is a very serious finding. The primary treatment of ARF is fluid therapy to re-establish hydration and expand the intravascular volume to induce diuresis. Dextrose 25% solution is a hypertonic solution which minimized oedema and enhancing ultra filtration of body fluid through osmotic diuretic effect (Chew and Gieg, 2006). Other treatment protocols focus on correcting electrolyte and acid-base disturbances. Fluid

therapy is important to increase kidney blood flow, correct and prevent dehydration and control abnormalities in serum electrolytes (Whittemore and Webb, 2005). Lactated Ringer's solution provides adequate acid–base buffering. Furosemide is the most common diuretic used in the treatment of renal failure (Uchino *et al.,* 2004). It is a loop diuretic that acts on the ascending loop of henley to prevent absorption of sodium and chloride, which causes increased ultra filtrate to form and thus increases urinary output (Solomon *et al.,* 1994). Uremic gastritis is a major cause of vomiting in patients with renal failure. Vomiting is usually controlled by administration of metoclopramide, which is antiemetic drug acting directly on the CRTZ as a D2 dopaminergic antagonist (Senior, 2006). Ranitidine have been recommended for control of uremic hemorrhagic gastritis because it blocks gastrin-stimulated gastric hyperacidity (Senior, 2006). Blood loss from gastrointestinal ulcerations, potentiated by uremic platelet dysfunction, contributes to anemia in present study. Supplementation with iron injection produces an initial erythroid response in most dogs (Cowgill *et al.,* 1998). Antibiotic should be given to prevent secondary bacterial growth because in acute renal failure, chance of bacterial growth is more due to deposition of waste product. In acute renal failure, diets should have restricted levels of phosphorus, sodium, and protein and contain enhanced fatty acid supplements (Allen *et al.,* 2000). *Boerhavia diffusa* is very useful for curing kidney diseases and it posses diuretic action (Murti *et al.,* 2010).

ARF is a serious disease with guarded prognosis. Prompt diagnosis and aggressive therapy can be utilized to prevent the conversion of ARF patient into CRF, and also to save the life of ARF patient.

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Haematobiochemical profile of dogs with prostatic affections

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Abstract

The present study was conducted on 10 dogs suffering from prostate affections to evaluate various haemato-biochemical changes. The blood and serum samples were estimated for Hb, TLC, DLC, platelet count, liver function tests, kidney function tests, total protein and albumin. The diagnosis was confirmed by clinical, radiographic ultrasonographic and Ultrasound guided fine needle aspiration biopsy (USG-FNAB) findings. In case of benign prostate hyperplasia (BPH), prostatitis, and prostatic carcinoma the blood SGPT and SGOT were moderately elevated. The alkaline phosphatase values were markedly elevated in animals suffering from prostatic carcinoma. The haemato-biochemical parameters alone are not diagnostic for dogs suffering from BPH and prostatitis. However, elevation of AKP values may be indicative of prostatic carcinoma when supported with clinical, radiographic and ultrasonographic findings

Keywords: Dogs, Haematology, Ultrasound guided fine needle aspiration biopsy.

Prostatic disorders are common in middle-aged and older sexually intact male dogs (Olsen *et al.* 1987) and have been categorized as hyperplasia, cyst, inflammation, primary and metastatic neoplasia. The diagnosis of prostatic disease in the past has been problematic and workers relied primarily on prostatic fluid analysis, commonly collected through prostatic massage, blind percutaneous fine needle aspirate and radiographic imaging (Olsen *et al.,* 1987). However, haemato-biochemistry is also considered as an important preliminary tool for proceeding towards the correct diagnosis and treatment protocol of prostatic affection. There is paucity of literature about the haemato-biochemical profile of dogs suffering from prostatic affections.

Materials and Methods:

The present study was conducted on 10 clinical cases of male dogs aged 1.5 - 10 years and body weight ranging from 9 to 36 kg presented at the small animal teaching hospital, GADVASU, Ludhiana with varying symptoms. The animals were subjected to systematic evaluation for diagnosis of disease conditions. Hematological and biochemical parameters including Hb (g/dl), TLC (x10^3 per µl), DLC (%), serum AKP, SGPT, SGOT (U/L), BUN, Creatinine (mg/dL), total protein and albumin (g/dL) were determined. Radiography and ultrasonography was performed in all the animals to evaluate the status of the prostate. Confirmatory diagnosis was made by ultrasound guided fine needle aspiration biopsy (USG-FNAB). The haemato-biochemical parameters were correlated with the disease conditions diagnosis.

Results and discussion

The animals were divided into following 3 groups based on clinical, haemato-biochemical radiographical, ultrasonographic and USG-FNAB findings.

1) Benign Prostate Hyperplasia (N=3)
2) Prostatitis (N=3)
3) Prostatic Carcinoma (N=4)

In all the animals suffering from prostatic affections, survey radiography showed that there was an increase in the soft tissue density caudal to the neck of the urinary bladder with cranially displaced and distended urinary bladder. Ultrasonographic findings showed that the prostate was enlarged with normal or generalized increase in echogenicity in case of BPH, mixed echotexture in prostatitis and hyperechoic/ mixed, non uniform echotexture mass were evident at the neck of urinary bladder in the region of prostate in case of prostatic carcinoma. Rectal temperature was within normal range and heart rate and respiration rate were moderately elevated in most of the cases (Table 1). Anorexia, hematuria, history of urolithiasis, constipation, and pasty faeces etc. are the common clinical findings in prostatic affections. Similar clinical signs were recorded by Singh (2006), Paclikova *et al.* (2006), Mahajan (2007) and Kraft *et al.* (2008) in

Table 1: The mean ± SE values of rectal temperature, pulse rate and respiratory rate in cases of prostatic affections.

Affections of prostate	Rectal Temperature (°F)	Respiratory Rate (Breaths/min)	Heart Rate (Beats/min)
USG-FNACBPH (N=3)	102.87±.406	42.00±3.055	98.00±5.774
USG-FNAC Prostatitis (N=3)	103.13±2.659	41.33±8.110	95.33±7.333
USG-FNACProstate carcinoma (N=4)	101.92 ±.522	40.00±3.162	96.00±5.228
USG-FNACNon Diagnostic sample (N=2)	102.80±.200	41.00±5.000	91.00±1.000

Table 2: Hematobiochemical parameters in prostatic affections.

Parameters	Prostatic affections			Normal reference ranges*
	Benign Prostate Hyperplasia (BPH)	Prostatitis	Prostatic Carcinoma	
Hb(X g/dL)Mean ± SE	9.43± 2.88	10.33± 1.29	9.00± 2.70	**12-18**
TLC($X10^3$/µL) Mean ± SE	22.03± 3.96	19.72± 4.48	15.52± 4.02	**6-17**
DLC (%) Mean±SE				
N (%)	78.67± 10.35	92.33± 2.03	87.50 ± 1.71	**60-70**
L (%)	14.00± 5.03	6.67± 1.33	12.50 ± 1.71	**12-30**
M (%)	1.67±0.33	0.33±0.33	-	**03-10**
E (%)	5.67± 5.67	0.67± 0.67	-	**02-10**
SGPT (µ/L)Mean ± SE	103.67± 8.95	131.00± 5.57	147.50± 20.56	**8.2-57**
SGOT (µ/L) Mean ± SE	147.00± 51.21	123.67± 12.73	118.25± 17.85	**8.9-49**
AKP (µ/L) Mean ± SE	229.67± 75.70	256.33± 23.68	296.25± 64.53	**10.6-101**
BUN (mg/dL)Mean ± SE	57.33± 33.41	100.93± 76.56	88.00± 44.87	**8.8-26**
Creatinine (mg/dL)Mean ±SE	1.83± 0.66	8.47± 7.62	4.65± 3.52	**0.5-1.6**
Total Protein (g/dL)Mean ± SE	6.70± 0.10	6.90± .06	6.30± 0.33	**5.5-7.5**
Albumin (g/dL)Mean ± SE	3.53±0.66	3.87± 0.03	3.40± 0.23	**2.6-4.0**

prostatic affections in dog.

In animals diagnosed with BPH (N=3) the blood SGPT, SGOT and AKP values were elevated (Table 2). The total platelet count was adequate. BUN and creatinine levels were normal in all except in one case in which BUN and creatinine level was markedly elevated (124 mg/dL and 3.1 mg/dL respectively). This could be attributed to concurrent renal failure diagnosed in this animal. Total protein and albumin level was within the normal range in all animals. In majority of cases there was mild neutrophilic leucocytosis which might be due to concurrent inflammatory conditions (Paclikova *et al.,* 2006). The blood Hb was normal in two dogs, but was very low in dog with renal failure. The prostate was found bilobed uniformly enlarged on per-rectal examination in all cases of dogs having BPH.

In case of prostatitis the blood SGPT, SGOT and AKP values were moderately elevated in all animals (Table 2). The increase in AKP value might be due to inflammation and degeneration of prostate cells (Paclikova *et al.,* 2006). Total platelet count , BUN, creatinine, total protein and albumin levels were within the normal range in all animals. In majority of cases there was neutrophilic leucocytosis as also reported by Hanson *et al*. (2001) and Paclikova *et al.* (2006). The prostate was found uniformly enlarged with smooth outer surface on digital rectal examination in all cases.

In case of prostatic carcinoma, the blood SGPT, SGOT values were moderately elevated in all animals but AKP value was markedly elevated in all animals (Table 2). The value of AKP was more than the upper limit of the normal range and such increases are usually indicative of the neoplastic conditions in dogs (Bush, 2002). Total platelet count was adequate in all the animals. In majority of cases there was marked neutrophilic leucocytosis. The blood Hb was within normal range in two cases and was low in rest of the two cases. The prostate was uniformly enlarged with smooth outer surface on per-rectal examination. Digital rectal examination of prostate revealed an enlarged, irregularly asymmetrical, usually non-painful, gland. Similar findings were also recorded by Zinkl (1999).

The haemato-biochemical parameters are not diagnostic for dogs suffering from BPH and prostatitis

unless correlated with clinical examination. However, elevation of AKP values may be indicative of prostatic carcinoma when supported with clinical, radiographic and ultrasonographic findings.

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Comparative evaluation of Johnin intradermal test and Gamma interferon assay in bovine paratuberculosis

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Abstract

Thirty seven animals from an endemic area of paratuberculosis were used to find the diagnostic sensitivity and specificity of Johnin intradermal test and gamma interferon assay. The diagnostic sensitivity and specificity of Johnin intradermal test over gamma interferon assay was 38.09% and 93.75%, respectively.

Keywords: Bovine, Diagnosis, Johnin Test, Paratuberculosis

Paratuberculosis in cattle is a chronic and progressive infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It is important to differentiate between conditions such as the infectious, infected and non-infected as infectious animals are an immediate risk for spread of MAP, whereas the infected animal constitutes a future risk of spread. Detection of non-infected animals is of particular importance in certification schemes (Nielsen, 2008). The present paper evaluates two tests in detection of MAP.

37 animals aged over 2 yr in paratuberculosis endemic area (26 crossbred cattle and 11 graded Murrah) were selected for diagnostic evaluation of gamma interferon assay and Johnin single intradermal test.

The single intradermal (SID) test was performed in the cervical area as described by OIE (2008) while for gamma interferon enzyme linked immunosorbent assay, BOVIGAM kit (Biocor animal health Inc., USA) was employed and the results were interpreted as positive and negative if the difference between the antigen stimulated plasma OD and the nil antigen control OD was < 0.1 and > 0.1, respectively.

Only 21 (11 crossbred cattle and 10 graded Murrah) were positive by gamma interference (γ- IFN) and 9 were positive by SID. The diagnostic Sensitivity and Specificity of SID over γ- IFN was 88.88% and 53.57%, respectively. The concordance between these two tests was 62.12. Kappa statistics were used to find the agreement between these two tests revealed value of 29.81 indicating that there was a fair agreement (Table 1).

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Single intradermal Johnin test was most popular and reasonably accurate although not sufficiently enough to be a dependable diagnostic method in individual animals because of the poor sensitivity. The test in the cervical area was more sensitive than the caudal fold which also suffers from its lack of availability if further tests were to be performed (Radostits *et al.,* 2007). Kallis *et al* (2003) found that in paratuberculosis-free herds, there was a low agreement between skin test and γ- IFN assay results *($\hat{e}$ = 0.29)* while in low prevalence herds the level of agreement was higher *($\hat{e}$ = 0.40)*. But in this study, the endemic herd showed a low agreement between these two tests ($\hat{e}$ = 0.29).

The sensitivity and specificity of SID over γ-IFN assay in infected paratuberculosis herd differ from the observation of (Kallis *et al.*, 2003). In our study the sensitivity and specificity was 38.09% and 93.75% respectively, probably due to the Johnin PPD used. Kallis *et al* (2003) indicated that difference in PPD antigens influenced SID and γ-IFN assay specificity and sensitivity.

Culling of CMI test-positives could be a cost-effective means of removing infected animals before they actually start faecal shedding. Positive skin test results with γ- IFN assay have been observed to have a high correlation when employed together. This observation has therefore been the basis of the study where in γ-IFN assay could effectively be combined with skin test and improves skin test specificity in the diagnosis of tuberculosis in cattle and humans (Whipple *et al.*, 2001; Pottumarthy *et al.*, 1999).

The low cost and ease of application of the skin test, and the possibility of confirmation offered by the γ- IFN assay are other arguments in favour of using

Table 1: Diagnostic evaluation of single intradermal test over gamma interferon assay

SID test/γ- IFN test	γ- IFN positive	γ- IFN negative
SID Positive	8	1
SID negative	13	15

(Sensitivity – 38.09%; Specificity – 93.75%; Concordance - 62.12; Kappa statistics – 29.81)

CMI tests for diagnosis and control of paratuberculosis (Kallis *et al.,* 2003). For paratuberculosis diagnosis, the Johnin skin test can be used with greater sensitivity if the animals are interpreted as positive when skin thickness increased >4 mm, is a specific and low-cost test for the early diagnosis of paratuberculosis in a majority of dairy herds.

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Therapeutic management of ketosis in bovine

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Abstract

The present investigation has undertaken to compare the efficacy of different therapeutic regimen in 40 ketotic cows. All the 40 ketotic animals are equally divided into gr T_1, T_2, T_3 and T_4 received 1 liter of 25% glucose iv followed by 500 ml iv once daily for next 2 days. Vit B Complex (B_1, B_6 and B_{12}) was added in gr T_2, T_3 and T_4 @ 10 ml daily with drip. In gr T_3 nandrolone (50 mg im as single dose) and in gr T_4 dexamethasone (5 ml im as single dose) were also given. The mean recovery time (days) was recorded highest in gr T_4 (1.7±0.26) followed by T_3 (2.1±0.23), T_2 (2.6±0.16) and T_1 (3.3±0.26). Frequency of relapse was highest in gr. T_1 (40%) in comparison to gr. T_2 (5%), T_3 (5%) and T_4 (5%). Therefore, hormonal therapy with dexamethasone or nandrolone in association parenteral glucose and B-complex (B_1, B_2 and B_3) gave excellent recovery rate and very less evidence of relapse.

Keywords: : Cow, Ketone bodies, Ketosis, Treatment

High incidence of clinical and subclinical ketosis causes economic loss to the dairy farmers due to loss of milk production as well as sharp drop in the SNF content of milk and failure of affected animals to return to normal production after recovery (Radostits *et al.*, 2000). Present paper reports comparative efficacy of different therapeutic regimen in the management of ketosis in crossbred cows at different stages of lactation.

Total 40 crossbred cows of 3-14 years age brought to Clinical Complex with the complaint of inappetence, pica and sudden fall in milk production (25-75%) were used. They were in different lactation numbers (1-7th), parturated 15-120 days back and suspected to be suffering from ketosis. The milk and urine samples tested were positive for ketone bodies. Blood glucose was estimated before and after therapy by standard method. These cows were randomly divided into four equal groups. Animals of all the groups received 1 liter of dextrose (25%) iv followed by 500 ml iv once daily for next 2 days. Vit B Complex (B_1, B_6 and B_{12}) was added in gr T_2, T_3 and T_4 @ 10 ml daily with drip. In gr T_3 nandrolone (50 mg im as single dose) and in gr T_4 dexamethasone (5 ml im as single dose) were also given. The efficacy of therapeutic regimens were evaluated on the basis of clinical response, time required for complete recovery, per cent recovery and post-treatment blood glucose values.

Urine and milk samples of 20 (50%) animals showed highly positive (+++), 10 (25%) showed moderately positive (++) and remaining 10 (25%) did not show any reaction with Rothera's test. Blood glucose levels were 31.37±1.34, 29.23±3.21, 30.11±1.54 and 28.87±0.85 mg/dl in gr T_1, T_2, T_3 and T_4, respectively and were comparatively low (Radostits *et al.*, 2000). Clinical examination revealed almost normal rectal temperature (101-103 ^{0}F), slightly elevated pulse (60-80/min) and respiratory (30-40/min) rates and reduced ruminal movements (1-3/min). A characteristic sweetish odour was also detected in the breath, milk and urine in most of the cases (Swain and Tripathy, 1987). Besides, 4 (10%) cows were showing wasting form of the disease and another 4 (10%) cows were showed nervous symptoms like head and nose pressing and vigorous licking of their own skin and mangers as well.

The low level of blood glucose in all the four groups could be attributed to the negative energy balance reflecting greater demand of glucose in the mammary gland (Anantwar and Singh, 1993). Hypocalcaemia can exert an additional depressive effect on endogenous glucose production; hence aggravate hypoglycaemia (Mandali *et al.*, 2002; Schlumbohm and Harmeyer, 2003). In ruminants glucose is synthesized from propionic acid and fulfills the requirement of glucose.

Animals of gr T_1 receiving only parenteral glucose recovered 100 per cent within 3.3±0.26 days. However, only three animals (30%) recovered after single therapy, whereas two animals (20%) required second dose and rest five animals (50%) recovered completely after third dose as evident by subsequent disappearance of clinical signs and gradual increase of milk production. Poor response in group T_1 could be due to glucose therapy alone could not maintain consistent blood glucose level and failed to restabilize disturbed body metabolism in ketotic animals (Mir and

Table1. Comparative efficacy of different treatments for the treatment of bovine ketosis

S.No.	Observations	gr T_1	gr T_2	grT_3	gr T_4
1.	Mean blood glucose level before treatment	31.37±1.34	29.23±3.21	30.11±1.54	28.87±0.85
2.	Mean blood glucose level after treatment	42.62±4.31	44.53±2.31	48.53±2.16	50.45±1.28
3.	Per centage recovery (%)	100	100	100	100
4.	Mean recovery time (days)	3.3±0.26	2.6±0.16	2.1±0.23	1.7±0.26
5.	Frequency of relapse (%)	40	5	5	5
6.	Recovery by single therapy (%)	30	60	75	80

Malik, 2003). Higher recovery rate in gr T_2 animals could be due to an additional treatment with B-complex. Animals of group T_3 given additional treatment with anabolic steroid (Nandrolone) showed very good response as compared to gr T_1 and T_2 which might be due to the faster elimination of ketone bodies from the blood 24-48 hr of treatment (Dhoble *et al.,* 2007). Recovery in group T_4 animals was earliest compared to the rest groups (Table 1) could be due to additional glucocorticoid (dexamethasone) therapy which increases blood glucose level within 24 hr by increasing the availability of gluconeogenic amino acids from increased protein mobilization (Odedra *et al.,* 1980). In addition, glucocorticoids accompanied by temporarily depression in milk yield, which may contribute to the recovery rate (Radostits *et al.,* 2000).

From this study, it can be concluded that hormonal therapy with dexamethasone or nandrolone in association with parenteral glucose and B-complex (B_1, B_2 and B_3) give excellent recovery rate and very less evidence of relapse.

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Evaluation of antioxidative potential of aqueous extract of *Mentha piperita* by electron transfer reaction assays

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Abstract

The antioxidant property of aqueous extract of *Mentha piperita* (AEMP) was studied by electron transfer reaction assays. It revealed substantial antioxidative property with respect to 2, 2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, reducing power on Cu (II) and total phenolics. However, chelating activity of AEMP was very poor.

Keywords: Antioxidative property, Extract, *Mentha piperita*

Mentha piperita extract has been used for treating loss of appetite, common cold, bronchitis, sinusitis, fever, nausea, vomiting and indigestion (Aktogan *et al.*, 2004) while its essential oil studied for antioxidative property (Mimica-Dukic *et al.,* 2003). AEMP has been reported to possess free radical scavenging capacity and antioxidant potential due to presence of phenolic acids (Damein *et al.,* 2003). The present study was conducted to investigate the antioxidative property of AEMP by electron transfer reaction methods.

The fresh green leaves of *M. piperita* at flowering stage were obtained from Medicinal Plant Research and Development Center, GBPUAT Pantnagar in month of April, 2007. Aqueous extract was prepared from these leaves as described by Damien *et al.* (2003) and Radoslaw *et al.* (2006). Antioxidative potential of AEMP was analysed by electron transfer reaction methods.

The level of total phenolics in extract was determined (Germano *et al.,* 2005). The scavenging effect of extract on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical (Singh *et al.,* 2005), chelating activity of aqueous extract on ferrous ions (Fe^{2+}) (Junctachote and Berghofer, 2005) and its reducing power.

The concentration of total phenolics in AEMP was 786.5 mg of gallic acid equivalent as calculated from regression equation obtained from the standard curve. This value was well within the range as reported by Damien *et al.* (2003).

DPPH scavenging activity of AEMP was concentration dependent and at 5, 10, 15, 20 and 25 µg/ml of AEMP, scavenging activity was 4.69, 9.48, 10.63, 11.02 and 12.26%, respectively which was slightly lower but comparable to gallic acid scavenging activity of 12.36, 13.22, 16.67, 18.10 and 19.67, respectively. The IC_{50} values of DPPH scavenging activity of AEMP was 113.30 µg/ml which was higher than the IC_{50} values of DPPH scavenging activity of gallic acid (99.544 µg/ml). The IC_{50} values of DPPH scavenging activity of AEMP was slightly lower as compared to the value reported by Demien *et al.* (2003). The lower IC_{50} values indicate a better free radical scavenging property compared to other *Mentha* varieties.

The chelating activity of AEMP at different concentrations of 0.10, 0.25, 0.50, 0.75 and 1.00 mg/ml was 1.460, 1.87, 9.28, 10.48 and 13.14% and these values were extremely low as compared to chelating activity of 0.02 mM EDTA (127.01%). Although AEMP exhibited an ability to chelate iron (II) ions in a dose dependant manner, the AEMP possesses very poor iron (II) chelating activity as compared to EDTA. This indicates that the amount of compound in AEMP to compete with ferrozine for iron (II) ions was less as compared to EDTA.

The reducing power of AEMP at different concentrations of 0.10, 0.25, 0.5, 0.75 and 1mg/ml were 14.50±1.31, 46.84±1.68, 64.85±3.91, 66.79±0.18 and 73.48±0.10%, respectively which was slightly lower but comparable with the reducing power of gallic acid as 27.40±2.38, 62.07±0.12, 76.91±0.19, 82.41±0.16 and

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85.39±0.02%, respectively at similar concentration. The results indicate that AEMP possesses substantially higher reducing power on Cu (II).

It revealed that AEMP has substantial antioxidative potential with respect to total phenolic content, free radical scavenging activity and reducing power.

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Prevalence of cardiac diseases in dogs in Gujarat state

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Abstract

Prevalence study of cardiac diseases in 1,095 dogs revealed that 84 (7.67%) had signs suggestive of cardiac diseases confirmed by electrocardiography. The highest prevalence was of cardiac arrhythmias (53 cases; 63.1%) and it was more in Pomeranian (30 cases; 35.7%). Cardiac diseases were more prevalent in animals of 0-3 yr age group (39 cases; 46.4%) and they had highest prevalence of cardiac arrhythmias (26 cases; 66.6%). Cardiomyopathy (7 cases; 26.9%) and heart worm (4 cases; 15.4%) was found to be more in adult dogs (9 yr and above).

Keywords: Arrhythmias, Cardiac disorders, Cardiomyopathy, Dog.

The cardiac diseases in canines have not been magnified through the research and clinical point of view and very meager information have been documented in India. Hence, the present study was aimed to survey the prevalence of cardiac problem in canines of Gujarat State.

Total 1095 dogs reported at Teaching Veterinary Clinical Service Complex (Zaveri Clinics) of the Veterinary College at Anand and Shri Surat Panjarapole Prerit Nandini Veterinary Hospital at Surat were screened for the prevalence of cardiac disorders and their relationship with breed, age, sex and season was evaluated (Kelly, 1984). Dogs found to have signs compatible with heart disease were subjected to further detailed clinical investigation including history and cardiac examination including auscultation of the heart, blood pressure, electrocardiographic and radiographic techniques.

Overall 84 (7.67%) cases of cardiac diseases were observed in dogs. Highest prevalence was of cardiac arrhythmias (53 cases; 63.1%) followed by cardiomyopathy (14 cases; 16.7%) and heart worm (7 cases; 8.3%). The prevalence of AHF (acute heart failure) and other (low ventricular complexes, ST segment depression, ST segment slurring) abnormalities was 3.6% (3 cases) and 8.3% (7 cases), respectively. Changkija (2007) noticed 56.67% cases of arrhythmias and conduction disturbances in dogs.

Fioretti and Delli (1988) reported similar findings in Italy where prevalence rate of heart diseases was 11% out of 7,148 dogs studied. This increase in the recognition of more cardiac diseases might be due to the advancement in the field of veterinary care since the 1950s and also changing attitude of dog owners towards the health of their pets. Although conduction disturbances may occur with primary heart disease or diseases primarily affecting the vagal activity, their frequent occurrence in the absence of detectable heart disease suggests vagal activity as an important factor in majority of cases.

The cardiomyopathy was present in 16.7% cases. Kibar and Alkan (2005) in a study of 30 geriatric dogs with suspected heart diseases evaluated clinically, radiographically and ultrasonographically the cardiac dilatation and hypertrophy of two or more chambers.

The over all prevalence of heart worm was 8.3%. Electrocardiographic changes such as atrial fibrillation (AF), T wave, low voltage complexes and sinus arrhythmias either alone or in combinations suggestive of cardiomyopathy have recently been reported in dogs with clinical dirofilariasis (Varshney *et al.,* 2008).

The cardiac problems were more (35.7%) in Pomeranian breed as compared to other breeds agreeing with the observations of Changkija (2007). The prevalence of specific cardiac diseases observed in Pomeranian breed were cardiac arrhythmias (56.6%), cardiomyopathy (16.7%) and heart worm (13.3%). The over all prevalence of cardiac problems was observed to be lower in Cocker Spaniel, Boxer, Japanese Chihuahua and Bull Mastiff (1.2% each).

The exact cause of these findings is not explainable, since in any geographical area, the breed prevalence may be affected by the preference of specific breeds by owners of that area or could be due to close association of this breed with humans may remained under stress in urban areas.

Cardiac abnormalities were higher (46.4%) in dogs up to 3 yr age followed by aging dogs of more than 9 yr old (31.1%). In young ones cardiac arrhythmias

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Table 1: Overall prevalence of different cardiac diseases in dogs

Disease	Cardiac arrhythmias (n= 53)				C	Cardio myopathy	Heart Worm	Others	Acute Heart Failure	Total
	A	B								
		B1	B2	B3						
Frequency	14	26	2	3	8	14	7	7	3	84
% of total	63.1					16.7	8.3	8.3	3.6	100
Prevalence% (CA)	26.4	49	3.8	5.7	15.1					100

A: Sinus Rhythm, B: Abnormalities of Impulse Formation, B1: Supraventricular, B2: Atrioventricular (AV) Junction, B3: Ventricular, C: Abnormalities of Impulse Conduction, CA: Cardiac Arrhythmias

were highest (66.6%). Herrtage (2003) stated that heart diseases were relatively common in dogs. The incidence of acquired heart diseases in dogs and cats increases with age. Miller *et al.* (1989) opined that approximately 25% of heart disease in dogs occur between the age of 9 and 12 yr, and 33% in dogs above 13 yr and older.

The prevalence of heart worm in the present study was found to be highest (15.4%) in adult and aging dogs (above 9 yr age group) agreeing with the observations of Varshney *et al.* (2008).

Of 84 dogs with cardiac abnormalities, 58 (69.0%) were male and 26 (31.0%) female. The prevalence of cardiac arrhythmias (38 cases; 65.4%) was more in males followed by cardiomyopathy (9 cases; 15.5%), heart worm (5 cases; 8.6%), other abnormalities (4 cases; 3.4%) and AHF (2 cases; 3.4%). Females had comparatively lower prevalence of cardiac arrhythmias (15 cases; 57.7%), cardiomyopathy (5 cases; 19.2%), other abnormalities (3 cases; 11.5%), heart worm (2 cases; 7.7%) and AHF (1 case; 3.8%). It was concluded that male dogs appear to be over-represented in Dalmatian DCM, which may suggest an X-linked trait although large studies have not been performed. Calvert *et al.* (1997) stated in their retrospective studies performed on populations of dogs with heart failure or sudden death attributed to DCM, males were affected nearly twice as often as females.

It appears that heart diseases are significant disease entity as a cause of morbidity in dogs and these should be taken into account during routine examination of a canine patient.

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Comparative efficacy of some anthelmintics in gastrointestinal nematodiasis in cows

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Abstract

Therapeutic efficacy trial of four different anthelimintics in clinical gastro-intestinal nematodiasis in lactating cows was carried out on the basis of faecal sample examination and per cent reduction in EPG at weekly intervals. Ivermectin and oxfendazole bolus were found 100% effective while N*igella sativa* and *Caesalpinia crista* showed 76.2 and 77.4% efficacy at the end of 28 days trial. Recovery was faster with ivermectin, compared to oxfendazole.

Keywords: Cows, Gastro-intestinal nematodiasis, Therapy.

High efficacy of a variety of chemical anthelminitic agents against the gastro intestinal nematodes in cows under varying geo-climatic and managerial conditions has been reported (Vatsya *et al.,* and Lombordo *et al.,* 1986). However, in view of the predominantly rural-based agrarian economy in India, increasing use of cheaper, yet potent, locally available anthelmintics is needed. The seeds and seed extracts of *Nigella sativa, Linn.* Kalajira and *Caesalpinia crista, Linn.* (Gataran) merit attention because of their several therapeutic properties including anthelmintic action (Anjaria, 2002).

This paper reports the comparative efficacy of various anthelmintics against naturally acquired infection of gastro-intestinal nematodiasis in cows on the basis of reduction.

Total 24 indigenous lactating cows in Dayodaya Trust Goushala, Tilwara village in Jabalpur district with established worm load of gastro-intestinal nematodes: Stronygles or mixed infection of Strongyles, *Strongyloides sp.* and *Eimeria sp.* and to a lesser extent *Trichuris* and *Moniezia sp.* were distributed randomly into 4 qual groups of 6 animals. Group T1 received ivermectin bolus* @ 200 mg/kg b wt single oral dose, Gr T2 *N. sativa*** seed powder @ 50 mg/kg b wt three consecutive daily oral doses-Gr T3 oxfendazole bolus @ 5 mg/kg* single oral dose, and Gr T4 *C. crista* seed powder*** @ 50 mg/kg b wt three consecutive days oral doses. Six healthy indigenous lactating cows with zero EPG count in three consecutive faecal samples served as control.

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The magnitude of infection was evaluated on the basis of sequential EPG counts (Soulsby, 1982), and the therapeutic efficacy of drugs were determined by the per cent reduction at different post-treatment intervals and pretreatment EPG count. Thus, anthelmintic efficacy (%) = a - b/ a x 100, where a , EPG count on day 0 (pre-treatment) and b = EPG count at a given interval (Mohapatra *et al.* 1990). The data were statistically analyzed with the completely randomized design (Snedecor and Cochran, 1994).

In gr T1, the minimum duration was required for total elimination of the gastro-intestinal nematode worm load, evidenced by zero EPG count attesting to 100% therapeutic efficacy on day 21. Similar observations were made by Saeki *et al.*(l995) and Vatysa *et al.* (2008). All animals in gr T2 responded to the anthelmintic principle(s) contained in *N. sativa* seed powder. However, the drug was effective only to the extent of 58.7% and 76.2% on day 21 and 28, respectively (Table 1). In gr T3, all animals responded well to oxfendazole, and the drug was effective to the extent of 97.4% and 100% on day 21 and 28, respectively. This finding is in agreement with the reports of Chahners (1985) and Lombordo *et al (*1986). In gr T4, all animals responded to the anthelmintic ingredients present in *C. crista* crude seed powder, but the drug was effective only to the extent of 67.9% and 77.4% on day 21 and 28, respectively (Table 1).

The seeds and seed extracts *of N. sativa and C. crista* have been used for anthelmintic and other properties in the indigenous system of medicine (Anjaria, 2002). Presumably, in the present study, the active principles present in these plant products have not been absorbed in adequate quantities required to maintain

Table 1: Faecal EPG count in cows receiving different anthelmintic treatment.

Treatment Group	Interval (day)				
	Pre-treatment	Post-treatment			
	0	7	14	21	28
T_1, Ivermectin	1466.7±66.3	116.7±19.1 (87.3%)	16.7±2.7 (98.2%)	0 (100%)	0 (100%)
T_2, *Nigella sativa*	1050.0±107.3	550.0±62.9 (47.6%)	500.0±55.3 (52.4%)	433.3±36.7 (58.7%)	250.0±25.6 (76.2)
T_3, Oxfendazole	1266.7±79.2	283.3±22.6	150.0±13.4	33.3±4.9	0
T_4, Caesalpimacrista	1400.0±90.7	816.7±79.1 (41.7%)	566.7±58.4 (59.5%)	450.0±45.9 (67.9%)	316.7±30.7 (77.4%)
T_5, Healthy Control	0	0	0	0	0

Figures in parentheses represent the therapeutic efficacy, evidenced by per cent reduction in the faecal EPG count

the optimal blood titres and cleanse the inactivated adult parasitic nematodes from the GIT and bring down the EPG count to the minimum.

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Clinical Article

Cryptosporidiosis in cattle of Bangalore district

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Abstract

A study was undertaken to screen calves and adult cattle from different dairy farms, animal shelters, abbatoirs and Veterinary hospitals in and around Bangalore for *Cryptosporidium* oocysts. The faecal samples collected from the dairy farms had a complaint of diarrhoea in neonatal calves, loss of weight and decreased body weight gain. Two species of *Cryptosporidium* were found to be prevalent *viz.*, *Cryptosporidium parvum* and *Cryptosporidium andersoni*. The prevalence of Cryptosporidiosis was higher in non diarrhoeic animals when compared to diarrhoeic animals and *Cryptosporidium andersoni* infection was more common than *Cryptosporidium parvum*. Out of three seventy five faecal samples ,forty eight samples were positive . Sheather's sucrose floatation technique detected more number of positive cases than the modified Ziehl-Neelsen staining technique.

Keywords: Cryptosporidiosis, *Cryptosporidium andersoni*, neonatal diarrhoea, *Cryptosporidium parvum.*

Cryptosporidiosis is one of the diseases whose causative agent is difficult to identify. It is an emerging zoonotic disease of global importance which needs more attention, since most of the time it goes undiagnosed. *Cryptosporidium* inhabits the microvilli of the epithelial surface of the gastrointestinal and respiratory tracts of a wide variety of vertebrates causing significant morbidity and mortality, which now represents the third major cause of diarrhoeal disease worldwide (Spano and Crisant,.2000).

It causes self-limiting watery diarrhoea in immunocompetent individuals but has far more devastating effects on immunocompromised individuals and in some cases can be life threatening due to dehydration caused by chronic diarrhoea (Caccio, 2005 and Chen et al., 2005).

Three hundred and seventy five faecal samples from the calves aged 0-2months, 2-6 months, 6 months-1 year and adults were collected from 12 different farms, animal shelters, abbatoirs and Veterinary hospitals in and around Bangalore and screened for the presence of *Cryptosporidium* oocysts. Sheather's sucrose floatation and modified Ziehl-Neelsen staining technique were employed for the detection of *Cryptosporidium* oocysts. Identification of oocysts was done by morphological characterization and micrometry as per Upton and Current (1985). The calves which were found positive for Cryptosporidiosis were classified into two categories *viz*., calves with diarrhoea (diarrhoeic calves) and calves with normal faecal consistency (non diarrhoeic calves).

The calves with diarrhoea exhibited symptoms like profuse watery diarrhoea mixed with little amount of blood, mucus and undigested milk clots, abdominal discomfort and anorexia. The perineal region, hind quarters and tail were soiled with dung. Mild to moderate dehydration was seen in calves. Appearance of mucus, blood streaks and undigested milk clots in the faecal samples of some calves in the present study was attributed to gastroenteritis and malabsorption. These observations were in agreement with Howard and Smith (1999) and Shobamani et al. (2006). Most of the calves were found to be concurrently infected with other parasites also such as *Eimeria*, *Strongyle* and *Monezia*.

The calves with normal faecal consistency had reduced weight gain, rough hair coat but the calves had normal appetite which was also reported by Shobamani et al. (2006). In the present study infection was higher in non diarrhoeic than in diarrhoeic calves which may be due to increased incidence of *Cryptosporidium andersoni* infection (Table 1). This is associated with reduced weight gain and decreased milk production. Enemark *et al*. (2002), Bukhari and Smith (1996) and Olson *et al.* (1997) had made similar observations.

Among the two techniques Sheather's sucrose floatation was found to be more specific and sensitive for the diagnosis of cryptosporidiosis. Out of 375 samples, 48 samples were positive for *Cryptosporidium* oocysts by Sheather's sucrose floatation method and 39 samples were positive by modified Ziehl-Neelsen staining technique. Sheather's sucrose floatation method gave significantly higher per centage of recovery even when the concentration of oocysts was low and no morphological alterations in the oocysts occurred during

Table 1: Prevalence of Cryptosporidiosis in diarrhoeic and non- diarrhoeic calves

Age group	No. of animals screened	No. +ve	Non-diarrhoeic			Diarrhoeic		
			No. Tested	No. +ve	% positive	No. tested	No. +ve	% negative
0-2 months	119	10	98	9	9.2	21	1	4.8
2-6 months	146	24	92	18	19.6	54	8	14.8
6months-1year	90	14	48	8	16.7	42	6	14.3
Adults	20	0	0	0	0	20	0	0
TOTAL	375	48	238	33	13.9	137	15	10.9

the floatation procedure. The sensitivity and specificity of Sheather's sucrose floatation was 81.25% and 96.42%, where as modified Ziehl-Neelsen staining technique had a sensitivity and specificity of 68.75% and 94.74% respectively.

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Intrathecal administration of tetanus anti toxin for treatment of tetanus in horse

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Abstract

A case of tetanus in a horse was treated with large doses of long acting penicillins along with tetanus anti toxins, tetanus toxoid, muscle relaxants, metronidazole, parenteral dextrose saline, antioxidants, nervine tonics and restoratives.

Keywords: Tetanus, intrathecal, tetanus antitoxin, horse, treatment

Tetanus is highly fatal, infectious disease of domestic animals characterized by hyperesthesis, tetany and convulsions. Although all species of domestic animals are susceptible to tetanus, horses are most sensitive to tetanus toxin (Radostits *et al*., 2000). The present work reports successful management of a case of tetanus in horse.

An eight year old non descript male horse was presented at the hospital with history of injury on fore legs around 15 days back and treated with some indigenous medicines. Clinical examination revealed normal rectal temperature (101.4° F), generalized muscular stiffness, closed mouth and raised tail. The detailed copro-haematological examinations were uncommital.

The animal was treated with long acting penicillins (Penidure LA® @ 48 lac IU deep im every 72 hrs), tetanus anti toxin @ 30,000 IU iv, tetanus toxoid (adsorbed) 10 ml im, magnesium sulphate (10%) 400 ml slow iv, dextrose saline (5%) 3 litres iv, metronidazole 2gm iv, nervine tonics, vitamin E and amino acids like choline chloride and dressing on the first day. The owner was advised to remove horse shoes.

Next day tetanus anti toxin @ 45,000 IU was given intrathecally after removing equal quantity of CSF followed by IV after 24 hrs. Acepromazine (Acepril® @ 2 ml im) was also given once daily for a week in addition to above treatment. The treatment continued for a period of 20 days. The animal was placed in an isolated dark room. With the above line of treatment recovery was uneventful in 20 days.

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An outbreak of ovine Chlamydial Keratoconjunctivitis and its treatment in sheep

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Abstract

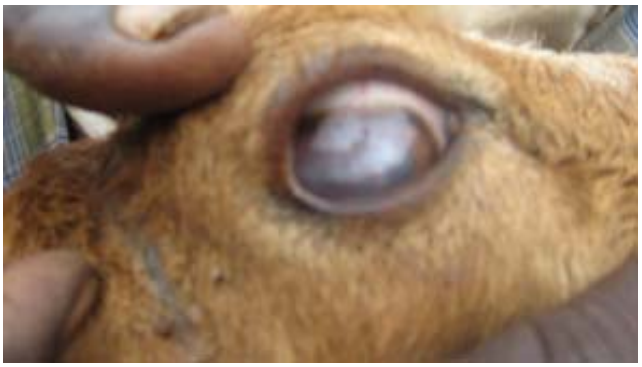
Outbreak of ovine Chlamydial keratoconjunctivites and its treatment in 428 local sheep of Kadapa district in Andhra Pradesh reported. Clinically the disease was characterized by conjuntival hyperemia, serous lacrimation, blephrospasm and cloudiness of cornea. Therapy revealed significant success.

Keywords: Ovine, Chlamydial, Keratoconjunctivitis.

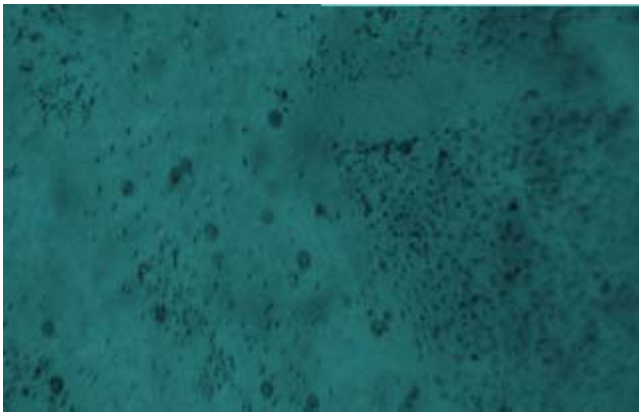
Ovine Chlamydial Keratoconjuntivities is a highly contagious infection of sheep that affects the eye and surrounding structures. It may cause temporary or in severe cases permanent blindness in affected sheep. The present paper reports an outbreak of ovine Chlamydial keratoconjunctivitis and its treatment in sheep of Kadapa district in Andhra Pradesh.

An outbreak of ovine chalmydial keratoconjunctivities was reported in 428 local sheep maintained in farm sector of different villages in Kadapa district of Andhra Pradesh. Clinically the disease was characterized by conjuntival hyperemia, serous lacrimation, blepharospasm and cloudiness of cornea. There was increased body temperature and animals often goes off feed due to the ocular pain associated with infection. Only adult sheep were affected. Although the morbidity was high and the mortality was nil. The local veterinarian began treatment with intramuscular long acting oxytetracycline, Vitamin A injections and topical preparations containing penicillin with poor clinical success rate. Culture was not attempted as previous treatment might have affected the isolation of the organism. But conjconctival swabs were collected from the affected sheep and stained with modified Ziel Nelson (ZN) method. The clinical cases were treated with intramuscular injection of tylosin (Tysin-vet, VET INDIA pharmacueticals, Hyderabad) 10mg/kg b.wt. The treatment was carried for 5 days.

Chlamydia species was identified in conjunctival swabs collected from the 428 sheep. On the basis of clinical signs and conjunctival swab staining results, clinical cases were diagnosed as ovine chlamydial keratoconjuntivitis. Clinical examination on 5th day revealed decrease in severity of clinical signs in both eyes. Subsequently over period of 7 days complete disappearance of corneal cloudiness with response to menace reaction was obvious in both eyes in all animals. Clinical signs observed in the present infection are in agreement with the findings of Andrews *etal*. (1987).



Ovine chlamydial keratoconjuntivitis



Modified ZN method Result: Chlamydia Species

Therapy of ovine Chalamydial keratoconjunctivitis as reported by Baysal *et al*. (1995) was adopted in this case under significant success.

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Mortality pattern in pigs based on postmortem examination

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Abstract

Based on postmortem (PM) findings in UAS piggery for the past eleven years (1983-1993) it was observed that 45.6 per cent of death were due to infectious cause viz., enteritis and pneumonia. From 1983-1993 there was a gradual increase in per centage of mortality over the years. Present study revealed unweaned piglets were more susceptible than weaned piglets for various causes of mortality. Mortality due to enteritis was higher in unweaned piglets and anaemia, physical trauma was higher in weaned piglets. Observation on sex predisposition on occurrence of various pathological entities indicated that males were more prone than females. Higher incidence of mortality was observed in S3 (June to September) season which could be attributed heavy rains, also higher rate of mortality was observed in S1 (January to February) and S3 seasons due to physical trauma which may be due to cold weather which makes animal less active and predisposing the young ones for trampling which leads to physical trauma.

Keywords: Enteritis, Post mortem, Pneumonia, Physical trauma, Weaned and Unweaned.

Among the livestock production activities for economic consideration, piggery has been receiving an increased attention. A variety of infectious and non-infectious factors are attributed to mortalities in pigs. A systematic approach of combating the problems faced in piggery units based on priorities assessed is of great importance to suit the local needs. The present paper reports mortality pattern in pigs based on postmortem data in piggery unit of college.

Autopsy records for the period of eleven years of piggery unit maintained by veterinary college, Bangalore were collected with respect to age, groups, sex, weaned, unweaned status and seasons. (S1 Jan-Feb, S2 March -May, S3 June-Sept, S4 Oct- Dec).

During the period of eleven years, total of 1363 mortalities were recorded for both the sexes and different age group of animals. Of these autopsy records were available for 996 piglets. Based on the postmortem diagnosis,the pattern of mortality was categorized into sex, age groups and seasons.

The cause of death was enteritis, pneumonia, inanition, anaemia, and physical trauma. Among total mortalities, enteritis was predominant cause of death in 328. Followed by pneumonia in 294, inanition in 183, anaemia in 78 and physical trauma in 113 animals.

Age wise distribution of the mortality pattern during the period revealed 85% mortality in unweaned and 14.93% in weaned piglets due to enteritis. The respective per centage due to pneumonia was 62.9 in unweaned and 37.0% weaned piglets. Among non infectious causes, inanition was attributed in 73.2% in unweaned and 26.8% in weaned piglets.

Anaemia was attributed as cause of death in 78.2% of unweaned and 21.8% of weaned piglets, while physical trauma was attributed in 76.1% of unweaned and 23.9% weaned piglets.

Season wise 289 mortalities (21.20%) in S1 season, 319 (23.40%) in S2 season, 465 (34.00%) in S3 season and 295 (21.56%) in S4 seasons were recorded.

289 mortalities in S1 season, 41 (20.39%) were due to enteritis, 66 (32.83%) due to pneumonia, 39 (19.40%) due to inanition, 20 (9.95%) due to anaemia and 35 (17.41%) due to physical trauma. Among 229 mortalities in S2 season, 95 (41.48%) were due to enteritis, 61 (26.63%) due to pneumonia, 34 (14.84%) due to inanition, 20 (8.73%) due to anaemia and 19 (8.29%) due to physical trauma. In S3 season, 116 (24.94%) death due to enteritis, 96 (20.64%) due to pneumonia, 61 (13.11%) due to inanition, 26 (5.59%) due to anaemia and 36 (7.74%) due to physical trauma were recorded. While in S4 season , 76 (25.76%) death were due to enteritis, 71 (24.06%) due to pneumonia, 49 (16.61%) due to inanition, 12 (5.06%) due to anaemia and 23 (7.79%) due to physical trauma.

The postmortem findings in 996 pigs indicated infectious causes viz., enteritis and pneumonia as cause of death in 622 (45.6%) piglets and 374 (27.43%) piglets revealed noninfectious causes viz., anaemia, physical trauma and inanition as primary cause of mortality in

piglets, which simulating the findings of Pillai and Thomas (1984) and Hellmers (1986).

The seasonal influence on mortality pattern in the present study indicated that higher mortalities were due to enteritis, pneumonia, inanition and anaemia during June to September months. This may be attributed to heavy rains with higher atmospheric relative humidity leading to a stress full situation. However, the highest mortality due to physical trauma were in January-February and October to December months. Cold weather has been considered to result in low activity of piglets which are more prone for physical trauma (Cabrera *et al.,* 1990).

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Prevalence of peste des petits ruminants (PPR) in small ruminants of Karnataka

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Abstract

Total 158 outbreaks, 2205 attacks and 407 deaths were recorded due to PPR in Karnataka and therefore 580 representative samples, comprising of nasal swabs, rectal swabs and tissue samples were forwarded for confirmation. By sandwich-ELISA kit 45 (7.76%) samples were positive. The disease caused 0.018% morbidity and 18.4% mortality.

Keywords: PPR, Prevalence, Sandwich ELISA.

Economic losses due to PPR alone in India have been estimated to be 1800 million Indian rupees annually (Bandyopadhyay, 2002). The sandwich-ELISA diagnostic kit has been found to be extremely useful for detection of PPRV antigen in clinical samples (Singh, 2002). This study was undertaken with an intention to detect PPRV in clinical samples using this kit and to derive estimates of overall prevalence of PPRV.

Total 158 outbreaks, 2205 attacks and 407 deaths due to PPR were recorded during 2006-08 from different districts of Karnataka. Total 580 samples were screened using PPR sandwich-ELISA kit for PPRV antigen detection as per the user manual provided by the Rinderpest laboratory, Division of Virology, IVRI, Mukteswar. The plates were read at 492 nm and the cut off value was calculated as given in the manual. Samples showing OD more than the cut off in both the duplicate wells were considered as positive, whereas, samples showing OD less than the cut off in both the duplicate wells were considered as negative. Further, a sample positive in one well but negative in the other duplicate well was considered as doubtful and retested.

Of 580 clinical samples tested by PPR sandwich-ELISA kit, 45 (7.76%) samples gave positive reaction.

The overall morbidity and mortality rates due to PPR during the period under report were 0.018% and 18.4%, respectively. Extremely low incidence rate of PPRV (1.11% in sheep and 2.07% in goats) was observed by Kumar *et al.* (2002) in India. Rao *et al.* (2001) recorded 16% morbidity during PPR outbreaks. Contrary to this, higher incidence of PPR with 60% morbidity rate was observed by Pawaiya *et al.* (2004) in Rajasthan.

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Detection of canine Parvovirus infection by HA and HI tests

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Abstract

Among 128 dogs with symptoms of vomiting, bloody diarrhoea, rise of temperature and dehydration, 58 suffered from parvovirus infection as diagnosed by clinical signs and confirmed by HA and HI tests. The HA and HI tests were found simple and rapid tests for detection of canine parvovirus infection through faecal samples.

Keywords: Canine parvovirus, HA, HI

The canine parvovirus (CPV) was first recognized in 1978 as the cause of two previously unknown disease syndromes of dogs, i.e. myocarditis and gastroenteritis (Appel *et al.,* 1978). Rapid and early diagnosis of the disease is essential (Pirjo *et al.,* 1986) due to its highly contagious nature. Faeces of dogs affected with parvovirus infection usually contain large number of virus particles during early stage of infection. Thus, identification of CPV in faeces is of high diagnostic value.

Total 128 faecal samples were collected in Hanks balanced salt solution, from dogs brought to Teaching Veterinary Clinical Service complex, Jabalpur Veterinary College with clinical signs of vomiting and diarrhoea. The samples were centrifuged at 10,000 rpm for 10 mins at 4^0C and the supernatant was used for detection of viral antigen.

Haemagglutination (HA) test was carried out as per the method of Carmichael *et al.* (1980) in 96 U bottomed microtitre plate. A 2- fold serial dilution of the test sample starting from 1:2 was made in phosphate buffered solution (PBS). Then 25 µl of 1% pig erythrocytes were added to all the wells. The plate was then incubated at 4^0C for 1 hr. The HA titre was expressed as the reciprocal of highest dilution of virus showing agglutination.

The hyper-immune serum (raised in rabbits) was inactivated at 56^0C for 30 mins in a water bath. A 1:10 dilution of serum in PBS was treated with 0.1 parts of 50% pig erythrocytes to remove non specific haemagglutinin. Serum was kept for 2 hr at room temperature and then centrifuged at 1500 rpm. Then 50 µl of PBS was dispensed into each well numbered from 1 to 12 and 50 µl of 1/10 diluted serum was taken into first well with the help of micropipette and mixed well. Then 50 µl mixture was taken from first well and dispensed into second well. This process was repeated till the second last well and discarded 50 µl from the last well and 50 µl of 4 HA dilution of antigen was added to each of the wells except the last two control wells. 50 µl of the 1% RBC suspension was added into all wells. The plate was shaked and incubated at 37^0C temperature for 45 min. The HI titre was expressed as the reciprocal of highest dilution of serum inhibiting agglutination.

Out of 128 faecal samples from clinical cases of dogs exhibiting haemorrhagic or non haemorrhagic gastroenteritis and tested by HA- HI test, 58 samples were reactive. The HA titre ranged between 1:64 to 1:512 whereas, only eight samples showed the titre as 1:1024. However, HI titre ranged from 1:320 to 1:1280. The HA-HI test was found highly sensitive when performed on clinical samples and it was cost effective (Rai *et al.,* 1994). Thus HA and HI tests are simple and rapid tests for detection of canine parvovirus infection in faeces.

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Efficacy of ayurvedic liniment against ticks of sheep and goats

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Abstract

The efficacy of 0.1, 0.2 and 0.5% aqueous emulsion of an ayurvedic liniment against ticks of sheep and goats was evaluated. At 0.1%, it gave 44.00% efficacy in sheep and 54.15% in goats. In sheep, 0.2 and 0.5% concentrations showed 73.31and 65.15% efficacy while in goats 66.83 and 72.47%, respectively on day 21. It was found fairly effective in controlling ticks in these animals.

Keywords: Goats, Sheep, Ticks, Treatment

Though chemical acaricides are effective in controlling ticks in livestock, many of them result in emergence of resistance, environmental pollution and health hazard to man due to passing of residues in milk and meat (Vatsya, *et al.,* 2006). Therefore, plant products to control ticks of livestock have been evaluated (Muraleedharan and Sahadev, 2007; Muraleedharan *et al.*, 2008). An ayurvedic liniment was evaluated against natural infestation of ticks of sheep and goats in the present study.

Tick infested sheep and goats were selected at random for the trials from the flock maintained in the farm. Ticks attached to the pinna of both ears were counted in all the animals before treatment as well as on day 7, 14, and 21 post-treatment. In the preliminary trial, 0.1% aqueous emulsion of ayurvedic liniment applied by cotton swab on ticks on the ears of 10 sheep and 10 goats only once and 5 sheep and 5 goats having tick infestations were kept as respective control. In trial II, 10 sheep and 10 goats, divided equally into two groups and each was treated with 0.2 and 0.5% liniment respectively. The per centage of efficacy was determined by using the formula: $[1-(T_2/T_1) \times (C_1/C_2)] \times 100$ where T_1 is pre-treatment and T_2 is post-treatment means of tick counts, while C_1 and C_2 are the corresponding values for the control group. The ticks collected from the ears were identified to generic level.

The ticks belonged to *Hyalomma* and *Haemaphysalis* species. The liniment showed reduction of ticks from the very next day of its application on the ear pinnae. At 0.1% strength, it was 44.00% effective on day 21 in sheep and 54.15% on day 5 in goats. In sheep, 0.2 and 0.5% concentration revealed 73.31 and 65.15% efficacy on day 21 while in goats 66.83 and 72.47% efficacy, respectively, were seen (Table1). The biological phenomena of dropping off and attachment of the stages of the life cycle of ticks in animals, besides allowing them to graze in natural and tick-infested surroundings would have some influence on the results. The plant extracts/oils available in liniment are used mainly for rheumatic pain in Ayurvedic Medicine. Some of its constituents like deodar oil, turpentine oil and eucalyptus oil have known to have ectoparasiticide action too. It was expected that these ectoparasiticide ingredients along with other constituents of the drug would have some combined beneficial effect in controlling the ectoparasites. That is also the reason to select this liniment for the present trials against ectoparasiticide. The efficacy of a single plant extract can be enhanced by judicious combination with others having similar action or their active principles which have adjuvant properties or toxic activity against ticks (Maske *et al.*, 2000). Many plants have tickicidal or repellent action either alone or in combination and the extracts of some of them is being used in proprietary formulations for control of ticks (Chhabra and Saxena, 1998). Further studies are warranted to find out the individual efficacy of such constituents of this liniment at various strengths, if they are not clearly known. The studies suggest that the ayurvedic liniment has fair effectiveness in controlling ticks on the sheep and goats.

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*Marketed by M/s Retort Pharmaceuticals Private Limited, Chennai-600 060 and each 25 ml contains Mahanarayana thailam 10 ml, Vatanashak thailam 2.5 ml, Deodar oil 2.5 ml, Nilagiri thailam 2.5 ml, Gandhapura thailam 2.5 ml, Tarpain thailam 2.5 ml, Mash thailam 2.5 ml and Kapoor 2.5 g.

Table 1. Efficacy of different strengths of ayurvedic liniment in sheep and goats

Trial No.	Animals	Treatment	n*	Dilution (%)	Day/ mean tick count				% efficacy
					0	7	14	21	
I	Sheep	Experimental	10	0.1	26.2	16.2	18.9	24.3	44.00
		Control	5	-	25.0	31.8	26.4	41.4	-
	Goats	Experimental	10	0.1	21.1	25.4	36.00	35.0	**54.15
		Control	5	-	14.2	16.6	15.2	23.4	-
II	Sheep	Experimental	5	0.2	47.2	31.6	19.2	21.0	73.31
			5	0.5	48.2	31.2	27.0	28.0	65.15
		Control	5	-	45.0	53.0	60.0	75.0	-
	Goats	Experimental	5	0.2	51.8	38.4	40.0	35.8	66.83
			5	0.5	53.0	22.8	28.6	30.4	72.47
		Control	5	-	60.0	82.0	90.0	125.0	-

** number of animals used ;**as on day 5 (mean tick count 13.9)*

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Phorate Toxicity in Cattle - A Case Report

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Abstract

Five cattle were presented with complaint of severe shivering, muscular tremors, staggering gait and respiratory dyspnoea. Animals were accidently got intoxicated with phorate, an organophosphate pesticide. Atropinization therapy was immediately started with atropine sulphate. Supportive therapy of corticosteroid, chlorpheniramine, dextrose saline, B-complex, and liver tonics were given. Clinical improvement was noticed within 30-36 hours suggesting that organophosphate toxicity in cattle can be managed successfully by atropinization therapy without the use of oxime reactivators.

Keywords: Phorate, Organophosphate toxicity, Cattle, Atropinization.

Phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) is a highly toxic organophosphate (OP) pesticide commonly used in agricultural practices to control insects, leaf hoppers, leafminers, and rootworms (WHO, 1988). OP compounds produce their toxic effects by binding with cholinesterase (ChE) enzymes, thereby inhibiting the catabolism of the acetylcholine. Toxicity is caused by overstimulation as a result of accumulation of acetylcholine at nerve synapses and at neuromuscular junctions (Plumlee, 2004). The major breakdown products (oxygen analogue) of phorate in mammals are more toxic and have greater anticholinesterase activity than phorate (Hayes, 1982). Atropine and Oximes such as pralidoxime are recommended antidotes for OP toxicity. Atropine blocks the effects of the excess acetylcholine at the neuromuscular junction. Oximes dissociate the OP-ChE bond and attach to OP compound, which allows for reactivation of ChE enzymes (Lorgue *et al.*, 1996).

In month of December, five non-descript cattle (two ox, two heifer and one cow) were presented to Govt. Veterinary Hospital, Sonbhadra, U.P., with signs of severe shivering, muscle twitching, tremors, incordination in gait, respiratory dyspnoea, excessive salivation, mild tympany and diarrhoea. Upon clinical examination heart rate and respiration rate were elevated but within the normal range and temperature was ranging between 100.0^0F to 102.5^0F. Tremors were observed first in the forelimbs and shoulders and then progressed over the whole body. The animals could walk for only a short distance, then fall and had considerable difficulty in rising. Condition in all the cases was progressive and finally all animals went into lateral recumbency. History revealed that the animals had accidently grazed potato leaves in field spread with phorate as pesticide and two other cows had died before owner could reach for treatment. All animals were in normal health before they grazed the phorate treated potato leaves. For diagnosis, history and clinical signs suggested that cattle got intoxicated with phorate.

Atropinization therapy was instituted immediately with atropine sulphate @ 0.25 mg/kg b wt. To shorten the response time, its 1/3rd dose was given slow iv along with 5% dextrose normal saline fluid and rest of 2/3rd dose was given sc at shoulder region. Treatment was repeated initially at 4 hrs to control the relapses and then at 6 hrs till the atropinization (dry mouth) was reached on the next day. Supportive therapy consisting of dexamethasone (0.05 mg/kg, im), chlorpheniramine injection (0.5 mg/kg, im), B-complexes and herbal liver tonics were also given. Atropine was given to control muscarinic effects and chlorpheniramine, an antihistamine, also had its own antimuscarinic effect. No oxime reactivators were used due to local non-availability of the drug. Moreover, they are ineffective once ageing occurs, and use of oximes is also controversial in the management of organophosphate pesticide poisioning (Bairy *et al.*, 2007). Clinical improvement was well appreciated in all animals after 30 hrs with gradual disappearance of nervous signs. Liver tonic (Liv-52 Vet, 15 ml bid, PO) was recommended for ten days. The present study suggested that atropinization therapy with the judicious use of fluid and other supportive therapy can manage organophosphate toxicity in cattle without the use of oxime reactivators.

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Meter	m	Centimeter	cm	Microlitre	ì l
Counts per minute	cpm	Millligram	mg	Cubic centimeter	cm^3
Millilitre	ml	Degree centigrade	^0C	Minute(s)	min
Degree Fahrenheit	^0F	Once a day	od	Decilitre	dl
Parts per million	ppm	Gram	g	Percent	%
Hour(s)	hr	Picogram	pg	Inch	in
Revolution per min	rpm	Intramuscular	im	Second(s)	sec
Intraperitoneal	ip	Square centimeter	cm^2	Intravenous	iv
Subcutaneous	sc	Kilo calories	kcal	Thrice a day	tid
Kilogram	Kg	Year(s)	yr	Twice a day	bid
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