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Research Article

Ultrasonography guided Thoracocentesis for the management of Traumatic pericarditis in cattle

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Abstract

The aim of this study is to explore the medical management strategy for traumatic pericarditis. A total of ten cows aged between 4 to 9 years were presented to teaching veterinary clinical complex of college of veterinary and animal Sciences Palampur (Himachal Pradesh) with the history of recurrent tympany and sharp decrease in milk yield. Clinical signs included increased in body temperature, laboured respiration, muffled heart sounds, edema of brisket region, anorexia and reluctance in walking. Blood samples were collected for haematological and biochemical estimation. Haematological examination showed marked decrease in haemoglobin (7.68 ± 0.35 gm%) and packed cell volume (26.7 ± 1.71%). There was significant increase in total leucocytic count (17.67± 0.61 x 10^3/µl) whereas differential leucocytic count showed marked neutrophilia (70.2 ± 2.20 %). Radiographic examination revealed presence of foreign body in two animals. Further 2-D sonography revealed the thickening of pericardium and fibrino purulent reaction in thoracic cavity. Sonography guided thoracocentesis revealed the purulent foul odour exudate. Treatment consisted of removal of exudate from thoracic cavity and lavaging with Normal Saline Solution mixed with broad spectrum antibiotics. Supportive treatment consisted of antiinflammatory, diuretics and antihistaminics but was not found to be very fruitful.

Keywords*:* Cattle, Traumatic pericarditis, Ultrasonography.

Disorders of fore-stomach in adult dairy cattle is attributable to multiple etiology such as dietary, inflammatory or mechanical. The bovine species does not have highly sensitive prehensile organs. As a consequence, they are prone to swallow sharp metallic objects such as nails and pieces of wires during grazing (Jones *et al.* 1996; Desiye and Mersha, 2012). During contraction and function of fore-stomach these sharp objects pearces adjoining tissues/organs resulting in inflammation and damage to these structures

Traumatic reticulo-pericarditis is one of such condition which is relatively common in adult dairy cattle over 2 years of age (Rebhun, 1995). It is likely that a predisposing factors , such as tenesmus or gravid uterus, causes migration of foreign body into the reticular wall (Rebhun, 1995). However the development of severe sequelae to the penetration of reticular wall depends on the characterstics of foreign as well as direction and extent of penetration (Radiostits *et al.*, 2007). The foreign body when penetrated diaphragm and pericardium, the cattle shows muffled heart sounds, increased jugular pulsation and brisket oedema secondary to congestive heart failure caused by pericarditis (Radiostits *et al.*, 2007). Ultrasonographic examination of normal bovine heart has already been described by Braun *et al.* (2001). The present study was carried out to know the clinical, haematological, biochemical and ultrasonographic changes in cattle affected with traumatic pericarditis and exploring the medical management strategy for traumatic pericarditis.

Materials and Methods

The study was conducted on a total of 10 clinical cases of cows aged between 4 to 9 years, presented at Teaching Veterinary Clinical Complex of College of Veterinary and Animal Sciences Palampur (Himachal Pradesh) with the history of recurrent tympany, anorexia, brisket edema and sharp decrease in the milk yield. All the animals were subjected to thorough clinical examination. Haematological and biochemical parameters including haemoglobin (Hb), packed cell volume (PCV), total leucocytic count (TLC), differential leucocytic count (DLC), Plasma AST, Total Plasma Protein, BUN and Creatinine were determined using standard techniques. All the cases were subjected to radiographic examination. The ultrasonographic examination was performed in the standing animal from left 3rd to 5th intercoastal space with 3.8 MHz volumetric probe using a Siemens Accuson PE ultrasound machine.

Ultrasonogram guided thoracocentesis was performed**.** Thoracocentesis was done using 18 G 8.75 cm spinal needle. Ultrasonogram of right fifth intercoastal space gave clear indication of depth of skin (cms.) and intercoastal muscles to be penetrated before the pleural cavity is reached.

Treatment consisted of removal of exudate from thoracic cavity and lavaging with Normal Saline Solution mixed with broad spectrum antibiotics. Supportive treatment consisted of anti-inflammatory, diuretics and anti-histaminic.

The data obtained were subjected to statistical analysis by using computer software Instat from Graphpad software, 2008. The mean values of different parameters between control and diseased groups were compared at 1% and 5 % level of significance using unpaired t test.

Results and Discussion

There was sharp decrease in milk yield, anorexia/inappetance and no effect of any treatment at field level and in some cases had recurrent tympany. Physical examination revealed increased temperature (102.8 °F – 104 °F), laboured respiration, muffled heart sounds, oedema of brisket region, distension/increased pulsation of jugular veins and reluctance in walking and abducted elbows (Fig. 1 & 2)

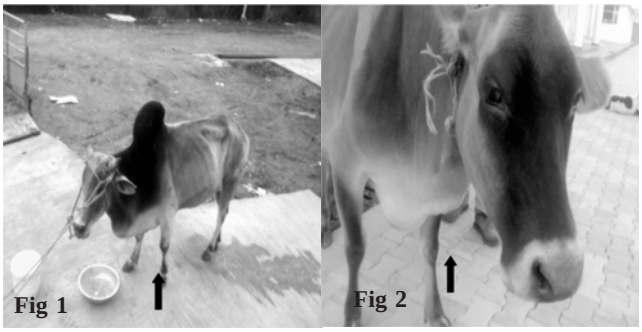

Fig 1&2: Animals showed apathy, poor body condition and brisket oedema

There was significant ($P<0.05$) increase in total leucocytic counts, neutrophilia and lymphopenia when compared to healthy cattle. There was significant ($P<0.05$) reduction in haemoglobin level in animals with traumati reticulo pericarditis (Table 1).

Significant increase in activity of AST but there was no significant change in levels of BUN and serum Creatinine between healthy and diseased cattle.

Table 1. Haematological changes(Mean ± S.E) in traumatic reticulopericarditis affected and healthy cattle

Parameter	Healthy Animals (n=10)	Affected Animals (n=10)
Hb (g/dl)	10.02± 0.26	7.65± 0.35*
PCV (%)	29.8± 0.93	26.7±1.71
TLC ($\times 10^3/\mu l$)	8.89± 0.48	17.67±0.61*
DLC		
Neutrophils (%)	32.6± 1.21	70.2 ± 2.20*
Lymphocytes (%)	65.7±1.11	28.2± 2.23*
Eosinophils (%)	0.7± 0.22	1.6± 0.47

* Represents significant difference between healthy and affected animals at 5% level of significance

However, hyperproteinemia was recorded in ailing animals as compared to healthy cattle (Table 2). Radiography revealed potential foreign bodies in two animals in the thoracic cavity (Fig. 3 & 4). 2-D sonography revealed the thickening of pericardium and fibrino purulent reaction in thoracic cavity. Deposits of fibrin tissue intersoersed with anechoic fluid pockets were seen close to pericardium. The fibrin threads were homogenous, echogenic with pericardial effusion and viewed as a mesh in the thorax. The wall of pericardium appeared thickened with thick deposits (Fig. 5 & 6).

Sonography guided thoracocentesis revealed presence of purulent foul odour exudates**.** Treatment consisted of removal of exudates from thoracic cavity

Table 2. Serum biochemical changes(Mean ± S.E) in traumatic reticulopericarditis affected and healthy cattle

Parameter	Healthy Animals (n=10)	Affected Animals (n=10)
AST (IU/L)	147.4 ± 9.136	255.3 ± 11.34*
Total Protein (g%)	7.46 ± 0.19	9.35 ± 0.63*
BUN (mg%)	17.54 ± 1.33	17.65±1.23
Creatinine (mg%)	1.12± 0.12	1.51±0.12

* Represents significant difference between healthy and affected animals at 5% level of significance

Fig 3&4: Arrows indicate presence of potential foreign bodies

and lavaging with normal saline solution loaded with broad spectrum antibiotics. Supportive treatment consisted of anti-inflammatory, diuretics and antihistaminics along with systemic antibiotics but treatment was not very effective.

In our study, animals exhibited characteristic clinical signs of traumatic reticulo-pericarditis such as increased temperature, laboured respiration, muffled heart sounds, oedema of brisket region, distension/ increased pulsation of jugular veins and reluctance in walking and abducted elbows. The clinical signs among animals differed because of variations in the severity of the disease. Similar observations were also reported by earlier workers (Imran *et al.* 2011).

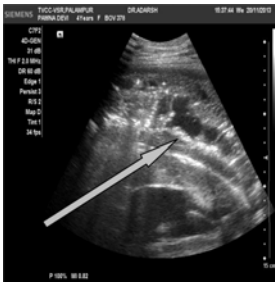
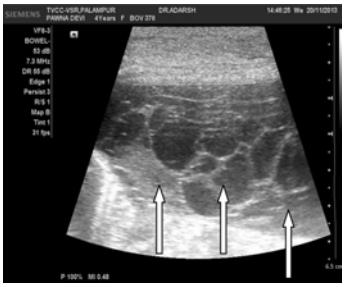

Fig. 5 & 6: Arrows indicative of shreds of fibrin in the thoracic cavity

Haematological examination demonstrated significant leucocytosis with neutrophilia and lymphocytopenia. These observations were in corroboration with the findings of Braun *et al.* (1993) and Imran *et al.* (2011). Leucocytosis with neutrophilia was indicative of inflammatory responses that might have been due to infection associated with the penetration of the reticulum and diaphragm. On the other hand there was significant lymphopenia, which might have been due to reduction in cellular immunity associated with the stress of penetration (Radiostits *et al.*, 2007).

The activity of AST was significantly higher in ailing animals as compared to control group indicating dysfunction of liver which are in agreement with findings of earlier workers (Ghanem, 2010; Athar *et al.* 2012). This may be due to soft tissue damage. Hyperproteinaemia was observed in ailing animals as compared to control animals. Some authors suggest that a total serum protein concentration greater than 10 g/dl is highly suggestive of TRP (Dubensky and White, 1983; Cavedo *et al.* 2004).

In the present study, potential foreign body could be detected in two cases only. Traumatic pericarditis in cattle is most commonly caused by penetrating metallic foreign bodies either migrating from reticulum or due to impailing (Sojka *et al.* 1990), yet foreign bodies are not seen radiographically in as much as 76% of pericarditis cases (Misk and Semeika, 2001).

It can be concluded that traumatic reticulo-peritonitis is a lifethreating condition in cattle. Diagnosis require a battery of test including haemato-biochemical, diagnostic imaging and above all clinical correlation. Madical management of traumatic reticulo-pericarditis has variable success and could not save life.

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Escherichia coli associated with bovine calf diarrhoea

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Abstract

The study was conducted to find out association of enterobacteria infection with diarrhoea in bovine calves. A total of 45 faecal samples were collected from 3-6 months old bovine calves showing diarrhoea and subjected to isolation and identification of enterobacteria. *E. coli* could be isolated from 35 samples, *E. coli* was found to be the major bacterial agent associated with cases of collibacillosis. *In vitro* sensitivity of the isolates was tested against six antimicrobial agent's viz. Gentamicin sulphate, Amoxyclav,cotrimoxazole, Chloramphenicol,cefotaxime and sulfadizine. Gentamicin was found most effective in controlling calf diarrhoea caused by *E.coli*

Keywords*:* Antibiogram, bovine calf, diarrhoea, *E.coli*

Calf scours is a complex disease, with many interrelated causes. Agent, host, and environmental factors collectively explain scours, and these factors interact dynamically over the course of time (David and Smith, 2007). Diarrhoeal diseases constitute a major health problem, causing heavy morbidity and mortality in calf of India (Sojka, 1971, Malik *et al.*, 2012). Neonatal calf diarrhoea causes severe impact on productivity of calf at its maturity (Gow *et al.*, 2005). Symptoms include diarrhoea, a rise in body temperature, weakness and lack of appetite. *E. coli* remain a predominant cause for the calf diarrhoea as "white" scour (Hemashenpagam *et al.*, 2009). Discontinuation or incomplete course of treatment and continuous indiscriminate uses of antibacterial drugs against diarrhoeal infection might have influenced to produce a new generation of virulent and resistant type of bacteria. Although routine laboratory isolation and drug sensitivity testing are expensive and impractical, the periodical check of the pattern of the drug sensitivity of organisms has great impact on success in therapy of calf diarrhoea. We therefore, attempted to identify the prevailing bacterial cause and their sensitivity to commonly available antibacterial in Indian market for managing calf diarrhea in an organized dairy farm.

Materials and Methods

Faecal samples 2 to 5gms and rectal swabs were collected from each diarrhoeic calf (cattle and buffalo) from Livestock Production and Management (cattle and buffalo) Farm, ICAR-Indian Veterinary Research Institute. Izatnagar between September 2012-2013 in sterile vials and were kept at 4°C for further processing.

Direct smear examination and concentration techniques (sedimentation and floatation) were done for identification of any parasitic cause (Jain and Jain, 2011). The samples were then inoculated onto different selective media i.e., MacConkey agar, Eosine Methylene Blue agar (EMB), Hektoen enteric ager (HEA) and incubated at 37°C for 24 hours. Primary characterization was done on the basis of cultural and morphological characters. For differential phenotypic characterization of pathogens biochemical tests were applied as per Bergey's Manual of determinative bacteriology (Holt *et al.*, 1994).

The agar plates were observed for typical colonies of *E. coli* with black centre showing metallic sheen in EMB agar and pink colonies (gram negative lactose fermenters) in MacConkey agar and black center with transparent colonies in Hektoen enteric ager (HEA). The positive colonies from the EMB agar plates and Hektoen enteric ager (HEA) were further confirmed by Grams staining, indole production, Methylene Red (MP), VogesProskaur's test, citrate utilisation, catalase, oxidase, urease and Pheny Pyruvic test reactions as described by Quinn *et al.*, (2002) and Holt *et al.*, (1994)..Out of 45 samples collected and processed from diarrhoeic calves, 35 samples yielded *E. coli* positive (77.77%). The Antibiogram patterns of the isolates were studied by using standard antibiotic discs (Hi-media). Antibiogram of isolates were carried out, as per the standard single disc diffusion technique according to Kirby-Bauer (Bauer *et al.*, 1996).

The data were analysed statistically using standard technique (Snedecor and Cochral 1994)

Results and discussion

On MacConkey agar the organisms in faecal samples developed pink colonies after 24 hours of post

[1]Division of Parasitology

incubation but no characteristic growth observed in Hektoen enteric ager. Out of the 45 isolates, 35 exhibited metallic sheen on EMB agar. All 35 isolates, when subjected to biochemical reactions revealed that they were positive to indole and methyl red tests while negative to VogesProskauer test, citrate utilisation, catalase, oxidase, urease and Phenylpyruvic test. It was further observed that none of the isolates produced H_2S onTriple Sugar Iron (TSI) agar slants. The isolates when subjected to sugar fermentation reactions revealed that they could ferment glucose, galactose, lactose and dextrose. The morphological observations revealed that isolates were gram negative Coccobacillary and Pleomorphic forms. They were also subjected to catalase and oxidase tests and all isolates were found catalase and oxidase negative. Finally, from the findings of different cultural, staining, biochemical examinations it may be concluded that the isolated organism was *E. coli*.

In vitro sensitivity test indicated that *E.coli* isolates were highly sensitive to Gentamicin Sulphate (94.28%), Amoxycillin Clavulanic acid (85.71%) and cotrimoxazole (74.28%)and intermediately sensitive to Chloramphenicol (68.75%), Cefotaxime (65.71%), and Sulfadizine (60%) and resistant to the rest of the antibiotics. The results of the sensitivity were shown in table 1.

E. coli appeared to be an important causative agent either alone or in combination with other bacteria in the aetiology of calf diarrhoea. Hussain and Saikia (2000) also found *E. coli* to be the causative agent in majority of the cases (73.12 percent). Sojka (1971) observed that in spite of involvement of many bacteria in neonatal calf diarrhea, about 50 percent cases were ascribable to *E. coli* infection alone. *E. coli* has been reported to be the first organism, which rapidly colonize the alimentary tract of new born in great number, that's why its frequency of isolation is greater than other bacteria (Smith and Halls, 1967). Our finding showed similarity with Purkayastha *et al.*, (2010) in biochemical examination all the isolates fermented dextrose, sucrose, fructose, maltose and mannitol with the production of acid and gas within 24-48 hrs of incubation. The isolates also revelaed positive reaction in MR test, negative reaction in VP test and differential results in Indole test.

Table 1: Sensitivity of different chemotherapeutic agents to *E. coli*. Serotypes

Name of Antibiotics	No. of isolates tested	No. of isolates sensitive	% of isolates sensitive
Gentamicin sulphate	35	33	94.28
Amoxycillin & clavulanic acid	35	30	85.71
Cotrimoxazole	35	26	74.28
Chloramphenicol	35	24	68.57
Cefotaxime	35	23	65.71

The Equivalent Average Morbidity Rate (EAMR) due to diarrhoea in calves was 8.33. Among the 35 *E. coli* isolates, 33 (94.28%) isolates were found sensitive to Gentamicin sulphate. *In vitro* sensitivity test indicated that *E.coli* isolates were highly sensitive to Gentamicin Sulphate (94.28%), Amoxycillin plus Clavulanic acid (85.71%) , cotrimoxazole (74.28%) and intermediately sensitive to Chloramphenicol (68.75%), Cefotaxime (65.71%) and Sulfadizine(60%) and resistant to the rest of the antibioticsAmikacin, Streptomycin, Cephtalexin, Norfloxacine, Erythromycin, Amoxicillin, Oxytetracycline. The results of our study were in concordance with Gitanjali (2005).As per the *in vitro* antibiogram all 35 diarrhoeic calves were treated with Gentamicin 5mg/kg body weight for 5 days along with anti- inflammatory drugs. All the calves recovered within 3 days of antibiotic treatment. During the lag period (2 days) between diarrhoea and antibiogram result based on previous research experience of calf diarrhoea, aminoglycoside antibiotics were used. Ramkumar (2012) reported that *E.coli* isolates were highly sensitive to Cefadroxil, Gentamicin Sulphate, Amoxycillin Clavulanic acid and Cotrimoxazoleand intermediately sensitive to Chloramphenicol,Cefotaxime,Ceftriaxone by Aniruddha and Rakesh (2009) reported that highest sensitivity of *E. coli* isolated from 116 diarrhoeicfaecal samples of calves was attributed to chloramphenicol (80.64%), followed by azithromycin (77.41%), Tobramycin (46.77%), Amoxicillin (35.48%), Co-trimeoxozole (35.48%), Enrofloxacin (22.58%), Cephalothin (12.90%), Chlortetracycline (12.90%), and Spectinomycin (8.06%).

The result of this study revealed that *E.coli* isolates were highly sensitive to Gentamicin Sulphate, Amoxycillin Clavulanic acid and cotrimoxazole and Amikacin, Streptomycin, Cephtalexin, Norfloxacine, Erythromycin, Amoxicillin, Oxytetracycline were found resistant to *E. Coli* isolate,that cause diarrhoea in calves. For better management of calf diarrhoea of *E.coli* origin Aminoglycosides are the best treatment choice and the

cost of the treatment is also less.

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Renal failure in Indian dogs : An epidemiological study

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Abstract

The present study was aimed to report prevalence of renal failure in canines. The overall clinical prevalence of renal failure in canine was 2.58% (236/9149). Retrospective study revealed, highest prevalence in Patna-6.82% (102) followed by Pantnagar-5.48% (100) and Jabalpur-0.58% (34). Breed wise prevalence of renal failure was highest in Labrador followed by German Shepherd and Pomeranian. Of 100 dogs affected with renal failure, acute renal failure (ARF) was 58% and chronic renal failure (CRF) was 42%. ARF and CRF was higher in >4-8 and >8 years of age, respectively. Pre-renal, renal and post-renal causes of renal failures were 20%, 57% and 23%, respectively. Renal failure affected dogs were identified as idiopathic, urolithiasis, leptospirosis and pyometra in 47, 23, 20 and 10%, respectively.

Keywords*:* Age, Breed, Canine, Prevalence, Renal Failure, Sex

Introduction

Renal failure is one of the most serious problems in canine and about 2-5% of dogs suffers from renal diseases .It is the third leading cause of death in dogs (Bronson, 1982 and Lund *et al.*, 1999). The prevalence is higher in geriatric dogs, with chronic renal insufficiency reaching a peak prevalence of 10% in veterinary clinical patients (Polzin *et al.*, 1992). The age of ARF patients ranging from 11 months to 15 years (average 6.9 years) both in male and female. Advance in diagnostic tools and awareness among pet owners make it possible to recognize the condition. Detailed status reports regarding its prevalence in animals, particularly in canine from different part of the country are laking. Therefore, the present clinical survey records the prevalence of renal failure in canine at some parts of India.

Material and methods

Total 9149 dogs were screened for renal failure at Pantnagar, Patna and Jabalpur during January, 2012 to December, 2013. Preliminary screening of dogs for renal failure was based on the patient's history, clinical signs and routine urinalysis. Furthermore, haemato-biochemical estimation, assessment of glomerular filtration rate (GFR), urine culture, Microscopic Agglutination Test (MAT) and imaging techniques (radiology and ultrasonography) were used for confirmatory and differential diagnosis of renal failure as pre-renal, renal (intrinsic) and post renal causes and finally as acute renal failure (ARF) and chronic renal failure (CRF). Moreover, retrospective data were collected from hospital records of the patient.

Statistical analysis: datawere analysed using standard techniques (Snedecor and Cochran 1994)

Results

Total 9149 canine cases, comprising of 5480 males and 3669 females were screened, and 236 (2.58%) were found have renal problems. The incidence of renal disorders was found higher in females (2.99 %) than males (2.29%). Dogs affected with renal failure was highest in older age (49.58%, 117/236) of >8 years followed by middle age (35.17%, 83/236) of >4-8 years and lowest in younger dogs (15.25%, 36/236) of less than 4 years of age. Region wise prevalence was highest in Patna (6.82 %) followed by Pantnagar (5.48%) and lowest in Jabalpur (0.58%). Of 100 dogs with renal failure at Pantnagar, the highest prevalence was recorded in Labrador- 18.00% (male-55.56% and female-44.44%) followed by German Shepherd- 16.00% (male-87.50% and female-12.50%) and Pomeranian- 16.00% (male-62.50% and female-37.50%) and lowest in Pug and Golden Retriever- 1% each (male-1.00% and female-0.0%). Among renal failure cases, acute renal failure (ARF) was observed in 58% (male-63.78% and female-36.21%) and chronic renal failure (CRF) in 42% (male 54.76% and 45.24%) cases. The incidence ARF was highest in >4 to 8 years of age (43.10%) and lowest in >8 years of age (25.86%), whereas CRF was highest in >8 years of age (80.95%) and lowest in <4 years of age (4.76%). The occurrence of pre-renal, renal and post-renal causes of renal failure was recorded in 20% (male-30% and female-70%), 57% (male-59.65% and female-40.35%) and 23% (male-86.96% and female-13.04%) of cases, respectively. Pre-renal (60%) and renal (50.88%) causes of renal failure was highest in older dogs and lowest (pre-renal-10% and renal-17.54%) in younger dogs, whereas in case of post renal failure, the highest incidence was recorded in younger dogs (47.83%)

and lowest in older dogs (21.74%).

The etiological factors involved in dogs affected with renal failure were urolithiasis, leptospirosis, pyometra and other unknown causes (idiopathic). The major causes were observed to be idiopathic (47%), which was highest (54.17%) in aged dogs and lowest (4.17%) in younger dogs. Urolithiasis was identified in 23% case and it was highest in younger age groups of dogs (47.83%) and lowest in aged dogs (21.74%). The incidence of leptospirosis (20.0%) was highest in middle age groups (45.0%) and lowest in old dogs (20.0%), whereas pyometra (10.0%) was highest in middle age groups (50.0%) and lowest in younger dogs (10.0%). Moreover, idiopathic renal disorders, leptospirosis and urolithiasis were observed more in male (54.17, 70.00 and 86.96%) than female (45.83, 30.00 and 13.04) dogs, respectively.

Discussion

The sex wise prevalence for renal disorders in canine was in accordance with the findings of Ahmed (2011). Higher prevalence of renal failure in male dogs could be due to more risk associated with urolithiasis in male than female due to several anatomic characteristics (Bjorling, 2003) and the presence of higher creatinine values in male than in female (Jergens *et al.*, 1987). Cystitis, pyometra and end-stage kidneys were highest in female (Houston *et al.*, 2003 and Kumar *et al.*, 2009). However, Shizuo (1995) and Tilley and Smith (2007) did not find relation of sex with renal failure in dogs. The age wise prevalence of renal disorders was closely related to Mallela *et al.* (2006), who reported that risk associated with renal disorders were more in dogs of older age (6-8 years). However, Kralova *et al.* (2010), Ahmed (2011) and Kavitha *et al.* (2013) opined that renal disorders are more common in senior dogs of above 8 years of age. Cowgill and Spangler (1981) also stated that frequency of renal failure in dogs increases with age as in human beings. Polzin *et al.* (1989) mentioned that 15% dogs of above 10 years of age were affected with renal impairment. Higher risk of renal failure associated with aged dogs could be due loss of nephron with the advancement of age. However, higher prevalence of renal disease in middle age groups of dogs could be due to greater incidence of leptospiral infection.

The prevalence of renal failure in dogs with respect to breed was in accordance with Mallela (2006) and Ahmed (2011) who reported highest renal disorders in Labrador and German Shepherd. The

Table 1: Area and sex wise prevalence of renal failure causes in canines

Breed	Dog showing renal failure											
	Pantnagar (N=100)			Patna (N=102)			Jabalpur (N=34)			Over all (N=236)		
	MA (FOD)	FA (FOD)	TA (FOD)	MA (FOD)	FA(FOD)	TA (FOD)	MA (FOD)	FA (FOD)	TA (FOD)	MA (FOD)	FA (FOD)	TA (FOD)
German Shepherd	14 (87.50%)	2 (12.50)	16 (16.00%)	5(31.25%)	11(68.75%)	16 (15.69%)	1.00(100.00%)	0.00(0.00%)	1.00(2.94%)	20(60.61%)	13(39.39%)	33(13.98%)
Labrador	10 (55.56%)	8 (44.44%)	18 (18.00%)	8(36.36%)	14 (63.64%)	22 (21.57%)	-	-	-	18(45.00%)	22(55.00%)	40(16.95%)
Doberman	1(33.33%)	2 (66.67%)	3(3.00%)	-	-	-	-	-	-	1(33.33%)	2(66.67%)	3(1.27%)
Dalmatian	2 (66.67%)	1 (33.33%)	3(3.00%)	1 (50.00%)	1(50.00%)	2(1.96%)	-	-	-	3(60.00%)	2(40.00%)	5(2.12%)
Dachshund	2 (66.67%)	1 (33.33%)	3(3.00%)	0(00.00%	1(100.00%)	1(0.98%)	-	-	-	2(50.00%)	2(50.00%)	4(1.69%)
Bhotia	3 (100.00%)	0 (00.00%)	3(3.00%)	-	-	-	-	-	-	3(100.00%)	0(0.00%)	3(1.27%)
Mongrel	1 (11.11%)	8 (88.89%)	9(9.00%)	3 (100.00%)	0(00.00%)	3(2.94%)	21(87.50%)	3(12.50%)	24(70.59%)	25(69.44%)	11(30.56%)	36(15.25%)
Pomeranian	10 (62.50%)	6 (37.50%)	16 (16.00%)	3(21.43%)	11(78.57%)	14 (13.73%)	5(83.33%)	1(16.67%)	6(17.65%)	18(50.00%)	18(50.00%)	36(15.25%)
Spitz	5 (38.46%)	8 (61.54%)	13 (13.00%)	12(40.00%)	18(60.00%)	30 (29.41%)	-	-	-	17(39.53%)	26(60.47%)	43(18.22%)
Lhasa Apso	2 (66.67%)	1 (33.33%)	3(3.00%)	0(00.00%)	1(100.00%)	1 (0.98%)	-	-	-	2(50.00%)	2(50.00%)	4(1.69%)
Pug	1 (100.00%)	0 (00.00%	1(1.00%)	4(44.44%)	5(55.56%)	9 (8.82%)	-	-	-	5(50.00%)	5(50.00%)	10(4.24%)
Rottweiler	1 (33.33%)	2 (66.67%	3(3.00%)	1(50.00%)	1(50.00%)	2 (1.96%)	-	-	-	2(40.00%)	3(60.00%)	5(2.12%)
Golden Retriever	1 (100.00%)	0 (00.00%	1(1.00%)	-	-	-	-	-	-	1(100.00%)	0(0.00%)	1(0.42%)
Great Dane	3 (100.00%)	0 (00.00%)	3(3.00%)	0 (00.00%)	1(100.00%)	1 (0.98%)	-	-	-	3(75.00%)	1(25.00%)	4(1.69%)
Saint Bernard	1 (50.00%)	1 (50.00%)	2(2.00%)	0(00.00%)	1(100.00%)	1 (0.98%)	-	-	-	1(33.33%)	2(66.67%)	3(1.27%)
Crossbred	3 (100.00%)	0 (00.00%)	3(3.00%)	-	-	-	-	-	-	3(100.00%)	0(0.00%)	3(1.27%)
Others	-	-	-	-	-	-	2(66.67%)	1(33.33%)	3(8.82%)	2(66.67%	1(33.33%)	3(1.27%)
Total	60/1057 (5.67 %)	40/767 (5.22%)	100/1824 (5.48%)	37/620 (5.97%)	65/873 (7.45%)	102/1496 (6.82%)	29/3803 (0.76%)	5/2029 (0.25%)	34/5832 (0.58%)	126/5480 (2.30%)	110/3669 (3.00%)	236/9149 (2.58%)

MA: Male affectedFA: Female affectedTA: Total affectedFOD: Frequency of distribution

Table 2: Breed wise frequency distributions of common renal disorders in dogs (N=100)

Breed	Renal failure	Acute renal failure	Chronic renal failure	Pre-renal causes	Intrinsic/Renal causes	Post-renal causes	Urolithiasis	Pyometra	Leptospirosis	Idiopathic
German Shepherd	16 (16.00%)	9(15.52%)	7(16.67%)	4(20.00%)	8(14.04%)	4/16(25.00%)	4/16 (25.00%)	1/16(6.25%)	3/16 (18.75%)	8/16(50.00%)
Labrador	18 (18.00%)	11(18.97%)	7(16.67%)	5(25.00%)	12(21.05%)	1/18 (5.56%)	1/18 (5.56%)	4/18(22.22%)	4/18(22.22%)	9/18(50%)
Doberman	3 (3.00%)	1(1.72%)	2(4.76%)	0(0.00%)	3(5.26%)	0/3(00.00%)	0/3(00.00%)	0/3(00.00%)	2/3(66.67%)	1/3(33.33%)
Dalmatian	3 (3.00%)	2(3.45%)	1(2.38%)	0(0.00%)	1(1.75%)	2/3 (66.67%)	2/3 (66.67%)	0/3(00.00%)	1/1 (100.00%)	0/3(0.00%)
Dachshund	3 (3.00%)	2(3.45%)	1(2.38%)	0(0.00%)	1(1.75%)	2/3(66.67%)	2/3 (66.67%)	0/3(0.00%)	0/3 (0.00%)	1/3(33.33%)
Bhotia	3 (3.00%)	1(1.72%)	2(4.76%)	0(0.00%)	3(5.26%)	0/3(0.00%)	0/3(0.00%)	0/3(0.00%)	2/3 (66.67%)	1/3(33.33%)
Mongrel	9 (9.00%)	4(6.90%)	5(11.90)	2(10.00%)	5(8.77%)	2/9(22.22%)	2/9 (22.22%)	1/9(11.11%)	1/9(11.11%)	5/9(55.56%)
Pomeranian	16 (16.00%)	7(12.07%)	9(21.43%)	5(25.00%)	7(12.28%)	4/16(25.00%)	4/16(25.00%)	2/16(12.50%)	0/16 (00.00%)	10/16(62.50%)
Spitz	13 (13.00%)	9(15.52%)	4(9.52%)	3(15.00%)	6(10.53%)	4/13 (30.77%)	4/13 (30.77%)	1/13(6.69%)	4/13 (30.77%)	4/13(30.77%)
Lhasa Apso	3 (3.00%)	2(3.45%)	1(2.38%)	0(0.00%)	2(3.51%)	1/3 (33.33%)	1/3 (33.33%)	0/3(0%)	1/3 (33.33%)	1/3(33.33%)
Pug	1 (1.00%)	1(1.72%)	0(0.00%	0(0.00%)	0(0.00%)	1/1(100.00%)	1/1(100.00%)	0/1(00.00%)	0/1 (00.00%)	0/1(00.00%)
Rottweiler	3 (3.00%)	3(5.17%)	0(0.00%)	0(0.00%)	3(5.26%)	0/3(00.00%)	0/3(00.00%)	0/3(00.00%)	0/3(00.00%)	3/3(100.00%)
Golden Retriever	1 (1.00%)	1(1.72%)	0(0.00%)	0(0.00%)	0(0.00%)	1/1(100.00%)	1/1(100.00%)	0/1(0.00%)	-	0/1(00.00%)
Great Dane	3 (3.00%)	2(3.45%)	1(2.38%)	0(0.00%)	3(5.26%)	0/3(00.00%)	0/3(00.00%)	0/3(0.00%)	0/3(00.00%)	3/3(100.00%)
Saint Bernard	2 (2.00%)	1(1.72%)	1(2.38%)	1(5.00%)	1(1.75%)	0/2(00.00%)	0/2(00.00%)	1/2(50.00%)	2/2 (100.00%)	0/2(00.00%)
Crossbred	3 (3.00%)	2(3.45%)	1(2.38%)	0(0.00%)	2(3.51%)	1/3(33.33%)	1/3 (33.33%)	0/3(00.00%)	0/3(00.00%)	2/3(66.67%)
Total	100/1824 (5.48%)	58/100 (58.00%)	42/100 (42.00%)	20/100 (20.00%)	57/100 (57.00%)	23/100 (23.00%)	23/100 (23.00%)	10/100 (10.00%)	20/100 (20.00%)	48/100 (48.00%)

Table 3: Sex wse prevalence of common renal disorder dogs

Sex	Acute renal failure	Chronic renal failure	Pre-renal causes	Intrinsic/Renal causes	Post renal causes	Urolithiasis	Pyometra	Leptospirosis	Idiopathic
Male	37/58(63.78%)	23/42(54.76%)	6/20(30.00%)	34/57(59.65%)	20/23(86.96%)	20/23(86.96%)	-	14/20(70.00%)	26/48(54.17%)
Female	21/58(36.21%)	19/42(45.24%)	14/20(70.00%)	23/57(40.35%)	3/23(13.04%)	3/23(13.04%)	10/10(100.00%)	6/20(30.00%)	22/48(45.83%)
Total	58/100(58.00%)	42/100(42.00%)	20/100(20.00%)	57/100(57.00%)	23/100(23.00%)	23/100(23.00%)	10/100(10.00%)	20/100(20.00%)	48/100(48.00%)

Table 4: Age wise prevalence of common renal disorders in dogs

Age	Renal failure (N=100)	Acute renal failure (n=58)	Chronic renal failure (n=42)	Pre-renal causes (n=20)	Intrinsic/ Renal causes (n=57)	Post renal causes (n=23)	Urolithiasis (n=23)	Pyometra (n=10)	Leptospirosis (n=20)	Idiopathic (n=48)
Up to 4 years	23/100(23.00%)	18(31.03%)	2(4.76%)	2(10.00%)	10(17.54%)	11(47.83%)	11(47.83%)	1(10.00%)	7(35.00%)	2(4.17%)
>4-8 years	47/100(47.00%)	25(43.10%)	6(14.29%)	6(25.00%)	18(31.58%)	7(30.43%)	7(30.43%)	5(50.00%)	9(45%)	20(41.67%)
>8 years	30/100(30.00%)	15(25.86%)	34(80.95%)	12(60.00%)	29(50.88%)	5(21.74%)	5(21.74%)	4(40.00%)	4(20.00%)	26(54.17%)

highest prevalence in Labrador could be due to the fact that this breed is more sensitive for pyometra, leptospirosis, systemic causes and other mixed conditions. German Shepherd and Pomeranian were found to be more sensitive for urethritis, urolithiasis, cystitis and other systemic conditions, whereas Dalmatian was more sensitive for urolithiasis. The breed wise relative differences in renal disorders might be due to geographical distribution and managemental practices.

The prevalence regarding renal disorders in the present study is corroborated with the findings of Ji-Young *et al.* (2010) and Kumar *et al.* (2011). Non-infectious causes like renal ischemia, hypotension, hypertension, drug induced nephrotoxicity and post-renal obstruction, and infectious causes like pyometra and leptospirosis are important causes of various renal abnormalities in dogs (Cowgill and Elliot, 2000 and Graner, 2007). Pyometra is one of the important causes of renal disorders in canines and the endotoxins produced in uterus due to pathogenic bacteria, reached to kidney via systemic circulation may cause interstitial inflammation and tubular atrophy, that finally lead to nephritis and renal inefficiency if untreated (Sato *et al.*, 2002 and Heiene *et al.*, 2007). The prevalence of canine leptospirosis has increased in recent years and its prevalence varies region to region and about >20% of healthy, client-owned dogs had exposed to *Leptospira* serovars (Stokes *et al.*, 2007). Acute renal failure is the most commonly recognized disease in dogs, accounting for more than 90% of reported cases of leptospirosis in range 2-6 years of age (Beckel *et al.*, 2005). Hepatic disease occurs concurrently in 10-20% acute renal failure dogs, but can also occur independently.

However, Harkin *et al.* (2003) mentioned that about 8.2% of dogs were shedding pathogenic leptospires, irrespective of the health status. Various systemic causes like cardiovascular, hepatic abnormalities and diabetes mellitus also responsible for renal damage, due to hypo-perfusion, renal ischemia and nephrosis (Sato *et al.*, 2002 and Remzi *et al.*, 2009). Excessive intake of protein rich diets may also lead to degeneration of nephrons and ultimately renal insufficiency in dogs (Polzin *et al.*, 1991).

Use of nephrotoxic drugs may be a contributing factor in 19-25% of cases of severe acute renal failure in critically ill patients (Mehta *et al.*, 2004 and Hoste *et al.*, 2006). Aminoglycosides, vancomycin and amphotericin B are the most commonly used drugs in septic patients, which cause acute tubular necrosis. Vancomycin results in nephrotoxicity in 6-30% of patients (Hidayat *et al.*, 2006). Amphotericin-B associated nephrotoxicity occurs in 25-30% of patients, with progressive increase in the risk of acute kidney injury with increase in cumulative dose (Habarth *et al.*, 2001). Surdyk *et al.* (2011) reported that the use of either NSAID in dogs with extracellular fluid volume depletion or in dogs receiving furosemide is deleterious to renal function during treatment. Many drugs, which are commonly used in the critical care setting, are associated with acute interstitial nephritis (NSAIDs, beta-lactams, sulphonamides, loop diuretics, thiazides, quinolones, cimetidine, allopurinol, proton pump inhibitors) and account for 3-15% of all drug induced ARF (Rossert, 2001). Iodinated contrast media are commonly used for diagnosis in critically ill patients, which causes significant morbidity and mortality due to contrast nephropathy (Maeder *et al.*, 2004). Toxins have attributed to renal failure in dogs due to ingestion of toxin-containing pet foods in dogs and cats (Jeong *et al.*, 2006; Brown *et al.*, 2007 and Burns, 2007). The relative differences in prevalence rate of various renal disorders in the present study might be due to several extrinsic factors like geographical, environmental and managemental practices.

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Bovine mastitis: a longitudinal study using generalized estimating equations (GEE) and random effect models

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Abstract

The present study deals with the binary longitudinal data of mastitis occurrence and non-occurrences and to study the effect of breed, lactation order and average yield per lactation (ALYP) of animals on the occurrence of mastitis. The data on the month wise occurrence /nonoccurrence of mastitis recorded for the period 2005 to 2014 from the dairy farm of Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh. The data on 225 dairy animals have been used in the present study. The analysis of longitudinal binary data can be done by generalized estimating equations (GEE) and random effects models. The study revealed that the effect of lactation order on occurrence of mastitis was significant ($p<0.05$). There is no effect of breeds (Vrindavani, Tharparkar and Murrah) and average yield per lactation on the mastitis occurrence. The interaction of breed, lactation order and average yield per lactation with time were non-significant indicating that the factors do not influence the mastitis occurrence over the time. The generalized estimating equations model proved to be superior to the random effect model as the error is less for every parameter in the GEE model.

Keywords*:* Binary logistic, generalized estimating equations, mastitis, random effect model

Introduction

Mastitis causes a great loss in productivity and influence the quality and quantity of milk yield (Singh and Sigh, 1994). Mastitis causes 30% reduction in productivity per affected quarter and a 15% reduction in production per cow per lactation (Radostits *et al.*, 1994). The disease generally involves interplay between management practice and infection. Among various infectious agents, bacterial pathogens have been known to be one of the most important causes of mastitis (Schalm *et al.*, 1971). Mastitis is a management related disease whose prevention and control depends on many factors. If management is improved; there is a reduction in the incidence of clinical mastitis and vice versa. As with most infectious disease, mastitis risk factors depends on three components i.e. exposure to the microbes, animal defense mechanism, and environment and management factors (Suriyasathaporn *et al.*, 2000). The statistical procedure were applied to longitudinal mastitis records (absence-presence) taken in the course of lactation using two different models for finding the probability of infection at any time *t* assuming conditional independence.

Material and Methods

The data on mastitis occurrence/nonoccurrence in cattle and buffalo have been recorded (Monthly) from 2005 to 2014 for a period of 10 years from dairy farm of Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, UP. A total of 225 dairy animals (75 animals each from Vrindavani, Tharparkar and Murrah) have been taken for the study.

Methods for the analysis of binary longitudinal data of mastitis

The analysis of longitudinal binary data can be done by using marginal and random models (Edwards, 1985 and Diggle *et al.*, 1994). The models were applied in the analysis of binary longitudinal data on mastitis disease occurrence. The marginal model was fitted by using generalized estimating equations (GEE) and the random effects models were fitted by using 'Proc GLIMMIX' procedure in SAS 9.3 (Zeger *et al.*, 1988). The parameters of interest were breed, lactation order (LO) and average yield per lactation (ALYP) of animals and mastitis disease occurrence.

Results and discussion

The present study was conducted to study the effect of breeds (Vrindavani, Tharparkar and Murrah) lactation order and average yield per lactation of animals on the milk production. To study the above effects we have used the generalized Estimating Equations (GEE) and Random effect models. The different parameters used are given in Table 1. The generalized estimating equations were fitted by using the following model.

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Fitting of logistic model

GEE logistic model: $y_{ij}(1/0)$ = infection indicator ~ Bin(1, δ_{ij}), logit(δ_{ij}) = $\hat{a}_0$ + $\hat{a}_1$Vrindavani + $\hat{a}_2$Tharparkar + $\hat{a}_3$Murrah + $\hat{a}_4$LO + $\hat{a}_5$ALYP + $\hat{a}_6$time ….(i). Table 2 indicates the model based covariance matrix and Table 3 indicated the empirical covariance matrix of different parameters used in GEE modeling. Two covariance estimates are similar, indicating an adequate correlation model. In GEE modeling, one has to specify the working correlation matrix in estimating the covariance of the parameter estimates. The specification of the working correlation matrix accounts for the form of within subject correlation of responses on dependent variables. The value of exchangeable working correlation was 0.3309 (Table 4).

We have applied GEE for studying the effect of different parameters on the occurrence of mastitis. In table 4 various measures of goodness of fit of the model are given. In table 5 the parameter estimates are given along with the standard errors. From the Table 5 we observed that: The estimated value of $\hat{\beta}_0, vrindavani = -0.1778$ $(SE = 0.4131)$ so the occurrence of mastitis was less in Vrindavani as compared to Tharparkar and Murraha breed. The estimated value $\beta_{0,}\ tharparkar = 0.6395$ $(SE = 0.4145)$ that represents that the occurrence of mastitis was more in Tharparkar breed as compared to Vrindavani and murrah breed. The estimated value of $\beta_{0,}\ LO = 0.2163$ $(SE = 0.1058)$ that indicates that with the increase in one lactation order the mastitis occurrence increase 0.2163 times than the previous lactation and the occurrence and non-occurrence of mastitis differ significantly ($p<0.05$) with lactation order. The estimated value of $\hat{\beta}_0, ALYP = 0.0397$ $(SE = 0.0692)$ that indicates that the occurrence of mastitis increases with increase in average lactation per yield. The estimated value of vrindavani breed with time $\hat{\beta}_1, vrindavani = -0.0001$ $(SE = 0.0032)$ so after adjusting for other parameters, the change rate in vrindavani cattle was 0.0001 which was more than Murrah and Tharparkar breed. The estimated values of Tharparkar breed with time $\hat{\beta}_1, tharparkar = -0.0020$ $(SE = 0.0021)$ so after adjusting for other parameters, the change rate of mastitis in Tharparkar was less than Vrindavani and murrah breed. The estimated value of Murrah breed with time $\hat{\beta}_1, Murrah = -0.0006$ $(SE = 0.0026)$ so after adjusting for other parameters, the change rate of mastitis in murrah breed was less than change rate of Vrindavani breed. The estimated value of lactation order with time $\hat{\beta}_1, LO = 0.0006$ $(SE = 0.0006)$ so after adjusting for other parameters, the change rate of mastitis increases with lactation order. The estimated value of average yield per lactation with time $\hat{\beta}_1, ALYP = -0.0000$ $(SE = 0.0003)$ so after adjusting for other parameters, the change rate of mastitis decrease with increase in the average milk yield per lactation.

Random effect model for mastitis occurrence

We have applied random effect model for studying the effect of different factors on the occurrence of mastitis. In table 6 various measures of goodness of fit of the model are given. In Table 7 the estimate of the random term is given along with the standard error. In Table 8 the parameter estimates of the fixed effects of random effect model are given along with the standard errors. From Table 8 we observed that the estimated value of $\hat{\beta}_0, vrindavani = -0.1272$ $(SE = 0.5433)$ so the occurrence of mastitis was less in Vrindavani as compared to Tharparkar and Murrah breed. The estimated value $\hat{\beta}_0, tharparkar = 0.6925$ $(SE = 0.4170)$ that indicates that the occurrence of mastitis was more in Tharparkar breed as compared to Vrindavani and murrah breed. The estimated value of $\hat{\beta}_0, LO = 0.2347$ $(SE = 0.09771)$ that indicated that with the increase in one lactation order the mastitis occurrence increase 0.2347 times than the previous lactation and the occurrence and non-occurrence of mastitis differ significantly ($p<0.05$) with lactation order. The estimated value of $\hat{\beta}_0, ALYP = 0.09870$ $(SE = 0.06895)$ that indicates that the occurrence of mastitis increases with increase in average lactation per yield. The estimated value of Vrindavani breed with time $\hat{\beta}_1, vrindavani = 0.000994$ $(SE = 0.002781)$ so after adjusting for other parameters, the change rate in Vrindavani cattle was 0.000994 which was more than Murrah and Tharparkar breed. The estimated values of Tharparkar breed with time, $\hat{\beta}_1, tharparkar = -0.00136$ $(SE = 0.001569)$ so

Table 1: The details of the different effects used in GEE model

	Parameter Information
Parameter	Effect
Prm1	Intercept
Prm2	Vrindavani
Prm3	Tharparkar
Prm4	Murrah
Prm5	Lactation order
Prm6	Average yield per lactation
Prm7	Vrindavani*time
Prm8	Tharparkar*time
Prm9	Murrah*time
Prm10	Lactation order *time
Prm11	Average yield per lactation *time
Prm12	Time

after adjusting for other parameters, the change rate of mastitis in Tharparkar was less than Vrindavani and murrah breed. The estimated value of Murrah breed with time $\hat{\beta}_1, Murrah = -0.00043\ (SE = 0.002179)$ so after adjusting for other parameters, the change rate of mastitis in murrah breed was less than change rate of Vrindavani breed. The estimated value of lactation order with time $\hat{\beta}_1, LO = 0.000846\ (SE = 0.000419)$ so after adjusting for other parameters, the change rate of mastitis increses with lactation order. The estimated value of average yield per lactation with time $\hat{\beta}_1, ALYP = -0.00016\ (SE = 0.000285)$ so after adjusting for other parameters, the change rate of mastitis decreases with increase in the average milk yield per lactation.

Genetic differences among breeds are known to influence disease resistance, and several studies have reported breed-dependent differences in prevalence of mastitis (Kelm *et al.* 2001). Increasing parity increased the risk of clinical mastitis in cows and buffaloes (Sharma, 2003; Kumar and Sharma, 2002; Sharma and Prasad, 2002; Whist *et al.*, 2006; Kavitha *et al.*, 2009). Sharma *et al.* (2007) conducted a study on 500 lactating buffaloes of different age, parities and stage of lactation at different organized or un-organized dairy farms, Chhattisgarh State and found that the higher prevalence of subclinical mastitis (SCM) in buffaloe was recorded in 5 to 9 years old animals and in 3rd and 4th parities. Older cows (>10 years) are at more risk (44.6%), particularly for subclinical mastitis (38.6%), than younger cows (23.6%) in which clinical mastitis was predominant (Biffa *et al.*, 2005).

Table 2: Covariance Matrix (Model-Based)

	Prm1	Prm2	Prm3	Prm5	Prm6	Prm7	Prm8	Prm9	Prm10	Prm11
Prm1	0.17441	-0.006798	-0.094	-0.01544	-0.01441	-0.000309	-0.000148	-0.000322	0.0000286	0.0000262
Prm2	-0.006798	0.12313	0.02312	0.008649	-0.01077	-0.000212	-0.000029	0.0000126	-0.000016	0.0000198
Prm3	-0.094	0.02312	0.10927	0.004443	0.006596	0.0001317	-0.000033	0.0001734	-9.10E-06	-0.000012
Prm5	-0.01544	0.008649	0.004443	0.00622	-0.000764	0.0000126	0.0000195	0.0000286	-0.000011	1.31E-06
Prm6	-0.01441	-0.01077	0.006596	-0.000764	0.002908	0.000046	0.0000143	0.0000262	1.31E-06	-5.27E-06
Prm7	-0.000309	-0.000212	0.0001317	0.0000126	0.000046	3.87E-06	1.33E-06	2.27E-06	-9.12E-08	-3.47E-07
Prm8	-0.000148	-0.000029	-0.000033	0.0000195	0.0000143	1.33E-06	1.26E-06	1.10E-06	-1.63E-07	-1.07E-07
Prm9	-0.000322	0.0000126	0.0001734	0.0000286	0.0000262	2.27E-06	1.10E-06	2.37E-06	-2.09E-07	-2.00E-07
Prm10	0.0000286	-0.000016	-9.10E-06	-0.000011	1.31E-06	-9.12E-08	-1.63E-07	-2.09E-07	9.49E-08	-1.18E-08
Prm11	0.0000262	0.0000198	-0.000012	1.31E-06	-5.27E-06	-3.47E-07	-1.07E-07	-2.00E-07	-1.18E-08	4.05E-08

Table 3: Covariance Matrix (Empirical)

	Prm1	Prm2	Prm3	Prm5	Prm6	Prm7	Prm8	Prm9	Prm10	Prm11
Prm1	0.32739	-0.005738	-0.17449	-0.03152	-0.02792	-0.001087	-0.000571	-0.001129	0.0001021	0.0000993
Prm2	-0.005738	0.17064	0.03093	0.009478	-0.01501	-0.000444	-0.000087	0.0000382	-6.934E-6	0.0000363
Prm3	-0.17449	0.03093	0.17178	0.008346	0.01386	0.0004276	-0.000040	0.0005451	6.8427E-6	-0.000050
Prm5	-0.03152	0.009478	0.008346	0.01120	-0.000260	0.0000896	0.0001012	0.0001039	-0.000042	-4.462E-7
Prm6	-0.02792	-0.01501	0.01386	-0.000260	0.004791	0.0001357	0.0000501	0.0000986	-7.792E-7	-0.000015
Prm7	-0.001087	-0.000444	0.0004276	0.0000896	0.0001357	0.0000104	4.2879E-6	6.8167E-6	-5.456E-7	-9.122E-7
Prm8	-0.000571	-0.000087	-0.000040	0.0001012	0.0000501	4.2879E-6	4.3578E-6	3.5229E-6	-7.057E-7	-3.135E-7
Prm9	-0.001129	0.0000382	0.0005451	0.0001039	0.0000986	6.8167E-6	3.5229E-6	6.767E-6	-5.823E-7	-6.256E-7
Prm10	0.0001021	-6.934E-6	6.8427E-6	-0.000042	-7.792E-7	-5.456E-7	-7.057E-7	-5.823E-7	3.1119E-7	-1.437E-8
Prm11	0.0000993	0.0000363	-0.000050	-4.462E-7	-0.000015	-9.122E-7	-3.135E-7	-6.256E-7	-1.437E-8	1.0671E-7

Table 4: Exchangeable working correlation and goodness of fit criteria for GEE Model

Exchangeable Working Correlation	
Correlation	0.3217315436
GEE Fit Criteria	
QIC	2603.1446
QICu	2558.0435

Risk of mastitis varies from breed to breed. High yielding cows are generally considered to be more susceptible to intramammary infection e.g. Holstein Frisian (HF), Jersey or HF and Jersey cross bred dairy cows are more susceptible to mastitis than Desi (Zebu) breeds of cows (Sharma, 2003). The incidence of mastitis is higher during just after parturition (first 2 months of lactation) and first 2-3 weeks of dry period and Corbett (2009) suggested that the highest number of clinical mastitis cases occurs during the first week of lactation, and that the lactating cow is more likely to develop clinical mastitis during the first three months of lactation than the remainder of the lactating period.

The generalized estimating equations model proved to be superior to random effect model as the error is less for every parameter in the GEE model. The random effect model overestimates the values with higher standard error.

Table 6: Measures of goodness of fit for random effect model and conditional distribution

Random Effect Model	
-2 Log Likelihood	2082.51
AIC (smaller is better)	2104.51
AICC (smaller is better)	2104.62
BIC (smaller is better)	2142.09
CAIC (smaller is better)	2153.09
HQIC (smaller is better)	2119.67
Fit Statistics for Conditional Distribution	
-2 log L(Response | r. effects)	1635.51
Pearson Chi-Square	1561.22
Pearson Chi-Square / DF	0.63

Table 7: Covariance Parameter Estimates

Covariance Parameter Estimates			
Cov Parm	Subject	Estimate	Standard Error
Intercept	newid	2.2146	0.2849

Table 5: GEE Parameter Estimates and Standard Errors of Estimates

Parameter	Estimate	Standard Error	95% Confidence Limits		Z	Pr > |Z|
Intercept	0.1886	0.5722	-0.9329	1.3100	0.33	0.7417
Vrindavani	-0.1778	0.4131	-0.9875	0.6318	-0.43	0.6668
Tharparkar	0.6395	0.4145	-0.1729	1.4518	1.54	0.1229
Murrah	0.0000	0.0000	0.0000	0.0000	.	.
Lactation order	0.2163	0.1058	0.0089	0.4237	2.04	0.0409
ALYP	0.0397	0.0692	-0.0959	0.1754	0.57	0.5659
Vrindavani*time	-0.0001	0.0032	-0.0064	0.0062	-0.02	0.9832
Tharparkar*time	-0.0020	0.0021	-0.0061	0.0020	-0.98	0.3275
Murrah*time	-0.0006	0.0026	-0.0057	0.0045	-0.24	0.8079
LO*time	0.0006	0.0006	-0.0004	0.0017	1.15	0.2484
ALYP*time	-0.0000	0.0003	-0.0007	0.0006	-0.09	0.9283
time	0.0000	0.0000	0.0000	0.0000	.	.

Table 8: Parameter Estimates and standard errors of estimates for the fixed effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	0.1385	0.5433	220	0.25	0.7990
Vrindavani	-0.1272	0.4718	2245	-0.27	0.7874
Tharparkar	0.6925	0.4170	2245	1.66	0.0969
Murrah	0	.	.	.	.
Lactation order	0.2347	0.09771	2245	2.40	0.0164
ALYP	0.09870	0.06895	2245	1.43	0.1524
Vrindavani*time	0.000994	0.002781	2245	0.36	0.7207
Tharparkar*time	-0.00136	0.001569	2245	-0.87	0.3846
Murrah*time	-0.00043	0.002179	2245	-0.20	0.8431
LO*time	0.000846	0.000419	2245	2.02	0.0438
ALYP*time	-0.00016	0.000285	2245	-0.56	0.5785

This study demonstrates the feasibility and advantage of a longitudinal analysis of sequential binary responses. The generalized estimating equations model proved to be superior to random effect model as the error is less for every parameter in the GEE model. The random effect model overestimates the values with higher standard error.

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Effect of *Calotropis procera* (leaf) and Amprolium supplementation on haematological parameters of broilers during mixed *Eimeria* species infection

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Abstract

Effect of *Calotropis procera* leaf powder on mixed *Eimeria* species infection in broiler chicks were studied. One sixty eight day old broiler chicks were randomly divided into seven groups each with two replicates of 12 chicks each. On 15th day of experiment, broilers of infected groups were infected by inoculating 1 ml suspension containing 50,000 sporulated oocysts of mixed *Eimeria* species. On 0, 5th, 10th and 15th days post infection blood from 3 broilers of each replicate was collected in heparinized vials for hematological parameters viz. Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocytic Count (TEC), Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC). It was observed that haematological parameters on 0 DPI and 15 DPI were non significant among all groups. On 5 and 10 DPI, Hb, PCV, TEC, MCH, MCHC, heterophils % and monocytes % were decreased, whereas MCV, TLC, lymphocytes % and eosinophils % were increased in infected groups than respective uninfected groups. Haematological variation due to coccidiosis was maximum in control infected group and maximum restoration of normal parameters was observed in 0.0125% amprolium supplemented group followed by 0.4% and 0.2% madar leaf powder supplemented group. Fom the results of present investigation, it is concluded that similar to standard anticoccidial amprolium, madar (*Calotropis procera*) leaf powder also has excellent potential to provide protection in broiler chickens against coccidiosis induced alteration in haematological parameters.

***Keywords**:* Amprolium, *Calotropis procera* leaf powder, Coccidiosis, Haematological parameters.

Introduction

Poultry is one of the fastest growing segments of the agricultural sector. In spite of threat of flu epidemics and cholesterol problem, there is tremendous growth in poultry sectors in India these days. Poultry farming is a beneficial occupation but sometimes even after giving balanced diet and good housing conditions, desired production is not obtained due to the occurrence of diseases. Among these diseases coccidiosis is one of the most dangerous diseases of poultry. It is caused by the intracellular protozoan parasite *Eimeria*, which undergoes its life cycle in the intestinal mucosa of the infected bird. According to Du and Hu (2004) in commercial poultry production incidence of coccidiosis can raised substaintially and can causes severe economic losses per annum . These losses are due to costs for treatment and prevention (Allen and Fetterer, 2002, Shirley *et al.*, 2005) and due to mortality, morbidity, impaired growth rate, temporary reduction of egg production in layers and poor feed conversion of chicken that survive outbreaks (Kitandu and Juranova, 2006). Coccidiosis is mainly controlled by using chemical coccidiostats administered in feed (Shirley *et al.*, 2005) but increase in resistant *Eimeria* field isolates (Yadav and Gupta, 2001, Usman *et al.*, 2011), limit use of these chemicals as satisfactory control measure. Along with above, the increasing regulations and bans on the use of anticoccidial drugs, high costs of developing new drugs enhanced the need for development of novel approaches and alternative control strategies for coccidiosis (Williams, 2006). Alternative control measures include vaccines for control of coccidiosis but high cost and chances of reversal of pathogenecity of these vaccines directed researchers in search of the new approach for control of coccidiosis such as use of natural products (Kayser *et al.*, 2003).

Calotropis procera, an important lant with diverse medicinal property mentioned in Ayurveda since time immortal. The plant belong to family 'Asclepiadaecae' and is known by various names like Swallow wort in English, Madar in Hindi and Alarka in Sanskrit. It has been widely used in the Indian, Unani, Arabic and Sudanese traditional medicinal system for the treatment of various diseases namely leprosy, ulcers, piles and diseases of the spleen, liver and abdomen (Sharma *et al.*, 2011). Looking to the medicinal properties of *Calotropis procera*, an experiment was conducted to study the comparative effect of supplementation of *Calotropis procera* leaf powder and

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a standard anticoccidial Amprolium on hematological profile of broilers during mixed *Eimeria* species infection.

Material and Method

Experiment was conducted in the College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar. Experiment was conducted for a period of 30 days. For the experiment, 168, one-day old commercial broiler chicks were randomly allocated to seven groups. The groups were designated as group I, II, III, IV, V, VI and VII. Each group had 2 replicates having 12 chicks each. Chicks of different replicates were kept in separate cages and maintained under similar managemental conditions. The chicks were housed in electrically heated and wire-floored battery cages. At the age of 5 days, vaccination against ranikhet disease and at 12 days, vaccination against gumboro disease were done using F_1 strain and Georgia strain vaccine, respectively. Feed for the chicks was brought from local poultry feed supplier and feed was free from coccidiostat. Broilers of group I and II were provided standard control diet without any supplement. In broilers of group III and IV, standard feed was supplemented with 0.0125% amprolium and broilers of group V and VI were provided with basal diet supplemented with 0.2% madar leaf powder. Broilers of group VII were provided standard control diet supplemented with 0.4% madar leaf powder.

In the experiment, at the age of 15 days, broilers of group II, IV, VI and VII were infected by inoculating 1 ml suspension containing 50,000 sporulated oocysts of mixed *Eimeria* species directly in the pharynx, using a long nozzled 2 ml plastic pipette. *Eimeria* spp. mixed culture contained *E. tenella* (80%), *E. necatrix* (10%), *E. acervulina* (6%), *E. maxima* (2%) and *E. mitis* (2%). Broilers of group I, III and V were inoculated with 1 ml plain water.

For estimation of haematological parameters, blood samples were collected from 3 broiler chicks of each replicate from wing vein at 15^{th}, 20^{th}, 25^{th} and 30^{th} day in uninfected groups and on 0, 5^{th}, 10^{th} and 15^{th} DPI in infected groups aseptically using sterilized disposable syringe and needles. These samples were kept in sterile glass tubes containing heparin as anticoagulant for haematological studies. Different haematological parameters estimated and calculated were haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Haemoglobin of blood samples was estimated using the method of Jain (1986) and results were expressed in gram per deciliter (gm/dl). PCV of the blood samples was estimated by microhaematocrit method and expressed in percent (%). The TEC and TLC were determined according to the method of Natt and Herrick (1952) and the results were expressed in millions /cubic millimeter (10^6/cu mm) and thousands per cubic millimeter (10^3/cu mm) respectively. DLC were estimated as per method described by Jain (1986) and number of lymphocytes, heterophils, monocytes, eosinophils and basophils were presented in %. The erythrocytic indices as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as per the following formulae given by Jain (1986).

Statistical analysis was carried out using one way ANOVA technique as described by Snedecor and Cochran (1994).

Results

Haematological parasmeters in broilers of different groups on 15^{th}, 20^{th}, 25^{th} and 30^{th} day of experiment or 0, 5, 10 or 15 days post infection (DPI) are presented in Table 1.

Haemoglobin concentration (Hb) of broilers of various groups on 15^{th} day of experiment was non significantly different among all groups showing no effect of various supplementations on Hb. On 20^{th} day of experiment, Hb differ non-significantly among all uninfected groups and it was minimum and significantly lower (P d" 0.05) from all other groups in broilers of group II (5.90). On 5DPI among infected supplemented groups significantly maximum Hb was recorded in broilers of group IV (9.60). Supplementation of 0.2 % madar leaf powder and 0.4% madar leaf powder also protect reduction in Hb value significantly. On 25^{th} day of experiment amid infected groups, Hb was non significantly different among all supplemented groups i.e. group IV (10.30), group VII (10.28) and group VI (10.04) but significantly lower and minimum Hb was recorded in broilers of unsupplemented control infected group (7.42). On 30^{th} day of experiment, Hb differ non significantly among

Table 1: Effect of different supplementations on Haematological parameters in experimental broiler chicks

Haematological parameters	Days	I (UC)	II (IC)	III (UA)	IV (IA)	V (UM)	VI (IM-1)	VII (IM-2)
Haemoglobin (g/dl)	15 Day (0 DPI)	11.43±0.25	11.38±0.48	11.24±0.25	11.28±0.25	11.58±0.71	11.53±0.44	11.64±0.76
	20 Day (5 DPI)*	11.52±0.51^{a}	5.90±0.05^{e}	11.29±0.23^{a}	9.60±0.65^{b}	11.65±0.13^{a}	8.25±0.09^{d}	8.90±0.15^{c}
	25 Day (10 DPI)*	11.60±0.48^{a}	7.42±0.05^{c}	11.33±0.13^{a}	10.30±0.13^{b}	11.71±0.36^{a}	10.04±0.05^{b}	10.28±0.08^{b}
	30 Day (15 DPI)	11.55±0.15	11.12±0.95	11.44±0.43	11.24±0.25	11.77±0.24	11.51±0.21	11.63±0.39
Packed Cell Volume (%)	15 Day (0 DPI)	30.95±0.76	31.02±0.55	30.88±0.15	30.83±0.23	31.10±0.57	31.17±0.48	31.21±0.15
	20 Day (5 DPI)*	31.06±0.78^{a}	21.62±0.22^{d}	30.85±0.35^{a}	27.84±0.34^{b}	31.28±0.28^{a}	26.42±0.22^{c}	27.13±0.49^{bc}
	25 Day (10 DPI)*	31.10±0.65^{a}	24.05±0.45^{c}	30.87±0.55^{a}	28.88±0.65^{b}	31.33±0.39^{a}	29.11±0.55^{b}	29.18±0.64^{b}
	30 Day (15 DPI)	31.11±0.65	30.65±0.76	30.90±0.63	30.82±0.75	31.18±0.66	30.95±0.45	31.07±0.61
Total Erythrocyte Count (10^6/cu mm)	15 Day (0 DPI)	2.97±0.25	2.96±0.15	2.92±0.35	2.93±0.25	3.01±0.11	2.99±0.34	3.03±0.45
	20 Day (5 DPI)*	2.98±0.22^{a}	1.98±0.32^{c}	2.96±0.22^{a}	2.57±0.23^{b}	3.06±0.07^{a}	2.49±0.09^{b}	2.52±0.16^{b}
	25 Day (10 DPI)*	3.01±0.05^{ab}	2.24±0.05^{e}	2.93±0.07^{bc}	2.68±0.02^{d}	3.07±0.04^{a}	2.79±0.11^{d}	2.81±0.07^{cd}
	30 Day (15 DPI)	2.98±0.15	2.89±0.25	2.96±0.23	2.91±0.24	3.03±0.26	2.98±0.15	3.00±0.25
Mean Corpuscular Volume (fl)	15 Day (0 DPI)	104.21±4.13	104.80±4.24	105.75±5.34	105.22±4.25	103.32±2.31	104.25±4.28	103.00±4.56
	20 Day (5 DPI)*	104.19±0.25^{d}	109.19±0.85^{a}	104.22±0.24^{d}	108.33±1.25^{b}	102.22±1.15^{e}	106.10±0.35^{c}	107.66±0.34^{b}
	25 Day (10 DPI)*	103.32±1.75^{cd}	107.37±2.25^{ab}	105.36±0.74^{abc}	107.76±1.38^{a}	102.05±1.14^{d}	104.34±1.25^{cd}	103.84±0.26^{cd}
	30 Day (15 DPI)	104.40±4.25	106.06±4.13	104.39±4.24	105.91±3.23	102.90±2.24	103.86±3.25	103.57±2.25
Mean Corpuscular Haemoglobin (pg)	15 Day (0 DPI)	38.48±1.24	38.44±0.75	38.49±0.57	38.50±0.25	38.47±0.46	38.56±0.54	38.42±0.35
	20 Day (5 DPI)*	38.66±0.35^{a}	29.80±0.25^{f}	38.14±0.34^{ab}	37.35±0.31^{bc}	38.07±0.45^{abc}	33.13±0.85^{e}	35.32±0.44^{d}
	25 Day (10 DPI)*	38.54±0.66^{a}	33.13±0.65^{c}	38.67±0.64^{a}	38.43±1.25^{a}	38.14±1.04^{a}	35.99±0.55^{b}	36.58±0.66^{b}
	30 Day (15 DPI)	38.76±0.85	38.48±0.73	38.65±0.71	38.63±0.74	38.84±0.84	38.62±0.76	38.77±0.85
Mean Corpuscular	15 Day (0 DPI)	36.93±0.25	36.69±0.76	36.40±0.57	36.59±0.71	37.23±1.15	36.99±1.04	37.30±0.48
Haemoglobin Concentration (%)	20 Day (5 DPI)*	37.10±1.10^{a}	27.29±0.80^{d}	36.60±0.76^{a}	34.48±1.15^{b}	37.24±1.14^{a}	31.23±1.15^{c}	32.81±0.80^{bc}
	25 Day (10 DPI)*	37.30±0.75^{ab}	30.85±0.54^{f}	36.70±0.35^{abc}	35.66±0.48^{cd}	37.38±0.86^{a}	34.49±0.48^{e}	35.23±0.85^{de}
	30 Day (15 DPI)	37.13±0.25	36.28±0.75	37.02±0.95	36.47±0.57	37.75±0.75	37.19±1.15	37.43±0.65f
Total Leukocyte Count (10^3/cu mm)	15 Day (0 DPI)	25.11±1.37	25.24±1.15	25.49±0.57	25.37±1.25	26.48±0.90	26.52±1.56	27.53±1.58
	20 Day (5 DPI)*	25.15±1.15^{d}	27.52±1.45^{abc}	25.56±0.67^{d}	26.24±1.14^{bcd}	26.51±0.91^{bcd}	27.86±0.97^{ab}	28.75±0.85^{a}
	25 Day (10 DPI)*	25.17±1.47^{b}	28.41±0.25^{a}	25.59±0.91^{b}	26.66±0.74^{b}	26.58±1.25^{b}	28.87±0.19^{a}	29.28±1.10^{a}
	30 Day (15 DPI)	25.18±1.33	26.75±1.00	25.62±0.91	25.80±1.01	26.63±1.07	27.25±1.16	27.91±0.93
Heterophils %	15 Day (0 DPI)	24.35±1.34	24.40±1.70	23.05±2.47	23.50±1.56	22.85±2.05	22.95±0.92	21.40±1.56
	20 Day (5 DPI)*	25.00±1.10^{a}	20.67±1.03^{de}	22.83±0.75^{b}	21.67±0.82^{cd}	22.00±0.63^{bc}	20.50±0.55^{e}	19.17±1.17^{f}
	25 Day (10 DPI)*	24.67±1.21^{a}	22.83±0.98^{bc}	23.17±0.75^{b}	22.00±0.89^{cd}	22.17±0.41^{bcd}	21.83±1.33^{cd}	21.16±0.96^{d}
	30 Day (15 DPI)	24.70±1.32	24.48±1.20	23.20±2.29	22.94±0.96	23.07±1.83	22.74±0.29	21.85±1.53
Lymphocytes %	15 Day (0 DPI)	69.90±0.54	70.12±1.40	71.23±1.99	71.27±1.92	71.68±2.02	71.90±2.47	72.34±1.56
	20 Day (5 DPI)*	69.00±0.63^{e}	73.50±0.55^{c}	72.00±0.89^{d}	72.83±0.75^{cd}	72.50±0.55^{d}	74.50±0.54^{b}	75.50±1.05^{a}
	25 Day (10 DPI)*	70.17±0.75^{c}	72.00±1.10^{b}	72.16±0.69^{b}	72.78±0.74^{b}	72.50±0.55^{b}	73.83±0.41^{a}	74.50±0.52^{a}
	30 Day (15 DPI)	69.93±0.56	70.20±1.51	71.25±2.06	71.37±2.05	71.68±2.02	72.10±2.19	72.74±1.58
Eosinophils %	15 Day (0 DPI)	2.17±0.41	2.11±0.10	2.18±0.40	2.26±0.18	2.50±0.55	2.43±0.35	2.66±0.14
	20 Day (5 DPI)*	2.48±0.50^{bcde}	3.33±0.52^{a}	2.50±0.55^{bcd}	3.00±0.63^{ab}	2.17±0.41^{def}	2.47±0.39^{bcdef}	2.83±0.75^{abc}
	25 Day (10 DPI)	2.43±0.29	2.83±0.41	2.56±0.46	2.67±0.52	2.46±0.16	2.45±0.27	2.78±0.20
	30 Day (15 DPI)	2.50±0.55	2.67±0.48	2.53±0.52	2.36±0.12	2.26±0.15	2.33±0.14	2.61±0.33
Monocytes %	15 Day (0 DPI)	2.67±0.18	2.63±0.21	2.58±0.30	2.55±0.25	3.00±0.61	3.19±0.80	3.34±0.71
	20 Day (5 DPI)*	2.66±0.14^{b}	2.50±0.55^{b}	2.67±0.52^{b}	2.56±0.48^{b}	3.33±0.51^{a}	2.33±0.56^{b}	2.50±0.55^{b}
	25 Day (10 DPI)*	3.00±0.63^{ab}	2.53±0.39^{c}	2.63±0.20^{bc}	2.50±0.47^{bc}	3.26±0.23^{a}	2.56±0.36^{c}	2.76±0.14^{bc}
	30 Day (15 DPI)	2.79±0.04	2.71±0.05	2.69±0.24	2.60±0.04	3.02±0.57	2.83±0.13	2.86±0.11

* Significant, a, b, c, d, e Means bearing different superscripts in a row differ significantly (P< 0.05)

all treatment groups.

On 15th day of experiment, PCV differ non significantly among all treatment groups. PCV (%) on 20th day of experiment differed non-significantly among all uninfected groups. PCV in broilers of infected amprolium supplemented group (27.84) and infected 0.4% madar leaf powder supplemented groups (27.13) differed non significantly. PCV was significantly lower and minimum in broilers of control infected group II (21.62). On 25th day of experiment, PCV in broilers of group IV (28.88), VI (29.11) and group VII (29.18) also had statistically non significant difference. Significantly minimum PCV was recorded in broilers of group II (24.05). On 30th day of experiment, significant difference was not observed among all treatment groups.

TEC (10^6/cumm) on 15th day of experiment was non significantly different among all treatment groups. On 20th day of experiment, TEC values differed non-significantly among all uninfected groups. TEC in all infected supplemented groups also differed non significantly but it was significantly minimum in broilers of group II (1.98). On 25th day of experiment, TEC values were non significantly different among all infected supplemented groups *i.e.* group IV (2.68), VI (2.79) and VII (2.81). TEC value was lowest in broilers of group II (2.24) and was significant to all group (P d" 0.05). On 30th day of experiment or 15 DPI, TEC values showed non significant differences among various treatment groups.

MCV on 15th day of experiment or 0 DPI, differed non significantly among all treatment groups.

On 20th day of experiment or 5 DPI was significantly maximum and significantly (P d" 0.05) higher in broilers of group II (109.19) and minimum in broilers of group V (102.22). MCV on 25th day of experiment or 10 DPI was non significantly different among group II (107.37), III (105.36) and IV (107.76) and was non significantly higher in group IV. Minimum MCV was observed in broilers of group V (102.05), which was non significantly different from MCV of broilers of group I (103.32), VI (104.34) and VII (103.84). On 30th day of experiment or 15 DPI, MCV values in broilers of various treatments had statistically (P d" 0.05) non significant differences.

MCH on 15th day of experiment or 0 DPI was non-significantly different among all treatment groups. On 20th day of experiment, MCH was non significantly different among. MCH in broilers of group IV (37.35) also had statistically (P d" 0.05) non significant difference to broilers of group III and V. MCH among infected madar leaf powder supplemented groups was significantly higher in broilers of group VII than group VI. Minimum MCH on 5 DPI was observed in broilers of group II (29.80). On 25th day of experiment, MCH was maximum in broilers of group III (38.67), which showed non significant difference to broilers of group I (38.54), IV (38.43) and V (38.14). MCH of infected 0.2% and 0.4% madar leaf powder supplemented groups showed non significant differences. On 30th day of experiment or 15 DPI, MCH of broilers of all treatment groups differed non significantly.

MCHC of broilers of all groups on 15th day of experiment showed non significant differences. On 20th day of experiment, MCHC was non significantly different among all uninfected groups. MCHC among infected groups was maximum in broilers of group IV (34.48), which showed non significant difference to broilers of group VII (32.81) and MCHC was significantly minimum in broilers of group II (27.29). On 25th day of experiment among infected groups, MCHC was maximum in broilers of amprolium supplemented groups *i.e.* group IV (35.66), which showed non significant difference to group VII (35.23). Both madar leaf powder supplemented infected groups *i.e.* 0.4 % (35.23) and 0.2 % (34.49) also differ non significantly whereas significantly minimum MCHC was recorded in broilers of group II (30.85). On 30th day of experiment or 15 DPI, MCHC of various groups differed non significantly.

TLC on 15th day of experiment or 0 DPI was non significantly different among all groups. On 20th day of experiment or 5 DPI, TLC was highest in broilers of group VII (28.75), which showed non significant difference to broilers of group II (27.52) and group VI (27.86). TLC of groups II (27.52), IV (26.24), V (26.51) and VI (27.86) also had non significant differences. Minimum TLC was noted in broilers of group I (25.15), which was non significantly different to broilers of group III (25.56). On 25th day of experiment or 10 DPI, maximum TLC value was recorded in broilers of group VII (29.28), which had non significant difference to broilers of group VI (28.87) and group II (28.41). Minimum TLC was recorded in broilers of group I (25.17), which was non significantly lower than group III (25.59), IV (26.66) and V (26.58). On 30th day of experiment or 15 DPI, TLC values of all groups showed non significant differences.

Heterophils per cent on 15th day of experiment or 0 DPI was non significantly different among all treatment groups. In all infected groups heterophil % was lower in comparison to respective control groups on 5 and 10 DPI. On 30th day of experiment or 15 DPI, herterophil % differ non significantly among all groups.

Lymphocyte % had no significant difference among all treatment groups on 15th day of experiment or 0 DPI. In all infected groups, lymphocyte % was more than respective control groups on 5 and 10 DPI whereas on 30th day of experiment or 15 DPI, however lymphocyte % was non significantly different among all groups.

Eosinophil % on 15th day of experiment was non significantly different among all groups. On 20th day of experiment or 5 DPI, maximum eosinophil % was shown by broilers of group II (3.33), which was non-significantly different from group IV (3.00) and group VII (2.83), and was minimum in broilers of group V (2.17), which showed non-significant difference from group I (2.48), III (2.50) and VI (2.47). On 25th day of experiment and 30th day of experiment all groups had non-significant difference in eosinophil%.

Monocyte % on 15th day of experiment or 0 DPI differed non significantly among all groups. On 20th day of experiment or 5 DPI, maximum monocyte % was exhibited by broilers of group V (3.33) and minimum by group VI (2.33), which differ non-

significantly to all other groups. On 25th day of experiment or 10 DPI, monocyte % was maximum in broilers of group V (3.26) which differed non-significantly to group I (3.00), while minimum monocyte % was recorded in broilers of group II (2.53), which is non-significantly different from all other groups except I and V. On 30th day of experiment or 15 DPI, all groups showed non- significant difference in monocyte %.

Discussion

There was non significant difference in Hb, PCV and TEC values of broilers of different treatment groups on 15th day of experiment, but these values were higher in madar leaf powder supplemented groups. Mossa *et al.* (1991) also found non significant increase in Hb and TEC in *Calotropis procera* aerial parts supplemented rats than the control unsupplemented. Kumar *et al.* (2013) noticed the anaemia removing and ulcer healing property of *Calotropis* sp.

On 20th day of experiment or 5 DPI, there was non significant difference in Hb concentration among different uninfected groups and all infected groups had significantly lower Hb than uninfected groups and was minimum in infected control group. Razzaq *et al.* (2003), Irizary-Rovira (2004), Singh *et al.* (2007) and Singh *et al.* (2013) reported significant reduction in Hb in infected groups. The probable reason may be the intestinal haemorrhage resulting from the liberation of second generation merozoites, which caused sloughing of intestinal mucosa with discharge of large amount of blood (Nayak and Rai, 1985). Among infected groups, broilers of amprolium supplemented group had minimum Hb concentration reduction and its mode of action is that it affects the second generation schizonts in the life cycle of *Eimeria* sp. due to its thiamine antagonist property (Bozkurt *et al.*, 2013). In madar leaf powder supplemented groups also Hb reduction was significantly lower than control infected group, this may be due to the anticoccidial property of *Calotropis procera* as discussed by earlier researchers (Mahmoud *et al.*, 2001, Zaman *et al.*, 2011). Wang *et al.* (1998) opioned that herbs provide nutrients such as proteins, essential amino acids, organic trace minerals etc. that influence the haematological profile. On 30th day of experiment or 15 DPI, Hb differ non significantly among all treatment groups. This may be due to the termination of haemorrhagic phase of coccidiosis in both infected control and supplemented infected groups, which results in the restoration of hematological parameters (Natt and Herrick, 1955).

On 20th day of experiment or 5 DPI, PCV values differed non significantly among uninfected groups and PCV was lower in infected groups than uninfected groups. Among infected groups PCV reduction was minimum in broilers of amprolium supplemented group and differed non significantly to infected 0.4% madar leaf powder supplemented group. Similarly Stephens (1964) observed that coccidiosis by *E. necatrix* caused decrease in packed cell volume and Stephens (1965) and Mukkur and Bradley (1969) explained that decreased PCV in coccidiosis is indicative of anaemia with acute blood loss due to discharge of 2nd generation of merozoites. Ogbe *et al.* (2010) also noticed that PCV reduction was significantly less in infected amprolium supplemented group than non supplemented control infected group whereas Zaman *et al.* (2011) recorded comparatively similar anticoccidial property of amprolium and herbal complex containing *Calotropis procera.*

On 20th day of experiment among all infected supplemented groups also TEC value differed non significantly indicating similar TEC reduction protection effect of amprolium and madar leaf powder whereas significantly minimum TEC was noticed in control infected group. In broilers of infected groups, TEC was lower than uninfected group. This reduction may be due to the loss of blood into GIT. Hein (1971), Irizary-Rovira (2004) and Wakenell (2010) recorded a significant reduction in total erythrocyte count, packed cell volume and haemoglobin in coccidiosis infected chicken. However different supplements as amprolium and madar leaf powder ameliorate the TEC reduction, similar results were noted by DU and DU (2002).

Increase in MCV and reduction in MCH and MCHC in infected groups in present study revealed macrocytic hypochromic anaemia in infected groups, similarly Singh *et al.* (2008b) also found that MCV, MCH and MCHC in coccidiosis infected groups resulted in macrocytic hypochromic anaemia from 5th to 7th DPI. Singh *et al.* (2007) also had similar findings as present study, he also recorded increased MCV along with decreased MCH and MCHC in infected control as well as infected supplemented groups than their respective uninfected groups on 6DPI.

TLC on 15th day of experiment or 0 DPI was non significantly different among all groups and was maximum in madar leaf powder supplemented group. Mossa *et al.* (1991) also recorded non significantly higher TLC in *Calotropis procera* aerial parts supplemented rats than the unsupplemented. Higher TLC value in infected groups may be due to increase in peripheral blood leukocyte in response to the infection with *Eimeria* spp. (Rose *et al.*, 1979). Similar observations have been made by Jaipurkar *et al.* (2004) using 3 indigenous herbal drugs against caecal coccidiosis.

On 15th day of experiment or 0 DPI, Heterophil %, lymphocyte %, eiosinophil % and monocyte % in broilers of all groups showed non significant differences but after introduction of infection heterophil % and monocyte % were decreased whereas lymphocyte % and eosinophil % were increased in infected groups. Adamu *et al.* (2013) revealed monocytosis, lymphocytosis, heterophilia and eosinophilia in broiler chickens infected with *E.tenella* and *E.brunetti*. Singh *et al.* (2007) and Singh *et al.* (2008a) also found the reduction in heterophil % and increment in lymphocyte % in infected groups than the respective uninfected groups on 6 DPI and 14 DPI.

Results of present experiment showed that haematological parameters among all groups were non significant on 0 DPI, indicating negligible effect of different supplementations on hematology during pre infection period, however after introduction of infection significant differences were noticed in infected groups as Hb, PCV, TEC, MCH, MCHC, heterophils % and monocytes % were decreased, whereas MCV, lymphocytes % and eosinophils % were increased in infected groups than respective uninfected groups. Haematological parameters restoration on 5 DPI and 10 DPI was maximum in infected amprolium supplemented group followed by madar leaf powder supplemented groups and concentration dependent effect of madar leaf powder was not recorded on all parameters. On 15 DPI haematological parameters showed non significant differences among all infected and uninfected groups.

It is concluded that haematological parameters restoration capacity of madar leaf powder and amprolium during *Eimeria* sp. infection indicates their excellent ability to control blood loss caused by coccidiosis, so it can be concluded that similar to amprolium, madar leaf powder supplementation also can be used to prevent coccidiosis in broilers prevalent in field conditions.

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In vitro antioxidant activity of seeds of *Entada phaseoloides* Linn. Merrill.

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Abstract

This study comprised of an *in vitro* antioxidant activity of the methanolic extract of the seeds of *Entada phaseolioides* that has been analyzed by 4 *in vitro* antioxidant models *viz.* Hydroxyl radical, Nitric oxide, Superoxide radical scavenging activity and Reductive ability assay. The methanolic extract of *Entada phaseoloides* exhibited a significant ascending trend of percentage inhibition with maximum activity at 60 ìg/ml and 80 ìg/ml concentration for all the four models of free radical scavenging activity. The results demonstrated that the seeds of *Entada phaseoloides* exhibit an excellent antioxidant activity which might be an alternative to the synthetic antioxidants available in the market.

Keywords*:* Antioxidant activity, *Entada phaseoloides*, Methanolic extract, Seed

Antioxidants play an important role in human health and nutrition, as they are known to protect the body against reactive oxygen species (ROS) (Martinez-Maqueda *et al.*, 2012). ROS are chemically reactive molecules containing oxygen which include free radicals such as superoxide (O^{2-}), hydroxyl radical (OH), peroxyl radical (ROO) as well as non-radical species such as hydrogen peroxide (H_2O_2). ROS are either formed as a natural byproduct from normal metabolism of oxygen or generated by exogenous sources such as ionizing radiation and have important roles in cell signaling and homeostasis (Devasagayam *et al.*, 2004). However, during environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically resulting in significant damage to cell structures, which is cumulatively known as oxidative stress. The generation of oxidative stress is harmful to the body and may cause peroxidation of membrane lipids leading to loss of membrane integrity and cell death, denaturation of proteins including enzymes and ion channels and strand breakage in DNA (Hussain *et al.*, 1987). Free radicals are highly reactive molecules derived from the normal metabolism of oxygen, or from exogenous factors and agents. They can attack unsaturated fatty acids in the biomembrane, resulting in membrane lipid peroxidation, decrease in membrane fluidity, loss of enzyme and receptor activities, and damage to membrane proteins leading to cell inactivation (Dong *et al.*, 2012).

The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, singlet oxygen quenchers and metal chelators (Joyce, 1987). Symmetry is maintained between the oxidants generated and the antioxidants produced naturally in the human body. However, due to overproduction of oxidants or inadequate supply of nutritional supplements, the equilibrium is hindered resulting in oxidative hassles. Now a days numerous crude extracts and pure natural compounds have been reported to possess antioxidant properties (Jayaprakasha *et al.*, 2003). As a consequence, dietary source in general is important for providing antioxidants, which helps in reducing the oxidative stress caused by free radicals. There has been increasing interests in natural antioxidants and their potential health benefits. Hence, the present work was undertaken to study the antioxidant activities of seeds of *Entada phaseoloides*.

Materials and methods

Plant materials

The dried seeds of *Entada phaseoloides* were collected from local market and identified by a taxonomist.

Extracts preparation

One kg of dried seed powder of *Entada phaseoloides* was dipped in 3 L methanol in a 5 L round bottom flask and the mixture was mechanically stirred at room temperature once daily for 3 days. Then filtered off the solid residue and concentrated the filtrate under vacuum at 50°C using rotary evaporator (Rotavapor R-210, Buchi). Again, the solid residue was dipped in sufficient amount of methanol for 3 days and is repeated for three occasions *i.e.* a total of 9 days in order to avoid any wastage of extract. The concentrated extract was dried in water bath for 1 hour at 50°C and finally 200 g of crude methanol extract was obtained achieving a percentage yield of 20.

Nitric Oxide Scavenging Activity

Free radical scavenging activity was estimated by nitric oxide scavenging test using sodium nitroprusside generating NO was compared with their parent compound according to the method described by Sreejayan and Rao (1997). Sodium nitroprusside was mixed with 1 ml plant extract of different concentrations (1, 3, 6, 9, 20, 40, 60, 80 µg/ml) prepared in phosphate buffer. The mixture was subsequently incubated at 25ºC for 150 min. This was followed by addition of 1 ml of Griess reagent to the incubated mixture. Absorbance was measured at 546 nm. Ascorbic acid was used as reference standard. Percentage inhibition was calculated as:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$$

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in the presence of the sample.

Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radical was measured by studying the competition between deoxyribose and the test extracts for the hydroxyl radical generated by Fenton's reaction according to the method reported by Halliwell *et al.,* (1987). The reaction mixture contained 0.2 ml plant extract of different concentrations (3, 9, 20, 40, 60, 80, 100, 110 µg/ml), 0.2 ml of EDTA (1.04 mmol/l), 0.2 ml of $FeCl_3$ (0.2 mmol), 0.2 ml of 2-deoxyribose (2.8 mmol), 0.2 ml of ascorbic acid (1 mmol) and 0.2 ml of H_2O_2 (10 mmol). After incubation at 37 °C for 1 h, 1 ml of cold TBA (2.8%) was added to the reaction mixture followed by 1 ml TCA (1%). The mixture was heated at 100 °C for 15 min and then cooled. The scavenging percentage was calculated according to the following formula:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$$

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in the presence of the sample.

Superoxide radical scavenging activity

Superoxide anion scavenging activity was measured according to Robak & Gryglewki (1998) with some modifications. The assay is based on the inhibition of the production of nitroblue tetrazolium formazon of the superoxide ion. The reaction mixture contained 1 ml NBT (156 µM), 1 ml NADH (468 mM) and 3 ml plant extracts of different concentrations (3, 9, 20, 40, 60, 80, 100, 110 µg/ml). The reaction was started by adding 0.1 ml PMS (60 mM). The mixture was incubated at 25ºC for 5 min and absorbance measured at 560 nm. The scavenging percentage was calculated according to the following formula:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$$

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in the presence of the sample.

Reducing power assay

Reducing power of plant extract is based on the ability of antioxidants to form coloured complex with potassium ferricyanide, TCA and $FeCl_3$ according to previous method by Jayaprakash *et al.,* (2001). In this test, 1 ml of different concentration of the plant extracts (100, 200, 300, 400, 500, 600 µg/ml) were added to 2.5 ml K_4FeCN_6 (1%) and 2.5 ml phosphate buffer (pH 6.6). The mixture was then incubated at 50ºC for 20 min. The reaction was stopped by adding 2.5 ml TCA (1%) and centrifuged at 3000 rpm. Then, 2.5 ml supernatant was added to 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%). Absorbance was measured at 700 nm. The percentage of superoxide radical scavenging activity was calculated as:

$$\%\text{Antioxidant activity} = [(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$$

Where $Abs_{control}$ is the absorbance of the control reaction and Abs_{test} is the absorbance in presence of the sample.

Statistical analysis

Data were presented as the mean (±SEM) of triplicate tests. The graphs were prepared using Graph Pad Prism 6 (version 6.03). Statistical analysis was performed with two-way analysis of variance (ANOVA).

Results and discussion

Nitric Oxide Scavenging Activity

Nitric oxide is spontaneously generated from sodium nitroprusside in aqueous solutions that reacts with oxygen (O_2) to produce nitrite (NO_2) and nitrate

(NO_3) which can be estimated by Griess reagent. Scavengers of NO compete with O leading to a decreased production of NO (Balakrishnan *et al.*, 2009). It was seen that the generation of NO was suppressed in a dose dependent manner by the extracts. The methanol extract of *Entada phaseoloides* showed good nitric oxide scavenging activity and had an IC_{50} value of 21.13 µg/ml almost comparable to the reference standard, which has IC_{50} value of 17.39 µg/ml indicating an excellent nitric oxide scavenging potential as represented in Fig:1.

Hydroxyl radical scavenging activity

Hydroxyl radical is an extremely reactive and highly damaging free radical species, which has the capacity to join nucleotides and cause strand breakage in DNA leading to possible carcinogenesis, cytotoxicity and mutagenesis (Moskovitz *et al.*, 2002). Ferric-Ascorbate-H_2O_2-EDTA, according to Fenton reaction forms hydroxyl radical that on generation reacts with deoxyribose to form thiobarbituric acid reactive substance (TBARS). On heating with TBA a pink chromogen is formed. Free radical scavengers compete with deoxyribose for hydroxyl radicals and thus cause a reduction in the color formation. The extracts showed inhibition of deoxyribose degradation in an increasing dose dependent manner. The methanol extract showed good scavenging activity with IC_{50} value of 9.42 µg/ml and that of standard is of 6.50 µg/ml as shown in fig:2

Superoxide radical scavenging activity

Superoxide anion is an initial free radical and a weak oxidant that ultimately produces stronger oxidative species such as singlet oxygen species and hydroxyl radicals (Stieff, 2003). The extracts showed a potent superoxide scavenging activity in a concentration dependent manner when compared with ascorbic acid standard at similar concentrations. Similar findings was also reported by Dong *et al.*, (2012) who observed potent antioxidant activity in the ethanol extract of stem of *Entada phaseoloides* when assayed by Superoxide radical scavenging assay. Methanol extract exhibited IC_{50} value of 10.25 µg/ml that of standard is of 10.71 µg/ml and no significance difference ($P<0.05$) was observed between them. Thus, the methanol extract of seeds of *Entada phaseoloides* showed the best superoxide radical scavenging activity as shown in Fig:3

Reducing power assay

The extracts of *E. phaseoloides* showed reductive ability by reducing Fe^{3+}ferricyanide complex

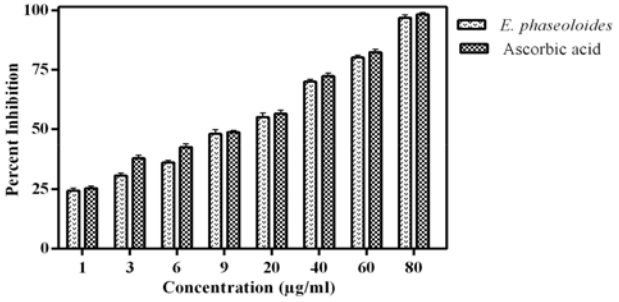

Fig.1 *Nitric Oxide Scavenging Activity* of *E.Phaseoloides*

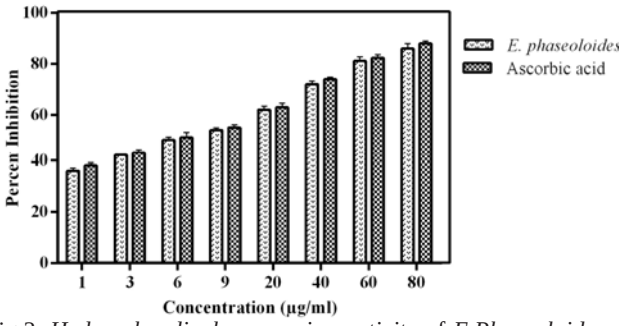

Fig.2. *Hydroxyl radical scavenging activity* of *E.Phaseoloides*

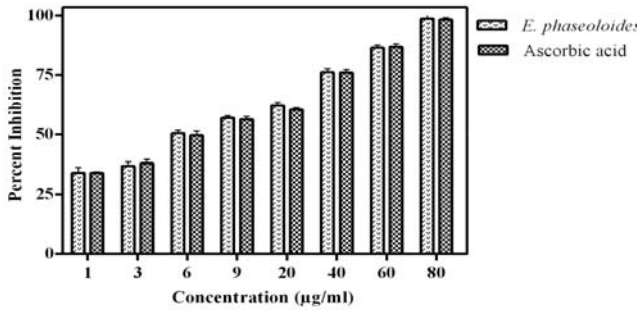

Fig.3. *Superoxide radical scavenging activity* of *E.Phaseoloides*.

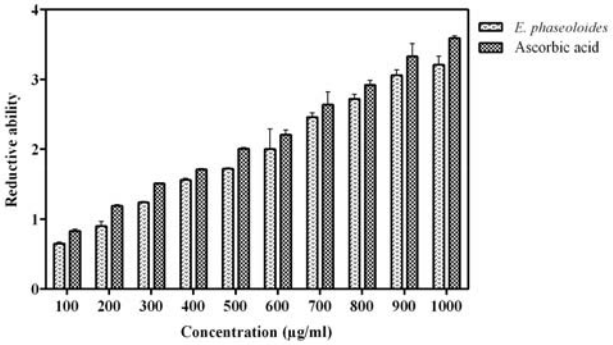

Fig.4. Reducing power assay *E.Phaseoloides*.

to Fe^{2+} (Oyaizu, 1986) in a dose dependent manner but reductive ability of the extracts increased with increasing concentration. Assessment of the reductive ability serves as an important indicator of the antioxidant potential. The reductive ability of the extracts might be due to the presence of reduction ions. The methanol extracts of *Entada phaseoloides* have shown potent reductive ability at various concentrations and is compared with that of the standard. No significance difference ($P<0.05$) was observed between them. However, Dong *et al.*, (2012) also reported a good reducing ability in the ethanol extracts of the stems of *Entada phaseoloides* as represented in Fig:4

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Visceral gout in commercial broiler chicks: an epidemiological study

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Abstract

The present investigation was undertaken to study different epidemiological aspects in visceral gout affected commercial broiler flocks. A total population of 6, 25, 603 broiler birds suffering from visceral gout were selected for this study with flock size ranging from 2,230 to 33,670 and a total mortality observed was 35,346 amounting to 5.65%. The per cent of mortality due to visceral gout increased with increase in flock size, during first week of age, when charcoal type of brooding was employed, with low as well as high brooder temperature and with poor brooder and litter management. A statistically significant (P<0.05) difference was observed between the mortality per cent due to visceral gout in flocks fed with different aflatoxin B1 and crude protein levels.

Keywords: Broiler chicks, Mortality, Visceral gout.

Visceral gout is a clinical manifestation of severe renal function disorder with hyperuricemia which may result in the precipitation of urate crystals on the visceral surfaces (Sodhi *et al.*, 2006). The gout is characterized by retention and build up of urates in tissues. The causes of gout are many which can be broadly categorized as nutritional and metabolic causes, infectious causes and other causes like mycotoxins. The present work was undertaken to explore different epidemiological factors affecting mortality in visceral gout in commercial broiler flocks.

Materials and Methods

The epidemiology of visceral gout was studied in 52 commercial broiler flocks located in and around Bangalore and Shimoga districts of Karnataka with a total population of 6,25,603 broiler bird. Information with respect to total mortality, flock size, age of onset, type of brooding employed, brooder management, brooder temperature and litter management were collected. Feed samples were collected from gout affected farms and analyzed for its aflatoxin B1 content, calcium content and crude protein content. Aflatoxin B1 content in feed samples was extracted as per Hesseltine *et al.* (1968) and was quantified using Thin Layer Chromatography (TLC) spot plate technique as outlined in AOAC, (1995). Crude protein in feed sample was determined by the Kjeldahl method as described by AOAC, (1995). Calcium estimation in feed sample was done by volumetric determination of calcium by titration of the oxalate with potassium permanganate as described by Scott, (1917).

Results

In the present study, total mortality observed due to visceral gout in these 52 flocks was 35,346 amounting to 5.65 % mortality with a range of 0.23 - 18.28 %.

The broiler flocks were classified on the basis of flock size as Small (< 5000), Medium (5001-10,000), Large (10001-20,000) and extra-large (> 20,000). The study revealed that mortality due to visceral gout was highest in extra-large flocks (7.21 %), followed by large flocks (5.42 %) then in medium sized flocks (4.54 %) and the least mortality was observed in small flocks (1.28 %). There was a significant (P<0.05) difference between mean mortality per cent of different flock size indicating that per cent of mortality due to visceral gout increases with increase in flock size.

Age wise mortality due to visceral gout during first week (0-7 days) of age was 6.92 % whereas during second week (8-14 days) of age was 5.07 % and there was a statistically significant (P<0.05) difference observed between mortality per cent in different age groups and a statistically higher mortality was noticed during first week of age.

Out of 52 commercial broiler flocks, 27 flocks were maintained under drum type of brooding, 13 flocks were maintained using charcoal type of brooding and 12 flocks were using electrical brooders. Mortality % were found to be 4.73, 8.10 and 4.65 with drum, charcoal and electric type of brooding respectively and there was a statistically significant (P<0.05) difference between flocks reared under different type of brooding. The results of this study indicated that mortality was highest when charcoal type of brooding was employed and least mortality was noticed in flocks maintained under electrical brooding.

Brooder management was classified as good, fair and poor on the basis of brooder house stock density, feeder and waterer distribution, distribution of chicks, brooder guard availability and presence or absence of rounded off corners. Mortality % due to visceral gout was found to be 4.76, 5.89 and 10.12 in flocks with good, fair and poor brooder management respectively. There was a statistically significant ($P<0.05$) difference between mortality per cent in flocks with different levels of brooder management indicating that maximum % of mortality was observed in flocks with poor brooder management and thus brooder management is one of the important factor influencing mortality due to visceral gout in commercial broiler chicks.

Brooder temperature was classified as high, optimal and low. The optimal brooder temperature for the first week and second week was considered (95±1°F) and (90±1°F) respectively. Mortality % due to visceral gout was 4.61 in flocks maintained under optimal brooder temperature whereas 6.30 and 7.81 % mortality was noticed in flocks maintained at high and low brooder temperature respectively. A significant ($P<0.05$) difference was noted with respect to mortality % and it was found to be high when the brooder temperature was low followed by high brooder temperature. This indicated that low as well as high brooder temperature increases the mortality in broiler chicks due to visceral gout.

Litter management was categorized as good, fair and poor on the basis of litter material condition, thickness of litter material and appreciable ammonia level in litter material. The mortality % in flocks under good, fair and poor litter management were 3.99, 4.82 and 9.71 respectively. A significant ($P<0.05$) difference was noted with respect to mortality per cent in different levels of litter management as poor litter management resulted in higher mortality rate due to visceral gout.

Aflatoxin B1 levels ranged from Traces - 0.100 ppm. These flocks were classified on the basis of aflatoxin B1 level in feed as Group I (Traces-0.02 ppm) and Group II (0.02-0.1 ppm), with 0.02 ppm being considered as the maximum permissible limit in broiler starter ration (BIS, 1992). Mortality % due to visceral gout observed was 5.0 and 5.97 in Group I and Group II respectively and a statistically significant ($P<0.05$) difference was observed between the mortality % in flocks with different aflatoxin levels.

Calcium levels ranged from 0.9 - 1.26 %. These flocks were classified on the basis of calcium level in feed as Group I (0 - 1.2 %) and Group II (1.21- 1.26 %) as per BIS, (1992) recommendation of 1.2 % inclusion in the broiler starter ration. Mortality % due to visceral gout was 5.65 and 5.63 in Group I and Group II respectively and no significant ($P>0.05$) difference was noticed when two groups were compared indicating that Ca level in feed up to 1.26 % had no influence on mortality due to visceral gout in broiler chicks.

Crude protein levels ranged from 14.51–22.00%. These flocks were categorized in to different groups based on crude protein level in feed as Group I (14-16 %), Group II (16-20 %) and Group III (20-22 %) based on recommendations of BIS, (1992) for 16-20 % crude protein as most optimal for 0-6 weeks broiler starter ration. Mortality % due to visceral gout was observed to be 5.02, 4.55 and 6.21 for Group I, Group II and Group III respectively. A significant ($P<0.05$) difference was observed between mortality % in flocks with different levels of crude protein level in the feed samples of broiler starter ration indicating that crude protein levels either less than 16 % or more than 20 % are associated with increased mortality due to visceral gout in broiler chicks.

Discussion

The earlier reports of mortality due to visceral gout in broiler chicks were 17.90 % (Rao *et al.,* 1993) and 8.85 % (Kumar *et al.,* 2008). The variation in mortality due to visceral gout observed in present study may be attributed to managemental practices adopted and different lines of broiler chicks studied.

The mortality due to visceral gout increased with increase in flock size. This observation agrees with the findings of Reece *et al.* (1981). Increase in mortality may be attributed to feed and water intake due to elevated plasma cortisone level (Pesti and Howarth, 1982).

Visceral gout in broiler chicks was noted during first and second week of age and was not recorded in flocks aged more than 2 weeks. This observation agrees with the findings of Uma *et al.* (1996); Kumar *et al.* (2008) and Singh *et al.* (2013). Chicks during the first week of age are in the process of adapting to the environment and are subjected to higher degree of stress (Singh *et al.,* 2013). Hence chicks are more susceptible to various disease conditions including visceral gout

(Kumar *et al.,* 2008).

The observation of higher mortality due to visceral gout in broiler chicks reared under charcoal type of brooding may be attributed to production of noxious gases such as carbondioxide and carbonmonoxide, when charcoal is burnt to provide heat to broiler chicks (Antony, 2012). Further, the initial brooding temperature remains comparatively high (Hassanuzzaman, 2004), resulting in dehydration of chicks and thus increasing the severity of visceral gout resulting in higher mortality. In drum type of brooding, gases produced due to burning of wood material is let out of the poultry shed through a chimney and this may be the reason for comparatively lower mortality. In electrical type of brooding no noxious gases are produced as only air is heated due to incandescent bulbs which spreads required amount of heat uniformly above a large area and avoids huddling of chicks directly under the brooder.

Deterioration in brooder management quality resulted in higher mortality due to visceral gout. This observation agrees with the findings of Caveny and Quarles, (1978) and Reece *et al.* (1980). The exposure of broiler chicks to ammonia concentration of more than 50 ppm during brooding period reduces feed efficiency (Caveny and Quarles, 1978) leading to starvation which lowers plasma concentration of hydroxy butyrates and increased uric acid concentration and increased protein catabolism (Kaneko *et al.,* 2008) resulting in increased mortality.

Optimal brooder temperature is very essential to maintain the health and performance of brooder chicks. Higher brooder temperature leads to dehydration which can lead to visceral gout (Jana *et al.,* 2009; Crespo and Shivaprasad, 2012). On the other hand low brooder temperature results in huddling of chicks, reduced feed and water intake and have impaired immune and digestive system and increased susceptibility to metabolic disorders like visceral gout (Fairchild, 2012).

There was a drastic increase in mortality rate in flocks with poor litter management. Poor litter management leads to accumulation of ammonia and carbon monoxide in poultry shed which affects normal ventilation of poultry house and reduces water intake. Reduced water intake can lead to increased incidence of visceral gout in broiler chicks. Inadequate water intake results in inefficient flushing out of urinary system thus increasing incidence and severity of visceral gout in broiler chicks (Singh *et al.,* 2013).

Higher aflatoxin B1 level in the broiler starter ration was found to be associated with increased mortality due to visceral gout in flocks. Aflatoxin is a cumulative nephrotoxin which affects normal renal excretory functions leading to visceral gout (Gupta *et al.,* 2002). Aflatoxicosis produces a primary lesion, kidney damage like, mild to moderate degree of congestion, multifocal areas of haemorrhages and tubular epithelial degeneration (Senthil Kumar, 2007; Mohmand, 2010).

Calcium level in feed up to the level of 1.26 % had no influence on mortality due to visceral gout in broiler chicks. This agrees with the findings of Wideman *et al.* (1993) who reported that plasma uric acid concentration are not directly related to calcium induced kidney damage leading to visceral gout. Further, kidney damage is noticed in broiler chicks when calcium content in feed is in the range of 2-3 per cent (Ansar *et al.,* 2004) and so no significant difference was observed when the calcium level in the broiler starter feed in the present study was in the range of 0-1.26 %.

The present study indicated that high as well as low crude protein levels in the broiler feed is associated with increased mortality due to visceral gout in broiler chicks. High levels of protein especially of animal origin in poultry feed have been reported to cause gouty nephritis in poultry (Nayak *et al.,* 1988; Rao *et al.,* 1993). High protein diet results in higher plasma uric acid concentrations (Hockings and Bernard, 1997) and increases kidney size and glomerular filtration rate, with subsequent glomerular injury, accumulation of mesengial deposits and glomerulosclerosis (Khan and Alden, 2001). On the other hand low dietary protein is reported to be associated with renal insufficiency due to renal lipidosis (Pollock, 2006). Impaired kidney functions results in excessive accumulation of uric acid in tissues (Eldaghayes *et al.,* 2010). Uric acid itself is not toxic but precipitated crystals can cause severe mechanical damage to tissues like kidney, heart, lungs and intestine.

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Benzimidazole resistance in parasitic nematodes of small ruminants in Odisha

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Abstract

The study was undertaken to detect the status of benzimidazole resistance in gastrointestinal nematodes of small ruminants. Five farms (three sheep farms and two goat farms) were selected based on the mean FEC of the animals, i.e. e"200 egg per gram (EPG) for conducting faecal egg count reduction test (FECRT) and egg hatch test (EHT). FECRT data showed resistance in OUAT goat farm and it was suspected in Satakania sheep farm. Daruthenga Sheep farm, Daruthenga Goat farm and Pokhariput Sheep farm expressed susceptiblility to BZs. Pre and post treatment faecal culture of pooled sample from each farm were also done which revealed *Haemonchus* species as the predominant species among the GI nematodes. EHT data showed resistance in two of the farms (OUAT Goat farm and Satakania sheep farm) and others were found susceptible to benzimidazoles.

Keywords*:* Benzimidazole, resistance, FECRT, Egg hatch test, Odisha

Anthelmintic resistance in parasitic nematodes is a serious threat to sustainable livestock production worldwide. *Haemonchus contortus* and *Teladorsagia circumcincta* are the two important species of parasitic nematodes of sheep which showed resistance to all broad spectrum anthelmintic classes (Kaplan and Vidya sankar, 2012). Benzimidazole (B_2) derivatives are broad spectrum anthelmintics because of their diverse biological activity and were effective clinical applications (Tonelli *et al.*, 2012). Due to intensive use of benzimidazoles since 1970s, resistance is very common to this group of anthelmintics (Silvestre and Humbert, 2000; Garg and Yadav, 2009). The common methods for diagnosis of BZ resistance in trichostrongyle parasites are faecal egg count reduction test (FECRT), egg hatch test (EHT), larval development assay (LDA), and AS-PCR (Coles *et al.*,1992). Though FECRT is the recommended test to surveys for resistance by WAAVP, it is time consuming and expensive. Therefore, *in vitro* tests like egg hatch test (EHT) and larval development assay (LDA) are now increasingly used as an alternate for large surveys of BZ resistance (Ancheta *et al.*, 2004).

Resistance to anthelmintics has been reported earlier from different parts of world (Acosta *et al.*, 2012 and Paraud *et al.*, 2009) including North India (Garg and Yadav, 2009, Rialch *et al.*, 2013). But there is no available report of resistance to anthelmintics in eastern part of India, particularly in the state of Odisha. Therefore, the present study was undertaken to investigate the status of BZ resistance in *H. contortus* of small ruminants reared in organized Govt. and private farms in Odisha.

Materials and methods

During the present study, seven sheep farms and five goat farms in and around Bhubaneswar were randomly screened for the presence of gastrointestinal nematodes (GIN). Out of these five farms were finally selected for conducting the faecal egg count reduction test and egg hatch test based on their mean faecal egg count (FEC) exceeding 200 eggs per gram (EPG). The selected flocks were not dewormed at least 15 weeks before conducting the tests.

FECRT was carried out as per the procedure given by Coles *et al.* (1992). Twenty animals of 3-6 months age from each flock were randomly selected and allocated into two groups (Treatment and Control group) of ten animals. Selected animals were not treated with any anthelmintics 15-20 weeks before the commencement of the test. On day zero (D_0), animals of treatment group were treated with Albendazole oral suspension (2.5% w/v) (Karnataka Antibiotics and Pharmaceuticals Ltd.) as per the manufacturer's recommended dose. Pre treatment FEC at D_0 and post treatment FEC at D_{10} of the selected animals was performed using modified Mc Master technique. For identification of resistant and susceptible species of GINs, faecal culture of the pooled faecal samples collected from selected animals of both treated and control group was done separately as per the procedure given by Sahai (1960) with some minor modifications. The identification of L3 larvae collected in Bearman's technique was done based on their morphology and morphometry (Van Wyk and Mayhew, 2013) and the

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percentage of *Haemonchus* larvae was counted.

Pooled faecal samples from each selected flocks were also collected to carry out EHT. These samples were either used within 3 hrs of collection or were stored an-aerobically as described by Hunt and Taylor (1989) and used within 7 days of collection. Nematode eggs were collected by repeated centrifugal floatation technique (Coles *et al.*, 1992) and the final concentration was made up to 250-300 eggs/100 µl of water. EHT was carried out as described by Le Jambre (1976) with some minor modifications (Taylor *et al.*, 2002).

Pure thiabendazole (50 mg) was taken in a 100 ml beaker to which 50 ml of dimethyl sulfoxide (DMSO) was added and thoroughly mixed to prepare a stock solution of concentration 1000 ppm of thiabendazole. Then a wide range of working solutions of TBZ were prepared by serial dilution of this solution to obtain final concentration of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 1 µg/ml. To each eppendorff tube, 20 µl of egg suspension and 10 µl of working solution of TBZ was added except the control tubes where 10 µl of DMSO 99 percent was only added. The volume in each tube was made up to 2 ml by adding distilled water. The test was carried out with two replicates for each drug concentration along with a control tube. After incubation at 27^0C for 48 hours, one drop of Lugol's iodine was added to check further embryonation of eggs and at least a total of 100 parasitic stages (dead or unembryonated eggs, and hatched larvae) were counted under an inverted binocular compound microscope at 10 X objective. The number of dead eggs and larvae at each concentration of TBZ was counted and hatching percentage was derived using the formula:

$$\text{Hatching \%} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs added}} \text{ X } 100$$

In FECRT, a reduction in faecal egg count less than 95% as well as lower 95% confidence level below 90 was taken as criteria to indicate the presence of anthelmintic resistant nematodes in the treated population (Coles *et al.*, 1992). In cases where only one of the two criteria was met, resistance was then suspected. In EHT**,** Effective Dose 50 (ED_{50}) value was calculated for the eggs by log probit analysis (Finney, 1971). Eggs having ED_{50} value exceeding 0.1 µg BZ anthelmintic per ml was indicative of resistance against BZ (Coles *et al.*, 1992).

Statistical analysis of the data obtained in the study was done as described by Snedecor and Cochran (1994). The t-test for paired samples having means with unequal variances was carried out (Using SPSS version 17.0). Variables with $p<0.05$, $p<0.01$ and $p>0.05$ were considered as statistically "significant", "highly significant" and "non-significant" respectively.

Results

Resistance to benzimidazole in the gastro-intestinal nematodes was found to be varied in different farm condition. The percentage reduction in mean faecal egg count among the five farms ranged from 90.63 (OG) to 97.44 (DG) (Table-I). Pre-treatment coproculture revealed predominantly L_3 of *Haemonchus* species, followed by *Trichostrongylus* spp., *Strongyloide*s and *Oesophagostomum*, while post treatment coproculture results showed that mostly *H. contortus* and *Strongyloides* survived albendazole (Table-II). ED_{50} values of Thiabendazole used for egg hatch test in different flocks of sheep and goat are given in Table-III.

High significant difference between the mean EPG of treatment and control group animals were found in this study. The correlation between the mean FEC of faeces with number of larvae hatched per gram of faeces in coproculture was found to be positive and significant

Table I: Farm-wise mean FEC, FECR% and resistance status

Sl. No.	Farm Name	Mean (±SE) of FEC		95% C.I.		FECR%	Remark
		Control (Xc)	Post* (Xt)	Upper	Lower		
1	SS	895 ±83.81	40 ±16.33	98	89	95.53	Suspected**
2	DG	585 ±69.14	15 ±7.64	99	92	97.44	Susceptible**
3	DS	530 ±62.89	20 ±8.16	98	91	96.23	Susceptible**
4	OG	480 ±47.26	45 ±20.34	96	75	90.63	Resistant**
5	PS	675 ±52.31	25 ±11.18	99	90	96.30	Susceptible**

N.B: FEC= Faecal Egg Count, FECR=Feacal Egg Count Reduction, C.I.= Confidence Interval, SE= standard error, **= Highly significant

Table II: Percentage of different species of Larvae in Coproculture (Pre and Post treatment)

Farm Name	No of Larvae counted		Percentage of Larval Count							
			Haemonchus species		*Trichostrongylus*		*Strongyloides*		*Oesophagostomum*	
	Pre*	Post*	Pre*	Post*	Pre*	Post*	Pre*	Post*	Pre*	Post*
SS	122	11	78.69	54.55	8.20	9.09	8.20	36.36	4.92	0
DG	111	5	82.88	20.00	6.31	40.00	6.31	40.00	4.50	0
DS	108	6	64.81	50.00	12.96	0.00	8.33	50.00	13.89	0
OG	109	26	71.56	73.08	10.09	3.85	9.17	23.08	9.17	0
PS	100	4	76.00	75.00	17.00	0.00	7.00	25.00	0.00	0
Total	550	52	74.91	61.54	10.73	7.69	7.82	30.77	6.55	0

N.B: Pre*-Pre treatment, Post*-Post treatment, SS-Satakania sheep farm, DG-Daruthenga goat farm, OG-OUAT goat farm, PS-Pokhariput sheep Farm.

Table III: ED_{50} values and the resistance status of different farms in EHT

Farm name	ED_{50} Values (µg/ml)	Resistance status
Satakania sheep (Ss)	0.129	Resistant
Daruthenga goat (Dg)	0.071	Susceptible
Daruthenga sheep (Ds)	0.089	Susceptible
OUAT goats (Og)	0.141	Resistant
Pokhariput sheep (Ps)	0.089	Susceptible

(r= +0.34).

Discussion

Benzimidazole group of drugs were the most commonly used, possibly because of their low cost as compared to other anthelmintics (Garg and Yadav, 2008 and Leignel *et al.*, 2010). The results of the present study revealed that, resistance developed among the parasitic nematodes of small ruminants was mostly due to *Haemonchus* species of nematode, which agrees with the findings of previous workers (Bakunzi, 2008). But three of the selected farms (Dg, DS and PS) also showed susceptibility towards BZ resistance in our study. This may be due to the possible under or over dosing of the selected animals (Vatta and Lindberg, 2006) because there was no weight estimates for determining the treatment dosages. As mentioned earlier, Anthelmintic resistance (AR) in small ruminants industry is an alarming situation throughout the world (Vatta and Lindberg 2006). Therefore, introduction of resistant strains from larger commercial farms to smaller farms might have been the source of detected AR, particularly in those animals that were never treated with anthelmintics by their owners.

The flocks of sheep and goats found either susceptible or resistant in FECRT were also found susceptible and resistant in EHT respectively except the Satakania sheep farm (suspected for resistance in FECRT, but found resistant in EHT) (Table-I and Table-III). These findings differed from those of Maingi *et al.* (1998) who found two third of BZ susceptible isolates in FECRT as resistant in EHT. The correlation between these tests is not always good. Maharshi *et al.* (2011) in Rajasthan while studying the status of BZ resistance in GIN of sheep from the organized farms and farmer's field using FECRT and EHT, observed a poor linear correlation between the two tests. The variations in results of different tests might be attributed to the fact that anthelmintic drugs act on adult worms in FECRT and eggs in EHT. Also immunological factors and the pharmacokinetics of drug in different hosts might cause the difference in results (von Samson-Himmelstjerna *et al.*, 2009).

In most part of the world including Odisha, it is obvious that farmers only treat the sick animals. This practice is encouraged as it leads to delay the AR development and thus considered as a control measure. This approach reduces the expenses in terms of money and labour used for anthelmintics application. Furthermore, "the proportions of resistant worms will be greatly diluted in refugia as the effectively dewormed animals will be voiding less number of resistant worms as compared to the number of susceptible worms voided by the untreated animals" (Varady *et al.*, 2011). Therefore, in spite of the use of BZs for such a long time, the development of resistant nematodes are not predominant in this study area. Furthermore, it is recommended that farmers should reduce their dependence on conventional anthelmintic drugs as the only method of control of GINs. Instead, one or more alternative approaches of nematode control along with selective use of anthelmintics must be adapted to delay

the BZ resistance.

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Short Communication

Clinico-haematological studies on Theileriosis in crossbred cattle in and around Bhubaneswar

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Abstract

Blood samples of 5237 numbers from clinically suspected cases of theileriosis were screened by blood smear examination for presence of piroplasms inside RBCs or Koch's blue bodies inside lymphocytes over a period of three years from March 2012 to February 2015 from areas in and around Bhubaneswar. The blood samples from all 3876 positive cases were examined for haematology and 93 serum samples from positive cases were used for estimation biochemical estimation. The important clinical signs observed were reduced appetite, anorexia, non remittent fever, enlargement of superficial lymph nodes, salivation, lacrimation, recumbence, pale conjunctiva, pale mucous membrane, dropped milk yield, debility, respiratory distress and coughing with nasal discharges. There was significant decrease in Hb%, PCV, TEC values and significant increase in Lymphocyte % in the affected cattle when compared to apparently healthy cattle. There was significant decrease in serum glucose, cholesterol, protein, albumin values and significant increase in blood urea nitrogen, creatinine, AST, ALT, total billirubin and direct billirubinwere in the affected cattle when compared to apparently healthy cattle. it may be concluded that monitoring of this disease through clinical signs and haematobiochemical alterations will be helpful for better diagnosis, prognosis and management of metabolic health status of the animal during the disease leading to increased production and reduced mortality in cattle in this region.

Keywords*:* Cattle, clinical signs, clinical biochemical, haematology, theileriosis,

Theileriosis has emerged as one of the fatal disease of exotic and crossbred cattle in recent years. The indigenous cattle in endemic areas, on recovery from natural mild infections, become resistant to reinfection and a persistent carrier state is developed. They are potentially dangerous to uninfected exotic and crossbred animals, by acting as reservoirs of infection (Brown, 1990). Crossbreeding and upgrading programmes resulting in enhanced milk production. Simultaneously, theileriosis has emerged as one of the fatal diseases of crossbred cattle threatening the sustainance of the success in white revolution (Singh, 2002). Therefore the present investigation was undertaken to study the clinical, haematological and biochemical changes in the naturally infected cattle which can be utilised for diagnosis, prognosis and for better management of metabolic health status of the animal during the disease.

Materials and Methods

Blood samples were collected from 5237 numbers of clinically suspected cases of theileriosis through the field veterinarians and also received at Department of Veterinary Pathology and Teaching Veterinary Clinical Complex referred by Veterinary clinicians over a period of three years from March 2012 to February 2015 from areas in and around Bhubaneswar. The suspected blood samples were screened for theileriosis by blood smear examination with presence of piroplasms inside RBCs indicating *Theileria annulata* and in few cases Koch's blue bodies inside lymphocytes in positive cases (Soulsby 1982).

The blood samples of all the positive cases were examined for estimation of Hb%, PCV, TLC, TEC, DC etc. by following methods described by Schalm, 1965. Serum samples were collected from 93 positive cases for estimation of glucose, ALT, AST, total protein, albumin, cholesterol, blood urea nitrogen, creatinine, total billirubun and direct billirubin by using biochemical kits supplied by Crest Biosystems, Goa in microlab 300 semiautomatic machine at TVCC, CVSc & AH. Blood and serum samples from 10 apparently healthy cows of the locality were also collected for estimation of normal average haematobiochemical values for comparison with that of affected cattle and significance study by using student's t test.

Results

Out of 5237 blood smear examined, 3876 were found positive and 1361 were negative for theileriosis on the basis of presence of piroplasms inside erythrocytes and in few cases Koch's blue bodies inside Lymphocytes. Most common clinical signs in the affected cattle were reduced appetite, anorexia, non

remittent fever, enlargement of superficial lymph nodes, salivation, lacrimation, recumbence, pale conjunctiva, pale mucous membrane, dropped milk yield, debility, respiratory distress and coughing with nasal discharges. Non remittent high fever was noticed in most of the cases. Nervous signs similar to trypanosomiasis like incoordination, trembling, circling movement, standing with head down, head pressing against wall or pole, falling down, bending of neck to one side were also observed in good number of cases. Few cases also showed change in feeding habits like not drinking liquid food or water, not eating solid food, pica, eating mud or soil. Few instances of abortion, diarrhea, bloody stool, swollen joints, swelling of legs, remaining standing and not lying down, allergy, alopecia and red eye and red urine were observed. The clinical signs were grouped as per manifestation in number of cases in Table 1.

Blood samples from 3876 affected and 10 apparently healthy cattle were examined for estimation of haematological parameters like Hb%, PCV, TEC, MCV, MCH, MCHC, TLC and DLC. There was significant decrease in Hb%, PCV, TEC, MCV, MCHC, Neutrophil percentage and nonsignificant decrease in MCH, TLC, Eosinophil, Basophil, Monocyte percentage in the affected cattle when compared to apparently healthy cattle. There was significant increase in Lymphocyte % in the affected cattle when compared to apparently healthy cattle. The average values of haematological parameters of affected cattle were compared with that of apparently healthy cattle (Table 2).

Table 1: Clinical signs observed in theileriosis positive cases

Clinical signs	No. of cases	%
Pale mucous membrane	2946	76%
Reduced appetite	2907	75%
Fever	1240	32%
Swollen lymphnodes	543	14%
Respiratory signs	271	7%
Nervous signs	155	4%
Drop in milk yield	232	6%
Depraved appetite	155	4%
Tick infestation	349	9%
others	39	1%

Table 2: Haematological parameters in affected and apparently healthy cattle

Parameters	Affected cattle	Apparently healthy
Hb(gm%)	8.56±0.03[a]	12.14±0.61[b]
PCV(%)	24.54±0.43[a]	31.32±1.58[b]
TEC(105/cumm)	4.72±0.10[a]	7.35±0.09[b]
MCV(fl)	31.62±2.53[a]	36.64±2.43[b]
MCH(pg)	17.86±0.74[NS]	21.52±0.62
MCHC(%)	56.41±0.79[a]	64.28±0.46[b]
TLC(per cumm)	7492±53[NS]	7517±102
Neutrophill(%)	35.85±0.22[a]	41.2±4.3[b]
Eosinophill(%)	3.89±0.06 [NS]	4.22±0.17
Lymphocyte(%)	58.87±0.22[a]	49.33±0.72[b]
Monocyte(%)	1.28±0.01 [NS]	1.51±0.03
Basophill(%)	0.29±0.02 [NS]	0.32±0.01

Serum samples from 93 positive cases and 10 apparently healthy cattle were used for estimation of biochemical parameters like glucose, cholesterol, total protein, albumin, globulin, blood urea nitrogen, creatinine, AST, ALT, total billirubin and direct billirubin. There was significant decrease in glucose, cholesterol, protein, albumin, globulin in the affected cattle when compared to apparently healthy cattle. There was significant increase in blood urea nitrogen, creatinine, AST, ALT, total billirubin and direct billirubin in the affected cattle when compared to apparently healthy cattle. The average value of biochemical parameters of affected cattle was compared with that of apparently healthy cattle (Table 3).

Discussion

Important clinical signs observed were reduced appetite, anorexia, non remittent fever, enlargement of superficial lymph nodes, salivation, lacrimation, recumbence, pale conjunctiva, pale mucous membrane, dropped milk yield, debility, respiratory distress and coughing with nasal discharges. Similar observations have been made by previous workers like Radostits *et al.* (1994), Roy *et al.* (2004), El-Deeb and Younis (2009), Masare *et al.* (2009), Khan *et al.* (2011) and Panda *et al.* (2011). There was significant decrease in Hb%, PCV, TEC, MCV, MCHC, Neutrophil percentage and significant increase in Lymphocyte % in the affected

Table 3: Mean values of serum biochemical parameters in affected and apparently healthy cattle

Parameters	Affected cattle	Apparently healthy
Glucose(g/dl)	60.51±0.4[a]	80.38±0.77[b]
cholesterol (mg/dl)	91.29±1.41[a]	125.1±1.54[b]
protein (g/dl)	6.08±0.07[a]	7.18±0.22[b]
albumin (g/dl)	3.04±0.05[a]	3.87±0.06[b]
globulin (g/dl)	2.95±0.007[a]	3.67±0.09[b]
BUN (mg/dl)	25.13±0.38[a]	17.53±1.01[b]
creatinine (mg/dl)	2.57±0.03[a]	1.84±0.12[b]
AST (IU/L)	213.4±4.78[a]	102.9±5.36[b]
ALT (IU/L)	31.33±0.77[a]	25.44±1.71[b]
Total billirubin(mg/dl)	2.33±0.03[a]	1.89±0.06[b]
Dir. billirubin (mg/dl)	0.63±0.02[a]	0.41±0.01[b]

cattle. Our findings are in accordance with reports made by Elissalde *et al.* (1983), Sandhu *et al.* (1998) and Singh *et al.* (2001) and Col and Uslu (2007). There was significant decrease in glucose, cholesterol, protein, albumin, globulin values. This might be due to liver damage. There was significant increase in blood urea nitrogen, creatinine, AST, ALT, total billirubin and direct billirubin in the affected cattle. Similar alterations in serum biochemical parameters also reported by Sandhu *et al.* (1998), Omer *et al.* (2003) and Aulakh and Singla (2006). The alterations in BUN, creatinine indicated kidney damage, possibly caused due to trapping of agglutinated damaged infected erythrocytes and lymphocytes in the glomeruli, resulting into glomerulo nephritis. Increase in AST and ALT values indicated degeneration and necrosis of tissues in affected animals. Increase in total bilirubin indicated enhanced phagocytosis of infected erythrocytes.

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Subclinical mastitis in cows of Gujarat

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Abstract

A total of 69 lactating cows {26 Kankrej, 35 triple cross (Kankrej x Jersey x HF) and 8 Gir} of an University livestock farm were screened for subclinical mastitis (SCM) by employing two mastitis markers viz. bacterial culture and electronic somatic cell count. Out of 266 quarters screened for SCM by bacteriological culture examination, 89 quarters were found to be positive. Among 796 quarter milk samples (qms) tested, the mean somatic cell count (SCC) of the healthy quarters was 155.6 x 10^3/ml whereas that of infected quarters was 1949.5 x 10^3/ml ($P<0.01$). The mean SCC of quarters infected with coagulase positive staphylococci (CPS) and coagulase negative staphylococci (CNS) pathogens was 1239.0 x 10^3cells/ml and 689.3 x 10^3 cells/ml respectively ($P<0.01$). Out of 796 qms subjected to SCC, 144 (18.09%) qms were identified as true positive whereas 369 (46.3%) qms showed true negative results. Besides, 66 (8.2%) and 217 (27.2%) qms were false positive and false negative respectively. The sensitivity and specificity of SCC was calculated to be 39.8 and 84.8% respectively. The compatibility between the results of SCC and bacteriological culture examination (reference test) was observed to be 64.4 per cent.

Keywords: Bacteriological Culture Examination, Somatic Cell Count, Subclinical Mastitis

Mastitis, an inflammation of the mammary gland, has a profound impact on milk production and milk quality leading to great economical losses to dairy industry in general and dairy farmers in particular. In India, the economic losses incurred due to mastitis have shown tremendous increase during the last five decades and lately the annual losses due to mastitis have been estimated to the tune of Rs. 7165.51 crore. Since mastitis affects the milk quality, its consequences are not just restricted to the dairy farm but expand beyond that. Increasing concerns among the consumers about the antimicrobial residues, antimicrobial resistance, milk quality and animal welfare further demand proper policies in place to effectively prevent and control mastitis.

The gold standard for determining udder infection status is by bacteriological culture examination. The invisible changes in sub-clinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), somatic cell count (SCC), pH and electrical conductivity (EC) test. These tests yield rapid as well as satisfactory results (Leslie *et al.,* 2002).

Milk SCC is a diagnostic figure for sub-clinical mastitis (IDF, 1999). Somatic cell count is an indication of the intensity of the cellular immune defense and it represents a marker of the sanitary state of the udder. The use of SCC to diagnosis udder diseases was the first widely used screening procedure and the marker has retained its position as the most reliable and specific test for mastitis diagnosis. The presence of sub-clinical mastitis (SCM) can be known only after laboratory examination of quarter milk samples (qms).

In the present study detection of SCM was tried using SCC and its sensitivity, specificity and accuracy were compared with the reference test in lactating indigenous and crossbred cows of an organized herd.

Materials and methods

The present study was conducted on a total of 69 lactating animals of Livestock Research Station (LRS) Anand Agricultural University, Anand during July-December, 2011. All the cows had apparently healthy quarters during the sampling period. The milk samples were collected from each quarter of individual cow for three consecutive days. Enumeration of bacteriological culture examination and SCC was done on foremilk fractions. After discarding first 2-3 streams of milk, approximately 40 ml foremilk samples were collected from each quarter during the milking time. After taking samples from each quarter, the cow identification number was marked on each tube as fore left (FL), fore right (FR), rear left (RL) and rear right (RR). The milk samples collected were brought in ice packs to the laboratory. Before carrying out the tests for screening the SCM, the milk samples were taken out of ice and kept at room temperature for 15-20 minutes.

Bacteriological Culture Examination

Loopfull of milk from foremilk sample from each quarter was streaked on blood agar plate (containing 5% sheep blood) and simultaneously on MacConkey agar plate for primary bacterial isolation and the plates were incubated at 37°C for 24 hours. Following incubation, the plates were examined for bacterial growth and the morphological characteristics of bacterial colonies were recorded. With the help of a loop, 2-4 identical colonies were picked up and transferred to nutrient/glucose agar slants, which were then incubated at 37°C for 24-48 hours. Characterization of bacterial isolates was performed as per the method described by Cowan and Steel (1970).

Somatic Cell Count (SCC)

The Fossomatic Minor is an electronic instrument which gives highly accurate counts of the number of somatic cell per ml of milk. It gives a total count of the cells, i.e. count both epithelial cells and leucocytes. SCC was carried out by using Fossomatic™ Minor cell counter (Foss Electric, Hillerod, Denmark) as described by Gonzalo *et al.* (2003).

Results and Discussion

Bacteriological Culture Examination

In the present study, out of 266 quarters screened, 89 quarters were found subclinically infected yielding 190 isolates. Of these 50 (56.1%) quarters yielded monomicrobic isolates and 39 (43.8%) quarters harboured mixed infection, which is close to the report of Patel (2001). Percentage of cows with the number of quarters showing subclinical infection, 22 (47.8%) cows had infection of two quarters which was followed by single quarter infection in 15 (32.6%) cows, 3 quarters infection in 6 (13%) cows, and all 4 quarters infection in 3 (6.5%) cows. More frequent infection of single quarter has also been reported by various workers (Patil *et al.*, 2000). Conversely, Dhote *et al.* (1999) observed much higher involvement of single quarter at 69.8% followed by two quarters (19.3%), and all four quarters (11.7%).

Somatic Cell Counts (SCC)

In the present study, out of the 266 udder quarters from 69 cows. 135, 100, and 31 quarters belonged to 35 triple cross, 26 Kankrej, and 8 Gir cows, respectively. Milk sample from each quarter was collected and subjected to SCC enumeration for 3 consecutive days. Thus, a total of 796 qms were subjected to SCC. Of these, 144 (18.09%) qms were found true positive, whereas 369 (46.3%) qms showed true negative results. Collectively, 64.4 per cent qms gave similar results as shown by the results of bacteriological culture examination. However, on the basis of somatic cell count 66 (8.2%) and 217 (27.2%) qms were categorized as false positive and false negative respectively. Like inflammation in other body parts, the infection of udder is also considered to be responsible for infiltration of polymorphonuclear (PMNs) cells at the site of infection. Chemical messengers or chemotactic agent released from leukocytes, normally present in milk or from damaged tissue, attract PMNs into milk in large numbers (Nickerson and Pankey, 1984). Thus, the end result of this process is an increase in the SCC in milk resulting from migration of PMNs to the site of infection. The function of PMNs in milk is to engulf and to digest the invading bacteria as a key defense mechanism in the udder (Harmon, 1994).

Mean SCC of healthy and infected quarters

The present study showed that the mean SCC in healthy and latent quarters was significantly ($P<0.01$) lower than in non-specific and specific mastitis infected quarters (155.59×10^3 and 243.36×10^3 Vs. 978.18×10^3 and 1949.48×10^3 cells/ml, respectively. Many workers have also observed significantly higher mean SCC in infected quarters than in healthy quarters (Leitner *et al.*, 2003; Verma *et al.*, 2009).

SCC of quarters infected with gram positive / CPS and CNS pathogens

Mean SCC of quarters infected with different CPS pathogens (*Staph. aureus, Staph. hyicus* and *Staph. intermedius*) was significantly ($P<0.05$) higher (1239.04×10^3/ml) than in quarters infected with CNS pathogens (*Staph. chromogenes* and *Staph. epidermidis*) (689.27×10^3/ml). Earlier, Patel (2001) also reported significantly higher mean SCC of quarters infected with different CPS pathogens than those infected with CNS pathogens.

Sensitivity and Specificity of SCC

The sensitivity of SCC as a mastitis marker was observed to be 39.8%. This was much lower than

reported by earlier workers (Sharma *et al.,* 2010). The specificity of SCC was recorded at 84.8% which falls in agreement with the observations of Choudhari (2000), who reported the same to be 84.4 per cent respectively.

Compatibility between reference test and SCC

In the present investigation, bacteriological culture examination was considered as the reference test and the compatibility of SCC was calculated by comparing its result with that of the reference test. The analysis of results obtained in the present study revealed that SCC, as a mastitis marker, showed 64.4% compatibility with the results of bacteriological culture examination which was close to the findings of Patel (2001). Earlier, Nauriyal and Pachauri (2004) reported compatability between the results 75.4% of SCC and reference test.

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Bronchoalveolar lavage fluid bacteriology in bovine respiratory disorders

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Abstract

"Endoscopic evaluation of Bovine Respiratory Disorders" was conducted on ten apparently healthy cattle and twenty-seven clinical cases with the objective of studying the incidence, haematobiochemistry, bronchoscopic and BALF bacteriological changes. Bacteriology of lavage revealed *Pasteurella spp., Staphylococcus spp.* and *Streptococcus spp.* were common isolates from control animals. *Pasteurella spp., Staphylococcus spp., Pseudomonas spp., Haemophilus spp.* and *Mycobacterium spp.* were the isolates from the clinical cases.

Keywords: Cattle, Bronchoscopy, Balf Bacteriology.

Bovine respiratory disorder (BRD) is one of the prime causes of morbidity and mortality in large domestic animals and a major source of economic loss to the farmers. Disorders of the respiratory tract of cattle are caused by a combination of infectious agents and predisposing causes such as inclement weather, the stress of weaning, transportation and poorly ventilated housing, each of which weakens the pulmonary defense mechanisms of the animals. In addition to routine clinical examination so many special diagnostic procedures namely radiography, thoracocentesis, Tran tracheal aspiration, nasal swab culture, pleuroscopy, ultrasonography and bronchoscopy are used in respiratory disorders. Bronchoscopy is one of the special techniques used in the diagnosis of respiratory disorders, and helps in direct visualization and collection of bronchoalveolar lavage fluid samples for identification of etiological agents and cytological studies.

Materials and methods

Ten apparently healthy animals and 27 clinical cases for this study were chosen from the animals that were brought to the Large Animal Clinic, Madras Veterinary College Hospital by subjecting to detailed physical examination, haematology and biochemical changes and subjected to Bronchoscopy (Thirunavukkarasu *et al.,* 2005). Bronchoalveolar lavage was performed in the affected lobe or lobes after infusing 30ml to 60ml of sterile normal saline. The fluid was retrieved out immediately by suction into the sterile disposable syringe or into the infant mucus extractor vial. Few ml of the collected fluid was transferred into a sterile screw capped vial for culture.

Broncho alveolar lavage Bacteriology

Direct microscopy

Stained smears of BAL can yield a considerable amount of information inexpensively and quickly. Lavage collected can be placed on microscopic slide for examination by carefully swabbing the lavage on the surface of slide. After the slide has dried, it should be fixed by heat and stained by Gram's method or Acid-fast method (ZN method).

Broncho alveolar lavage Culture

All the samples were inoculated in nutrient broth and incubated at 37°C for 6 hours to 8 hours until turbidity developed. Subsequently the samples streaked on nutrient agar, blood agar enriched with 5 per cent sheep blood, MacConkey's agar and Chocolate agar. The agar plates were incubated at 37° C for 24 hours. Presumptive and definitive identification of the isolates were determined by colony morphology, colony characteristics, Gram's reactions, and standard bacteriological identification schemes (Quinn *et al.,* 1994; McKiernan *et al.,* 1984).

Results and discussion

Out of 27 samples 2 were positive for acid-fast bacilli (ZN-positive) in their broncho alveolar lavage. In Control animals out of ten broncho alveolar lavage samples single type of bacteria was isolated from seven animals. Three samples had no growth. Out of 7 isolates *Pasteuralla spp.* was 30.00 per cent, *Staphylococcus spp.* was 20.00 per cent and *Streptococcus spp.* was 20.00 per cent of cases.

In Clinical cases out of 27 broncho alveolar lavage samples cultured single type of bacteria were isolated in twenty-three samples, two type of bacteria were isolated in two samples and no growth in two samples. Out of 27 clinical cases *Pasteurella spp.* was isolated from 44.44 per cent of cases, *Staphylococcus spp.* was isolated from

14.81 per cent of cases, *Pseudomonas spp.* was isolated from 11.11 per cent of cases, *Actinomyces spp.* was isolated from 11.11 per cent of cases; *Haemophilus spp.* was isolated from 11.11 per cent of cases and *Mycobacterium spp.* was isolated from 7.41 per cent of cases. The culture results show that a variety of organisms present in the respiratory tract of both clinical cases and controls.

In the present study out of 27 broncho alveolar lavage sample cultured single bacterial species isolated in 23 samples and two bacterial species isolated in two samples. Out of 27 bacterial isolates 66.67 per cent (18/27) were gram negative i.e., *Pasteurella spp., Pseudomonas spp.*, and Haemophilus *spp.* Gram positive bacteria were isolated in 25.93 per cent (7/27) i.e., *Staphylococcus spp.* and *Actinomyces spp.* Acid-fast bacilli were isolated in 7.14 per cent i.e., *Mycobacterium spp. Pasteurella spp.* was the common isolate in clinical cases. Allan *et al.* (1985), Barbour *et al.* (1997), Allen *et al.* (1992, 1992) and Rohn *et al.* (1998) have isolated *Pasteurella spp.* in animals infected with respiratory diseases. The clinical observation is in concurrence with findings of the earlier workers.

Pasteurella spp. are commensals on the mucous membrane of the upper respiratory tract of animals. *Pasteurella spp.* with various stresses, including concurrent viral infections, predisposing to infection. *P. haemolytica* produces a soluble leucotoxin that has a role in breaching the lung's primary defense mechanism by its action on the alveolar macrophage and other leucocytes in cattle. *Staphylococci* colonise the nasal cavity, skin and mucous membranes and can be transient in the respiratory tract. The staphylococci are pyogenic and are associated with suppuration. The pathogenic staphylococci produce toxins and enzymes. This enzymes and toxins are responsible for pathogenicity. *Pseudomonas spp.* produces a number of protein exotoxins and endotoxins that are responsible for infections. *Actinomyces* are present on mucous membranes of oral cavity or naso pharynx of cattle. *Actinomyces spp.* causes pyogranulomatous reactions in animals. The source of mycobacterium is usually infected animals. Local multiplication of mycobacterium occurs in respiratory tract and resistance to phagocytic killing allows continued replication and pathogenicity. *Haemophilus spp.* are commensals of mucous membranes, most commonly upper respiratory tract. *H.somnus* produces serofibrinous and/or suppurative lesions in lungs, often in mixed infection with other agents.

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Cutaneous streptothricosis in cattle of Kerala

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Abstract

The present study was undertaken to study the clinical epidemiology dermatophilosis in cattle. Dermatophilosis was confirmed in 82 cattle based on direct microscopical detection and cultural isolation of *D. congolensis* from the clinical specimens. Affected animals revealed characteristic exudative dermatitis lesions with formation of thick and horny scabs, crusts and fissures with matted hair at their bases. Out of the 82 animals, only 10 animals had extensive generalised lesions in different parts of the body. The lesions were most common on lower part of hind limbs and forelimbs. Papular and crusted lesions were also noted on udder, perineum and inguinal region in the present study. Less frequently the lesions were also reported in other regions such as ventral abdomen, flank, head, ears, tail, axilla and back. In severe cases lameness, mastitis and reduction in milk yield were observed depending on the areas affected. The knowledge on the appearance of lesions and distribution of lesions will help in an early and correct diagnosis under field condition thereby prompt treatment and control can be attempted to reduce the economic losses to the farmers.

Keywords*:* Cattle, Clinical signs, Cutaneous streptothricosis, Dermatophilosis,

Cutaneous streptothrichosis or Dermatophilosis is a skin infection of domestic, aquatic and wild animals caused by *D. congolensis*. It is one of the four major bacterial diseases which affect cattle and other animals in the tropical and subtropical regions (Hashemi Tabar *et al.*,2004). It causes severe economic losses to cattle farmers through inferior quality of hide, reduced milk production, weight loss, cost of treatment and culling of severely affected animals. The disease in cattle is a chronic dermatitis and could occur in any part of the body and occasionally become generalised. Accurate diagnosis and early treatment are found to be useful for better clinical recovery from the condition. Understanding clinical signs at varying stage of disease can help in accurate clinical diagnosis and better and early therapeutic management of the condition. Hence the present study was undertaken to recent clinical forms of the disease during various stages of disease and areas of skin affected in naturally occurring cases of dermatophilosis in cattle.

Materials and methods

Hundred cattle of different age groups and breeds presented with different dermatological lesions on various parts of body were subjected to detailed clinical, bacteriological, mycological and parasitological examinations. Impression smears and smears made from the under surface of moistened scabs were stained with Gram's and Giemsa methods and were examined (Quinn *et al.*, 1994). The scabs were subjected to cultural isolation in sheep blood agar under 10% carbon dioxide in a candle jar as per the method described by Haalstra (1965). The isolates obtained were identified as per the methods described by Cowan (1974). The skin scabs and scrapings were subjected to direct microscopical examination with 10% potassium hydroxide to rule out any fungal or mange infections. Dermatophilosis was confirmed in 82 cattle based on direct microscopical detection of *D. congolensis* and cultural isolation from the clinical specimens. The spectrum of clinical signs observed were noted and type of lesions and distribution of lesions on various parts of the body of these animals were recorded and presented.

Results and discussion

Clinical examination of the affected animals revealed characteristic exudative dermatitis lesions with formation of thick and horny scabs, crusts and fissures with matted hair at their bases suggestive of dermatophilosis in 82 cattle (Plate 1). Similar types of lesions were described by most of the workers irrespective of the species of the animals affected. (Koney, 1996; Quinn *et al.*, 2011).The formation of thick and horny scabs in dermatophilosis was attributed to repeated cycles of invasion and multiplication of the organism in to the epidermis, rapid infiltration of neutrophils, regeneration of epidermis and invasion in to regenerated epidermis (Ambrose *et al.*, 1999). There was excessive matting of hairs with the exudates from the lesions and the hairs were unaffected which were

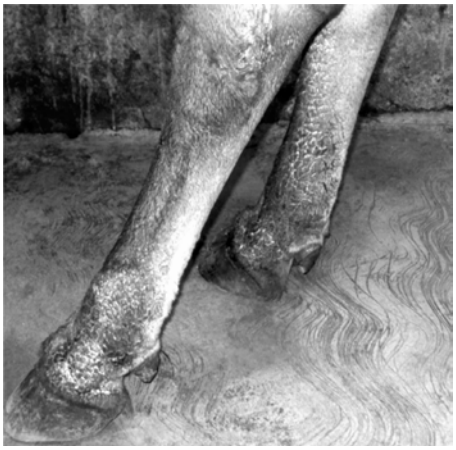

Plate 1. Exudative dermatitis lesions with thick and horny scabs, crusts and fissures

Plate 2. Matting of hairs with exudates presenting paint brush appearance

Plate 3. Detached scabs with typical paint brush appearance

Plate 4. Extensive generalised lesions

Plate 5. Crusted lesions were also noted on udder, perineum and inguinal region

Plate 6. Hypochromic hairs in the areas of healed lesions

penetrating through the scabs holding them in place. The hairs on the affected part were matted with exudates presenting paint brush appearance (Plate 2). The detached scabs with hairs also resembled typical paint brush appearance (Plate3) as described by Koney (1996) and Decostere *et al.* (2002).

Removal of these scabs was painful during the early stages of disease and resulted in raw hyperaemic surface with oozing of serosanguinous exudates as reported by Wernery and Ali (1990). During the healing stages the scabs were easily detached from the lesions leaving a normal skin with alopecia. As the lesions were healed and hairs started to grow, the areas appeared hypochromic initially and appeared to be normal by four to six weeks. There was no scar formation at the site of lesions.

Out of the 82 animals, only 10 animals had extensive generalised lesions in different parts of the body (Plate 4). Of these 10 animals, six animals were in advanced stage of pregnancy, three animals were in early stage of lactation and one in the third month of lactation. The stress and immunosuppression during these stages might have resulted in generalised diseases suggested by Ambrose *et al.* (1999).

The lesions were noticed most common on lower part of hind limbs and forelimbs. Koney (1996). Frequent finding of lesions on the lower limbs might be due to prolonged wetting of these areas due to standing in water logged areas for prolonged period as occurred during grazing of animals in the paddy fields. Papular and crusted lesions were also noted on udder, perineum and inguinal region in the present study (Plate 5). The lesions were with thick scabs in all regions, but in udder and inguinal region the lesions were mostly moist with thin scabs and folded skin as described in OIE manual

(2008). Less frequently the lesions were also reported in other regions such as ventral abdomen, flank, head, ears, tail, axilla and back.Variations in the frequency of lesion distribution usually correlated with those areas of skin predisposed to infection as stated by Quinn *et al.* (2011).

The clinical data such as temperature, pulse, respiration, rumen motility, appetite and milk production were normal in majority of animals. But those animals with generalised lesions had depression, inappetance and reduced milk yield.

Lameness due to oedema and pain from severe lesions on the limbs was reported in nine animals (Plate 6). Pruritus was reported only in six cases, which was shown by licking and scratching the affected area. But in majority of animals there was no history of pruritus.

Reduction in the milk yield was recorded in 16 lactating animals especially in those affected with generalised disease and those with lesions on the udder. Three cows developed mastitis subsequent to udder lesions and two animals were culled due to permanent loss of production. Loss of weight was reported in three cases with generalised lesions. Two animals developed myiasis as a complication of lesions on legs.

No fatality was reported among the cases included in the present study. The present study clearly indicates the impact of the disease on animal health and production. The knowledge on the appearance of lesions and distribution of lesions will help in an early and correct diagnosis under field condition thereby prompt treatment and control can be attempted to reduce the economic losses to the farmers.

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Skin and subcutaneous tissue neoplasms in Canine : an epidemiological study

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Abstract

In the present investigation, 25 cases of skin and subcutaneous tissue neoplasms were classified in relation to various epidemiological parameters canine population, feeding/diet, body weight (Kg), housing pattern and breeding history. The animals were also evaluated clinically for their body condition, location, size, weight and consistency of tumour mass were also recorded. The effect of neoplastic conditions on various physiological parameters like rectal temperature, heart rate and respiration rate has also been recorded in this study. The genital region (36%) and mammary gland (24%) were most commonly affected by skin and subcutaneous tumours. The dogs with non-vegetarian diet (44%) and body weight ranges from 0-10 kg affected with skin and subcutaneous tumours were fifteen (60%) whereas confined dogs (52%) and female of earlier breeding history (parity) were more prone to skin and subcutaneous tumours. On clinical examination, dogs with healthy body condition (80%), tumours with non-pedunculated (broad based) (72%) and hard consistency (60%) along with tumours mass weight ranging from 0-50 kg were found in of eleven cases (44%) which contributed maximum number of skin and subcutaneous tumours. In lumpectomy (group I and II) and lumpectomy plus vincristine sulphate and methotrexate (group III), there was a significant increase ($P<0.05$) in the heart rate upto 60 mins and after lumpectomy and antineoplastic treatment it decreased by 60-120 mins post treatment. The body temperature showed a non-significant decrease while the respiration rate showed a non-significant increase between 30 to 60 mins in all the treatment groups at various intervals.

Keywards: Benign, Canine, Malignant, Genital neoplasm, Mammary neoplasm.

Different types of tumours have been reported to occur in dogs. Skin has been reported to be the most common site of tumours in dogs and other domestic animals (Dayananda *et al*, 2009). Moriello and Rosenthal (1990) reported that the incidence of skin tumours in dogs was 7.28% further observed that female dogs had higher incidence of skin neoplasms than the male dogs. Canine transmissible venereal tumors (TVT) are cauliflower-like, pedunculated, and nodular, papillary, or multilobulated in appearance. They may be transplanted to adjacent skin, oral, nasal, or conjunctival mucosae. The present article is aimed to provide data regarding occurrence and of skin and subcutaneous tumours in canines in Durg (Chhattisgarh) and their adjoining areas.

Materials and method

The present study was conducted on clinical cases of 25 dogs presented with the history of neoplastic growths at various sites during the period from October 2010 to September 2011 at the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Anjora, Durg (C.G). Particulars of animals like feeding/diet, body weight (Kg), housing pattern and breeding history viz. parity in female/earlier breeding in male were recorded by detailed anamnesis. Clinical parameters of animals like general condition of animal, visual examination of the tumour mass, consistency of tumour, location of the tumour and their size/weight were noted in predefined record sheets.

Evaluation of physiological parameters consisted of recording the rectal temperature, heart rate and respiration rate before and 30, 60, 90 and 120 minutes during surgical interventions and treatment. For, this the dogs were divided into three groups for comparing the three different treatments. In group I, fifteen dogs which were affected with benign tumours were treated surgically. In group II, five dogs which were affected with malignant tumours were treated surgically while remaining five dogs of group III which were affected with malignant tumours were treated by lumpectomy together with antineoplastic therapy (Vincristine Sulphate @ 0.025mg/kg body weight I/V followed one week later by Methotrexate @ 0.3mg/kg body weight) intravenously at weekly interval. The data obtained were subjected to statistical analysis as per the procedure described by Snedecor and Cochran (1994).

Results and discussion

Dogs with non-vegetarian diet were found to be the most affected with skin and subcutaneous tumours 11 (44%) followed by mixed diet 8 (32%) and vegetarian

diet 6 (24%). This study revealed that a majority of the cases were in between 0 to10 kg accounting for fifteen (60%) cases, followed by seven (28%) dogs having body weight between 20 to 30 Kg and three (12%) dogs with 10 to 20 Kg body weight. Out of 25 dogs, seventeen females (68%) and eight male (32%) with earlier breeding history.

Out of total twenty five dogs positive for skin and subcutaneous tumours, twenty (80%) were in good body condition, five (20%) cases were in poor health condition. Majority of skin and subcutaneous tumours 18 (72%), non-pedunculated (broad based) only 7 (28%) were pedunculated. Majority of tumour mass were hard in consistency accounting for fifeteen (60%) cases. Further only ten (40%) cases showed soft consistency of tumour mass. In the present study, the weight of tumour mass was found to be in the range of 0 to 300 grams.

During therapeutic intervention rectal temperature in all the groups of animals showed a non-significant decrease between 60 to 120 mins. The mean decreased values of rectal temperature ranged from 100.96 ± 0.28 ^{0}F to 101.89 ± 0.12 ^{0}F in different groups of animals at various intervals. Heart rate in all the groups of animals showed a significant ($P< 0.05$) increase upto 60 mins. followed by decrease between 60-120 mins. in group II and group III. The mean increased values of heart rate ranged from 72.50 ± 2.30 beats/min to 82.96 ± 0.85 beats/min. in different groups of animals at various intervals. Respiration rate in all the groups of animals showed a non-significant increase between 30 to 60 mins after lumpectomy. The mean increased values of respiration rate ranged from 27.83 ± 1.95 per min. to 36.0 ± 2.30 per min. in different groups of animals at various intervals (Table I).

Most of the dogs presented with tumour were in good physical condition. No correlation could be established or traced out with literature between feeding habit and the prevalence of the tumours. No correlation could be established with the body weight, housing pattern and prevalence of skin and subcutaneous tumours and no literature could be traced out in this regards. However, hormonal imbalance, inbreeding and poor nutrition have been postulated for causation of tumours covering the entire body.

Canine are more susceptible to cancer than any other domestic animals. A higher incidence of tumours in canines could be attributed to their population and species involvement (Singh *et al.,* 1991). Most of the cases had recovered uneventfully with good surgical wound healing except cases of high grade malignancy. The reason for delayed healing might be due to large surgical wound, poor health condition, decreased immunity, improper management by the owners and post operative infections. The occurrence of non-pedunculated (broad based) neoplasms was more than pedunculated tumours in this study. This finding corroborates well with those reported by Vani *et al.* (2007). They also found pedunculated cauliflower like growth at the level of lower incisor in two cases and a Pomeranian bitch with a solid mass at the left angle of the mouth.

Mostly oral tumours and venereal tumours were soft in consistency. Similar findings were also reported by Gupta and Tiwari (2009), who found that the weight of neoplasms ranged from 20 gms to 1.25 kg in canine.

Table I : Physiological observations before and after treatment in dogs affected with skin and subcutaneous neoplasms.

Parameters	Groups (n=15 in I and n=5 in II,III)	0 min.	Post operative time interval (min.)			
			30	60	90	120
Rectal Temperature (^{0}F)	I	101.25±0.08	101.21±0.11	101.78±0.13	101.01±0.14	101.89±0.12
	II	101.68±0.11	101.48±0.10	101.56±0.07	101.76±0.19	101.5±0.14
	III	101.76±0.09	101.48±0.20	100.96±0.29	101.48±0.11	101.46±0.11
Heart rate (beats/min.)	I	72.50±2.30	75.80±1.86*	76.50±1.34*	72.60±1.50	72.80±1.20
	II	77.50±2.00	81.50±0.34*	82.80±1.46*	76.58±1.32	77.10±1.20
	III	75.40±2.36	81.20±0.68*	82.96±0.85*	74.24±1.84	75.90±1.24
Respiration Rate (permin.)	I	28.93±2.83	29.46±2.49	30.6±3.57	29.53±1.53	27.83±1.95
	II	33.6±1.67	35.6±2.28	36.0±2.30	34.2±1.28	32.8±1.28
	III	28.6±1.72	29.8±1.06	30.0±1.04	29.8±1.39	28.8±1.39

* $P<0.05$= Significant at 5% level

The increase in the heart rate could be due to the cardio-stimulatory effect of ketamine. Ravi Kumar *et al.* (2000) reported non-significant rise in the mean respiration rate for varying period and attributed this rise in respiration rate due to stress of surgery or stress of antineoplastic drug. Thus, the transient increase in the respiration rate might be due to stimulatory action of ketamine on the respiration center or stress of antineoplastic drug therapy.

Based on these observations it can be concluded that occurrence of skin and subcutaneous neoplasms probably depends upon population of breeds under various geographical areas. However no single cause can be pointed out for occurrence of neoplastic conditions on body. Still, the tumours are found on those aspects of body surfaces which are exposed to sunlight. The occurrence was recorded in higher proportions in dogs kept indoors with non vegetarian diets and having good body condition ageing between 6 to 10 years.

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Outbreak of Classical Swine Fever in an organised pig farm in Assam

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Abstract

An outbreak of classical swine fever (CSF) in an organized pig farm having the history of vaccination against CSF with lapinized CSF vaccine was recorded in Assam during 2009.All age groups of pigs were affected and percentage of morbidity in piglets, growers and adult pigs were 97.30, 96.00 and 92.06 respectively. The percentage of mortality was the highest (95.50) in piglets followed by the mortality percentage in growers (52.00) and adult pigs (19.05).The overall morbidity, mortality and case fatality were 96.13%,76.45% and 79.53% respectively during the outbreak.

Keywords*:* Pig, Classical Swine Fever, Sandwich ELISA, Mortality.

Classical swine fever (CSF) is a highly contagious, disease of pigs. The disease is caused by classical swine fever virus (CSFV), an enveloped, single-stranded RNA virus of the genus Pestivirus and family Flaviviridae (Moennig, 2000). The disease may run an acute, sub-acute and chronic depending on host factors, age of the animals, and the virulence of the virus. The disease is less severe in adult pigs than young animals (OIE, 2008). Occurrence of CSF in different parts of India has been reported by several workers (Saini *et al.* 2000, Sarma *et al.* 2007, Sarma *et al.* 2008). Occurrence of CSF in vaccinated pigs in Assam, Meghalaya and Nagaland was also recorded in June to August 2005. Mortality of 23-50% of the affected pigs was recorded during the outbreaks (Sarma, 2007). The present investigation describes an outbreak of classical swine fever in an organized pig farm. This study was carried out at the All India coordinated Research Project on Pig and Mega Seed Project on Pig, Assam Agricultural University, Khanapara, Guwahati. The AICRP on Pig is maintaining different genetic groups of pig *i.e.* 87.50% Hampshire, 75% Hampshire and Mega Seed Project on pig maintained 50% Hampshire, T&D, Pure Hampshire. On 19th October' 2009 two of the castrated pigs showed high rise of temperature and died on the second day. On observation it was found that few grower pigs also showed the symptoms of high rectal temperature followed by anorexia and two of the affected pigs died. Within one week the symptoms were noticed in several pigs. Following thorough postmortem examinations of the dead animals, it was suspected for classical swine fever. For the final diagnosis clinical samples like spleen, lymph nodes, tonsils and kidney and serum samples from some of the unaffected pigs were collected and examined for the presence of CSF virus specific antigen in the clinical samples by double antibody sandwich ELISA (Sarma *et al.*, 2008) and antibodies in the serum samples by indirect ELISA (Sarma and Sarma, 1996) at the ICAR National Fellow Project, Department of Veterinary Microbiology, College of Veterinary Science, AAU, Khanapara, Guwahati. The morbidity, mortality and case fatality rates were also calculated according to standard methods.

A total of 310 pigs were in both the projects on day of incidence of the disease, out of which 222 were piglets (0-2months), 25 were growers (2-8 months) and 63 were adult (over 8 months, pregnant and lactating) animal irrespectively of sex. There was a record of vaccination of the pigs in the month of July'09 with the lapinized CSF vaccine (Institute of Veterinary Biologicals, Khanapra, Guwahati, Assam) in adult and growers but the new born piglets were not vaccinated as they are in preweaning stage and few are just weaned.

The diseased initially appeared in grower pigs of the AICRP on pigs, which are kept in the corner side pens of the house. The animals might have picked up the infection from a nearer pig unit, where the pigs brought from villages were kept. A few piglets of the project were subsequently affected and died without any symptoms except higher body temperature. Within one week the disease spread to the pigs of the Mega Seed Project on pig. The disease continued in both the projects for a period of one month.

In the initial phase of the illness, the condition was treated symptom-atically with antibiotics (eg, enrofloxacin and sulphadimidine) and multivitamin injections, but mortality continued. Majority of the affected piglets showed the symptoms of high rise of rectal temperature (higher than 40°C), huddling,

Table 1: Morbidity, mortality and case fatality of pig due to Classical Swine Fever outbreak

Age Group	Total Population in Farms	Morbidity		Mortality		Case fatality
		Affected	(%)	Died	(%)	(%)
Piglets(0-2 months)	222	216	97.30	212	95.50	98.15
Growers(2-8 month)	25	24	96.00	13	52.00	54.17
Adults (over 8 months)	63	58	92.06	12	19.05	20.69
Total	310	298	96.13	237	76.45	79.53

incoordination of movement and died with 4-5 days. The adult pigs showed the symptoms of high temperature, anorexia, lethargy, conjunctivitis, weakness of hind legs, incoordination and few died after 12-15 days and some are recovered. Few growers and adult pigs showed typical haemorrhages of the skin, on the base ear, abdomen and the inner side of the limbs with posterior paralysis and few showed constipation followed by diarrhoea. A total of 7 pregnant sows delivered still born foetus.

On post-mortem examination, extensive haemorrhage in intestine, mesenteric lymph nodes, kidney, urinary bladder and spleen in adults and pinpoint haemorrhages in kidney of the dead piglets were noticed. The lungs become congested and haemorrhagic. In addition, necrotic foci or "button" ulcers were also found in the intestinal mucosa of few of the grower and adult pigs.

On laboratory investigations all the clinical samples of the affected piglets were found positive for CSF virus antigen in the sandwich ELISA test. The serum samples examined from the pigs, which were not affected at the initial stage showed the presence of very low level (< 1:16 titre) of CSF virus specific antibodies.

The overall morbidity, mortality and case fatality rates in farms were found to be 96.13%, 76.45% and 79.53% respectively. In case of piglets the morbidity, mortality and case fatality rates were found to be 97.30%, 95.50% and 98.15% respectively. The corresponding rates in growers were 96.00%, 52.00% and 54.17% and in adults were 92.06%, 19.05% and 20.69% respectively. The morbidity and mortality were found to be higher in case of piglets as compared to growers and adults. The higher rates of morbidity and mortality in piglets might be due to immature immune system and absence of protective antibody level against CSF virus as the piglets were not vaccinated against CSF. Six piglets of a sow which were not affected during the outbreak might be due to high maternal antibody level against CSF virus. Lower morbidity and mortality in growers and adults might be due to the vaccination against CSF. There are also reports (OIE Terrestrial Manual, 2008) that the mortality due to CSF is more in piglets compared to adult and growers. Outbreaks of CSF in vaccinated pigs particularly after lapinized virus vaccination has also been reported by earlier workers (Barman *et al.* 2003; Sarma 2007; Jindal *et al.* 2008,) and findings of the present study also suggested for improvement of the vaccine.

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In vitro efficacy of some herbal extract and cypermethrin on *Lymnaea* snails

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Abstract

Two different herbal extract i.e. *Azadirachta indica* (neem) leaves and *Pongamia glabra* (Karanj) seed and cypermethrin were used alone or in combination at different concentration to know the efficacy of these compounds on *Lymnaea* snails. *In vitro* efficacies of herbal molluscicides revealed that neem leaf powders kills all the snails in 24 and 12hours at 5% and 10% dilution, respectively while, karanj seed powder showed 100% efficacy in 12 and 6 hours interval at 5% and 10% dilution respectively. Whereas, combined effect of both herbal extract with cypermethrin showed significant effect (100%) efficacy at 1 hr interval on snails.

Keywords*:* Herbal, Extract, Cypermehrine, *Lymnaea*, Snails

Snails are the intermediate hosts of several trematodal parasites (*Fasciola*, *Paramphistomes* and *Schistosoma* spp.) which affect livestock.These parasites are transmitted by various aquatic snails viz. *Lymnaea auricularia*, *L. luteola, Indoplanorbis exustus* and *Gyraulus convexiusculus* (Liu *et al*.2010,). Chemical compunds i.e. copper sulphate, sodium pentachlorophenate; aluminum sulphate, thiodicarb and niclosamide etc. are commonly used for controlling snail population. But these chemicals have adverse effect on the aquatic flora and fauna, toxic substances from these compounds eventually enter into their systems and finally enter in food chain. (Pant and Singh, 1983; Hodson, 1988; Johal and Dua, 1995). In India, several workers have reported phytomolluscicides activity of different plant extract (Singh and Singh, 1999). Therefore the present study was undertaken to find out the efficacy of cypermethrin and different herbal extract for control snails.

Materials and methods

Cypermethrin (w/w, 10%) purchased from Dhanuka Agritech Limited, were diluted in 1 liter of distilled water in different flasks in the dilution of 1:10,000 and 1: 20,000 by dissolving 0.01 ml and 0.005 ml, respectively.

The dried leaves of neem (*Azadirachta indica*) and karanj seed (*Pongamia glabra*) were pulverized separately in an electrical grinder until they turned into powder. After that both preparation were suspended in separate mortar and pestle, dilution were made by mixing 5% and 10% of neem leaf powder and karanj seed powder separately in 1 litter of distilled water kept in flask.

For combination neem leaf powder 10% + Cypermethrin 1:10,000 and Karanj seed powder 10% + Cypermethrin 1:10,000 were used separately against snails.

Apparently 25 normal *Lymnaea spp*. snails were kept in each dilution as mentioned above. Simultaneously a control group having the same volume of water and number of snails were kept separately. For assessing the efficacy of different molluscicides the live and dead snails count were observed separately at different intervals.

Results

With 5% dilution of neem leaf powder, no snails died upto 3 hours, while 4(16%), 17(68%) and 25(100%) snails were found dead at 6, 12 and 24 hours, respectively. Whereas, in 10% dilution of neem leaf powder, 2(8%), 7(28%), 11(44%) and 25(100%) mortality were observed at 1, 3, 6 and 12 hours, respectively.

Similarly, 5% dilution of karanj seed powder, 1(4%), 6(24%), 21(84%) and 25(100%) mortality were observed at 1, 3, 6 and 12 hours, respectively. Whereas 10% dilution showed 4(16%), 11(44%) and 25(100%) mortality at 1, 3 and 6 hours, respectively

Cypermethrin showed 8(32%), 19(76%) and 25(100%) efficacy in 1: 10000 dilution at 1, 3 and 6 hours, respectively. While in 1:20000 dilution, 3(12%), 9(36%), 21(84%) and 25(100%) efficacy were observed at 1, 3, 6 and 12 hours, respectively. Combination of herbal extract with Cypermethrin showed great efficacy. Combination of cypermethrin + neem leaf powder killed 22(88%) and 25(100%) snails in 1 and 3 hours, respectively, whereas, Combination of cypermethrin (1:10000) + karanj seed powder (10%) showed 100% efficacy against *Lymnaea* snails within 1 hour.

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Table-1 *In vitro* molluscicidal efficacy of herbal extract and cypermethrin against *Lymnaea* spp.

Sl. No.	Different compounds	Dilution used	Percent mortality in hours					
			1	3	6	12	24	48
1	NLP	5%	N	N	4(16%)	17(68%)	25(100%)	-
		10%	2(8%)	7(28%)	11(44%)	25 (100%)	-	-
2	KSP	5%	1(4%)	6(24%)	21(84%)	25 (100%)	-	-
		10%	4(16%)	11(44%)	25(100%)	-	-	-
3	CYP	1:10,000	8(32%)	19(76%)	25(100%)	-	-	-
		1:20,000	3(12%)	9(36%)	21(84%)	25(100%)	-	-
4	CYP+NLP	1:10,000 + 10%	22(88%)	25(100%)	-	-	-	-
5	CYP+KSP	1:10,000 + 10%	25(100%)	-	-	-	-	-
6	CONTROL	Water	N	N	N	N	N	N

N = No mortality

Discussion

The goal of present study was to know the molluscicidal effect of different herbal extract and cypertmethrin at differtent concentarion. The reason for using these compounds was that they are cheap and easily available in rural areas. In present study, Neem leaf powder showed 100 percent efficacies in 5% and 10% dilution at 12 to 24 hours. *In vitro* molluscicidal efficacy of neem leaf powder in different dilution has also been reported by Devi *et al.* (2007) and Alam *et al.* (2010). Azadirachtin, a complex tetranortriterpenoid limonoid an active component of neem seed may be responsible for the molluscidal effect. Methanolic extracts of Karanj seed powder also showed 100 percent mortality within 6 to 12 hours. Cypermethrin showed very good result against snails in 1:10000 and 1:20000 dilutions within 6 to 12 hours. Singh and Agarwal (1990) reported that the cypermethrin had very high degree of molluscicidal activity against snails. Combination of cypermethrin + neem leaf powder and cypermethrin + karanj seed powder showed 100% mortality within 1 hour, may be due to chemical substance present in cypermethrin, neem and Karanj, which acts on nerve terminals of snails, and thus snails died very fast in combination of chemical and herbal compounds. In snails, cypermethrin significantly reduced acetylcholine esterase, cytochrome oxidase and lactic dehydrogenase activity in the nervous tissue, cause repetitive discharge at the nerve terminal and blocks the electron transport chain as reported by Singh and Agarwal (1990). There is lack of information on this type of study. Hence the results obtained could not be compared with the findings of others.

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Effect of *Trigonella foenum-graecum* (Fenugreek) seeds on performance of lactating buffaloe having subclinical mastitis

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Abstract

Subclinical mastitis is a major hurdle in the production of milch animals. Many studies have been undertaken in the past to overcome this menace using antibiotics, nutrient supplements, homeopathic remedies and herbs, with varying results. This study was undertaken to explore the therapeutic efficacy of *Trigonella foenum-graecum* seed's powder in treatment of subclinical mastitis and its effect on production traits. Powdered fenugreek seed was administered @ 100gm of grounded Methi seeds (*Trigonella froenum-gracum)* per Os for 15 days. Changes in production traits namely milk yield (L), fat (%), solids not fat (SNF, %) and total solids (%) were recorded before and after treatment. The therapeutic efficacy of methi in treatment of SCM was adjudged based on variation in somatic cell count and was compared with untreated control. A recovery of 83.33% was recorded in treatment group. . An increase of 35.37% milkyield and decline in somatic cell count was also recoverd.

Keywords: Sub Clinical Mastitis, Buffaloe, *Trigonella foenum-gracum.*

Buffaloe are the backbone of dairying in India. In recent years attempts to upgrade nondescript low yielding native buffaloes with Murrah breed have not only resulted in increase of graded Murrah buffaloes with high milk potential but also made our native buffaloes more susceptible to udder affections like mastitis in the absence of proper healthcare and management. Amongst the several barriers in achieving the production targets, mastitis is the major threat. It is estimated that mastitis alone accounts to about 70% of all avoidable losses incurred during milk production. Average decrease in milk yield due to clinical and subclinical mastitis was estimated to be 50% and 17.5%, respectively (Singh and Singh, 1994).

In order to restore the animal productivity and to optimize the milk production in individual animals for better profits, various drugs, herbal preparations, hormones, mineral supplements and feed additives have been tried with variable results (Ramesh *et al.*, 2000). The use of synthetic antibiotics is being increasingly discouraged because their presence in dairy milk may have potential downstream effects on population health and the Agri-food chain (Taga *et al.*, 2012). World Health Organization has recommended all the member countries to actively promote native medicines of their respective country (Komboj, 2003). The use of conventional plant products described in ancient literature in modern medicine suffers from the fact that scientific evidence and explanations are lacking. The present study, therefore, is an effort to investigate the potential of commonly used and frequently mentioned herb *Trigonella foenum-graecum* seed's powder in management of subclinical mastitis in buffaloes.

Materials and methods

The study was undertaken on twelve animals which were detected positive for subclinical mastitis. These lactating buffaloes were divided into two groups of six animals each. The following regimens of treatment was followed.

FSG (Fenugreek supplemented group)	100gm of grounded Fenugreek seeds (*Trigonella foenum-gracum*) per Os × 15 days
CON (Untreated control)	Untreated control

Response to the treatment was studied by collecting milk samples from all the twelve animals before and after treatment on zero, 5th, 10th, 15th day post treatment. Response to the treatment was adjudged on the basis of somatic cell count, California mastitis test, white side test and few milk traits. Changes in production traits namely milk yield (L), fat (%), solids not fat (SNF, %) and total solids (%) were also recorded before and after treatment.

The therapeutic efficacy of methi in treatment of SCM was adjudged and was compared with untreated control. The results were analysed statistically to record

any significant change as per the method described by Snedecor and Cochran (1968).

Results

Animals in FSG group treated with methi showed 83.33 % recovery. The mean SCC decreased from 4.2±0.22 ×10^5 cells/ml to 2.17×10^5 cells/ml on day 15 (Table 1). All the recovered animals were negative for California mastitis test and white side test after 15 days. The SCC started declining from day 5 post therapy and significant decline was observed on day 10^{th} onwards. The milk production increased from 4.58±0.24 lit on day 0 to 6.2±0.33 on day 15. An overall increase of 35.37% was recorded (Table 2). An increase in fat (%), Solid Not Fat (%) and total solids (%) was observed but was statistically non significant (Table 3). In comparison of treated groups, none of the animals in untreated control group recovered from SCM. An increase in SCC (x 10^5 cells/ml) from 4.38 ±0.34 to 4.93± 0.60 was however recorded. The milk yield in this group decreased from 4.26± 0.24 to 4.00±0.29. The decrease in milk yield could be due to deterioration of SCM state as depicted by an increase in Somatic Cell Count. A statistically non significant decrease in fat (%) and total solids (%) was recorded in untreated group.

Supplementation with Fenugreek seeds (*Trigonella foenum-gracum*) seeds proved to be an effective remedy to cure subclinical mastitis.

Discussion

The seeds of fenugreek contain many phyto chemical compounds such as choline, trigonella diosgenin, vamogenin, trigogenin and neotigogens. Together these compounds attribute for the medicinal properties of fenugreek. This prized spice is an excellent source of minerals like copper, potassium, calcium, iron, selenium, zinc, manganese and magnesium. It is also rich in many vital vitamins that are essential nutrients for optimum health including thiamin, pyridoxine, folic acid, riboflavin, niacin, vitamin A and Vitamin C (USDA National nutrient data base).

Earlier Abo El-Nor *et al.,* 2007 recorded a significant and gradual increase in milk production in buffaloes fed 200 gm fenugreek seeds /head/day and also observed improvement in digestibility co-efficient of lactating buffaloe fed different levels of fenugreek seeds. Tomar *et al.*, (1996) suggested that the fenugreek seed stimulates feed intake in dairy cattle, resulting in a significant increase in milk production. The improvement in digestibility could be justified on the basis of that fenugreek seeds contain saponins which stimulate anaerobic fermentation of organic matter that improve efficiency of utilization of nutrients (Abo El-Nor *et al.,* 2007). In addition fenugreek seeds increased bacterial number in the rumen of lactating cows (Valdez *et al.*, 1986; Ali *et al.*, 2005). The improvement in milk

Table 1. Mean Somatic Cell Count (×10^5 cells/ml) in different treatment groups

Groups	0 Day	5 Day	10 Day	15 Day
FSG	4.2±0.22	2.97*±0.20	2.88*±0.19	2.17*±0.19
CON	4.38±0.34	4.41±0.32	4.75±0.46	4.93±0.60

*P (<0.05)

Table 2. Mean milk yield in different treatment groups

Treatment groups	0 Day	5 Day	10 Day	15 Day	% Variation
FSG	4.58±0.20	5.13±0.18	6.11*±0.45	6.20*±0.45	35.37 increase
CON	4.26±0.24	4.26±0.24	4.1±0.29	4.00±0.29	6.10 decrease

*P (<0.05)

Table 3. Changes in production traits in different groups

	FSG		CON	
	Before treatment	After treatment	Before treatment	After treatment
Milk yield (L)	4.58 ±0.24	6.2±0.33	3.58±0.19	3.33±0.17
Fat (%)	6.14±0.3	6.32±0.25	6.27±0.22	6.19±0.26
SNF (%)	9.28±0.32	9.44±0.37	9.04±0.19	9.10± 0.21
Total solids	15.46±0.41	15.76±0.39	15.31±0.36	15.29±0.35

production can be attributed to the fact that fenugreek seeds may contain some active components stimulating the hypothalamus or directly to pituitary gland leading to release of prolactin (Basha *et al.*, 1987) and also due to higher value of nutrients digestibility. Increased dry matter intake in lactating buffaloes fed different levels of fenugreek seeds was reported by Petit *et al.*, (1993) and Abo El-Nor (1999). Petit *et al.* (1993) reported that an isolated steroidal saponin fraction of fenugreek seeds increase feed intake and motivation to eat in normal rats. Secondly, the boosting effect of fenugreek seeds supplementation might also be attributed to the fact that fenugreek seeds increase the appetite for food (Borca *et al.*, 2000). Although Abo El-Nor (1999) suggested that fenugreek seeds may be have an effect on hypothalamus gland to stimulate hungriness center in the brain and increase the desire for eating. No reports are however on its effect on somatic cell count and subclinical mastitis. Flavonoids of fenugreek have been observed to possess antioxidant activity (Moskaug *et al.*, 2005), that would have resulted in decrease in somatic cell count. These properties of improved milk production and potent immuno-potentiation may be the reason for improved therapeutic efficacy

The result of our study suggests that Fenugreek supplementation effectively treat the subclinical mastitis in buffaloe and can be a potent substitute to the commercial therapeutic agents.

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Hematological and biochemical changes in canine Leptospirosis

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Abstract

The study was undertaken to analyse the hematological and biochemical changes associated with canine Leptospirosis. A total of 150 dogs with signs of Leptospirosis were considered. Blood and urine PCR was performed using hap1 primer. The blood was analysed for hematology and biochemical parameters. Out of 150 dogs, 47 were confirmed to be infected with Leptospires based on PCR results. There was neutrophilic leukocytosis, lymphopenia, monocytopenia and eosinopenia in confirmed cases. A reduction in RBC, Hb, PCV and platelet counts were observed. Elevated ALT, ALP, GGT, total protein, globulin, bilirubin, BUN, creatinine and decrease in albumin in confirmed cases. Neutrophilic leukocytosis was present in all and anaemia in 50 per cent of the confirmed dogs.

Keywords : Lymphopenia, Monocytopenia, Neutrophilic, Zoonotic.

Leptospirosis is one of the most important zoonotic disease affecting man and wide animal. This is more so in the tropics where leptospires sp. survive better in the warm humid conditions and remain viable for weeks in neutral or slightly alkaline water or soil (Ellis, 1984). It has been reported in over 150 mammalian species. In the developed countries, the incidence of the disease has come down substantially but contrastingly, the incidence appears to be increasing in developing countries like India. Further studies on clinical aspect of canine Leptospirosis is limited in India. Considering these facts, present study was undertaken to ascertain the hematological and biochemical changes associated with canine Leptospirosis.

Materials and methods

A total 150 dogs presented or referred to Veterinary College Hospital with the history of fever lethargy, vomiting, anorexia, melena, icterus, abdominal pain, polyuria and polydipsia from September 2012 to August 2013 were selected as subjects for the present study. Dogs positive for at least one PCR (either blood or urine) were regarded as positive.

Blood was collected from clinical cases in clean sterile vials containing EDTA for haematology and in another vial with clot activator for serum separation. The haematological parameters were estimated using Auto analyser/cell counter. Differential leukocyte count was enumerated using stained blood smears as described by Schalm *et al.* (1975). Serum ALT, ALP, BUN, creatinine, GGT, bilirubin, total protein and albumin were estimated using biochemical analyzer. Globulin was estimated as described by George and Kingsley (1939).

Globulin = Total protein – albumin.

Haematological and biochemical results were compared with reference range (Tilley and Smith, 2000).

Results and discussion

Out of 150 dogs included in the study 47 were positive and 103 were negative by PCR. All the 47 (100%) confirmed cases had leukocytosis, 45 (95.74%) dogs had neutrophilia. Out of 47 confirmed cases 37 (78.72%) and 10 (21.27%) had lymphopenia and normal lymphocyte count, respectively. Thirty (63.82%) and 17 (36.17%) dogs had monocytopenia and normal monocyte count respectively. All confirmed dogs had normal basophil count. Thirty two (68.08%) and 15 (31.91%) had eosinopenia and normal eosinophil count respectively. Twenty six (55.31%) and 21 (44.68%) had normal and subnormal RBC count respectively. Twenty three (48.93%), 23 (48.93%) and one (2.12%) had subnormal, normal and above normal haemoglobin level respectively. Twenty four (51.06%), 22 (46.80%) and one (2.12%) had normal, subnormal and above normal PCV respectively. Out of 47 cases 23 (48.93%), 18 (38.29%) and 16 (34.04%) cases respectively, had normal, subnormal and above normal platelet count.

Among the confirmed cases 26 (55.31%) and 21 (44.68%) had normal and above normal ALT level, respectively. Out of 47 dogs eight (17.02%), 30 (63.82%) and nine (19.14%) dogs had below normal, normal and above normal total protein, respectively. Out of 47, 24 (51.06%), 21 (44.68%) and two (4.25%) dogs had below normal, normal and above normal albumin, respectively. Six (12.76%), 30 (63.82%) and 11 (23.40%) dogs had below normal, normal and above

normal globulin, respectively as compared to reference range (2.3-4.5g/dl). Among the confirmed cases 27 (57.44%) and 20 (42.55%) dogs had normal and above normal ALP, respectively. Out of 47 confirmed cases 34 (72.34%) had normal and 13 (27.65%) dogs had above normal GGT. Nine (19.14%) dogs had normal and 38 (80.85%) dogs had above normal total bilirubin. Nine (19.14%) cases had normal and 38 (80.85%) dogs had above normal direct bilirubin. Thirty (63.82%) had normal and 17 (36.17%) dogs had above normal BUN. One (2.12%), 28 (59.57%) and 18 (38.29%) dogs had below normal, normal and above normal creatinine, respectively.

In the present study, leukocytosis, neutrophilia, lymphocytopenia, monocytopenia and eosinopenia were observed. Eighteen (38.29%) of confirmed dogs had thrombocytopenia. The findings were in accordance with Chandrasekaran *et al.* (2011). Neutrophilic leukocytosis could be due to inflammatory and coagulatory abnormalities in Leptospirosis as opined by Greene *et al*. (1998). Eosinopenia may occur due to stress or inflammatory response. In the present study, more than 40 per cent of confirmed cases had below normal RBC, PCV and Hb. Accelerated consumption of platelets can be due to widespread activation of the coagulation system or endothelial damage. Resulting in moderate to significant thrombocytopenia (Karen, 2010).

In the present study there was elevated ALT, ALP and GGT hyperproteinaemia, hypoalbuminemia and heperglobulinemia, increased total and direct bilirubin concentration. Similar findings have been recorded by Chandrasekaran *et al*. (2011). The endothelial damage, subsequent thrombosis and possible disseminated intravascular coagulation seen in acute disease may contribute to hepatic damage as reported by Shawn (2009). In the study, leptopirosis confirmed dogs had increased BUN and creatinine, Which is in agreement with Geisen *et al*. (2007) Azotemia reported in the present study could be due to renal dysfunction as a result of tubule-interstitial nephritis. In the present study, liver dysfunction was more than renal failure in cases of Leptopirosis. Age may be the contributing factor for hepatic dysfunction in more number of positive cases when compared to renal failure. The present study concludes with the findings that neutrophilic leukocytosis was present in all and anaemia in 50 per cent of the confirmed dogs. Alteration in liver enzymes were more than the kidney parameters.

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Canine babesiosis in south Gujarat : an hospital based study

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Abstract

The overall prevalence of 15.81% was recorded with *Babesia gibsoni* as the dominant strain in canines in the south Gujarat. Labrador and Pomeranian breeds of dogs accounted for highest number of cases while males showed higher predisposition (67.20%) than females (32.80%). The disease was recorded in all age groups varying from < 4 months to >72 months of age. The clinical picture in naturally occurring cases remained diverse and was categorised as general state, gastrointestinal, nervous, cardiac and respiratory forms. Majority (92.22%) of the dogs had clinical signs attributable to the general state.

Keywords*: Epidemiology, Canines, Gujarat, Babesia gibsoni*

Babesiosis is one of the important tick borne diseases of domestic and wild canidae, caused by intraerythrocytic piroplasms of the genus *Babesia*. It is well established clinical entity in the tropical and subtropical world (Levine, 1973; Soulsby, 1982) including India and now emerging as an important clinical disease in cosmopolitan cities (Varshney *et al.*, 2008).

Dogs brought at the Nandini hospital, Surat for treatment of illness were used as subject for this study. Of the 1986 dogs presented during study period, 518 ailing dogs with various systemic signs were clinically examined and subjected to peripheral blood examination for haemoprotozoan infection. Of the ailing 518 dogs, 314 were positive to *B. gibsoni* infection making the prevalence of the disease as 60.62% among the ailing dogs and 15.81% among the dog population attended the hospital for various health reasons.

Varshney and Dey (1998) reported a prevalence rate of 0.66% of canine babesiosis in the population at Referral Veterinary polyclinic, Bareilly. Saud and Hazarika (2000) observed prevalence rate of 21.68% in the dogs examined during the study period. Senthil Kumar *et al.*(2009) reported an incidence of *B. gibsoni* infection as 9.8% in dogs in Chennai. Varshney *et. al.* (2009) earlier reported high prevalence (78.8% of the 103 pups examined) of canine babesiosis at Surat. The variations in the prevalence could be ascribed to variations in geo-climate, sample size, study period, breed and age. Increasing urbanization and improved living standards in India has led to a positive trend in keeping pet animals. The climate of Western and Southern India, is getting hotter and humid for a longer time due to global warming favours for the propagation of arthropods which in turn increases the risk of arthropod borne infections in the country. In India babesiosis has been reported from many states from Assam (Saud *et al.*,2000) to Tamil Nadu (Harikrishnan *et al.;* 2002; Selvaraj *et al.*, 2010) and UP(Varshney and Dey, 1998).

In the present study, babesiosis was recorded in 15 different breeds of dogs with higher predisposition in Labradors, (26.43%), Pomeranians (22.61%), non-descript (15.28%) followed by German Shepherds (9.55%), Rottweilers (6.68%) and Great Danes, (4.14%). The rest of the cases were contributed by Doberman, Golden Retriever, Lhasa Apso, Cocker Spaniel, Daschund, Pug, Mastiff, St Bernard, and Boxer to the tune of 2.86, 2.86, 2.22, 1.91, 1.59, 1.27, 0.95, 0.95 and 0.63% respectively.

Chaudhari, (2006) recorded higher predisposition in Pomeranians, German Shepherds and non-descript breeds while Bashir *et al.* (2009) recorded highest number of cases in non-descript breeds. It is however difficult to gauge the actual breed wise prevalence from the present study as the total populations of each breed in the area was not known. Breed preference of owners is also different in different areas and hence the variation in the prevalence rate of babesiosis in different breeds may be due to variation in the population of the breed in the area and different attitudes of the owners in the care and management of their pets. Thus the higher incidence in Labradors in

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this study could be due to the fact that the number of Labradors may be more in the canine population in Surat. The present study was hospital based hence prevalence rate in non-descript may not match with the earlier surveys. (Gadahi *et al.* 2008; Bashir *et al.*2009).

Predisposition of the males was found higher (211, 67.20%) than females (103, 32.80%). The overall sex ratio (male/female) was 2.05:1. This is in accordance with findings of Mathe *et al.* (2006) who noted that male dogs were more susceptible to babesiosis than females. Fernandes *et al.* (2009) and Van Zyl (1995) also reported a higher incidence in males (59.18 and 57%) compared to females (40.82 and 43% %).Since *B.gibsoni* can also be transmitted due to non-vectored transmission by blood exchange during fighting and biting (Jefferies *et al.*, 2007; Bostrom *et al.*, 2008), direct dog-to-dog transmission may be an important route of infection of *B. gibsoni* in all localities where it was found (Irwin, 2007). This may explain the higher number of males affected as inter-dog aggression is more common in male dogs than in female dogs.

Babesiosis was recorded in all age groups from < 4 months to >72 months of age. The youngest dog with babesiosis was 30 days old. Most number of cases were seen in the dogs less than 4 months of age (0-4 months) with 28.34% prevalence followed by middle aged dogs, (13-36 months) with 25.80% prevalence. Prevalence of babesiosis in dogs in age group 5-12 months was 21.02% and that in age group 37-72 months was 13.06%. The least number of cases was seen in the geriatric age group i.e. dogs older than 72 months with only 37, 11.78% of the total cases. This difference though was statistically non-significant. Similar findings have been reported (Selvaraj *et al.* 2010; Varshney *et al.*2003).

Varshney*et al.* (2009) studied 130 puppies and found that 78.8% were suffering from babesiosis while Irwin (2005) stated that puppies were more severely affected than adult dogs. However Senthil Kumar *et al.* (2009) recorded highest prevalencein adult dogs (> 3 yrs).

The clinical picture in naturally occurring cases of babesiosis remained diverse with wide variability in clinical signs. The signs were categorised as general state, gastrointestinal, nervous, cardiac and respiratory. Majority (92.22%) of the dogs had clinical signs attributable to the general state. Rectal temperature was elevated in 64.44%, (174),hypothermia in 7.77%, (21) and remaining 27.77% of the dogs had normal body temperature. Thus it was apparent that pyrexia was not a concordant sign in canine babesiosis due to *B. gibsoni*, contrary to the common belief. It was interesting to note that severely septicaemic dogs had subnormal temperature. The fact that normal rectal temperature was also recorded in infected animals, pyrexia should not be taken as a thumb rule in babesiosis and the disease can be suspected even when there is no elevation of temperature if other clinical indicators are present.

All the cases of babesiosis in the present study were due to *B. gibsoni* and no case of *B.canis* was recorded during the study period. In an earlier study Varshney *et al.* (2009) also reported a preponderance of *B. gibsoni* infection (94 pups, 97.9%) as compared to *B.canis* (2 pups, 2.1%) at Surat (South Gujarat). Chaudhari (2006) recorded *B. gibsoni* in 99.53% of the cases in his study.

In India, both *B. canis* (Kalra and Singh, 1984; Varshney *et al.*, 2004) and *B. gibsoni* (Verma *et al.*, 1989; Varshney *et al.*, 2003) are prevalent with geographical variations. *B. canis* is more prevalent in southern regions of the country, whereas *B. gibsoni* is more prevalent in the northern states. The present study revealed that *B. gibsoni* is the predominant strain of Babesia in Gujarat.

Concurrent infection with *E. canis* was found in 4.78% of the cases. Concurrent infections are quite common (Mathewman *et al.*, 1993; Chou, 1995; Varshney and Dey, 1998; Kumar, 2004) which make the clinical picture more complex leading to treatment failures.

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Comparative efficacy of *keetguard* (herbal acaricide), amitraz and deltamethrin against *Sarcoptes scabiei* mite infestation in black bengal goats

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Abstract

The study was conducted to evaluate the comparative efficacy of acaricides *viz*., keetguard (herbal acaricide), deltamethrin and amitraz in naturally infected *Sarcoptes scabiei* mites in Black Bengal goats. A total of 32 confirmed clinical cases of scabies in Black Bengal goats were divided into 4 groups. Out of 4 groups; Group A as disease control were given weekly tap water bath for 3 consecutive weeks whereas Group B, C and D were treated externally with keetguard (herbal acaricide), deltamethrin 12.5% and amitraz 12.5%, respectively at their recommended dose rate at weekly intervals for 3 consecutive weeks. keetguard (herbal acaricide) and amitraz-treated goats revealed both faster clinical (28 days) and parasitological (14 days) recovery however; deltamethrin-treated goats did not show complete clinical/parasitological recovery even after application of drug for 3 consecutive weeks. keetguard (herbal acaricide) and amitraz-treated goats revealed faster reduction in the mite density.

Keywords: Amitraz · Black Bengal goats, Deltamethrin, keetguard (herbal acaricide), *Sarcoptes scabiei*

Ectoparasitic infestation often causes severe economic constraints in goat production across the world. Amongst the ectoparasites, *Sarcoptes scabiei* causes major economic threat in terms of poor quality meat, low production of milk and make a major trade embargo in leather industry. It is a deep borrowing mite and is of zoonotic importance. The disease commonly known as scabies is characterized by intense pruritus, hyperkeratitis, alopecia as well as general erythema of the skin. Apart from dermatological symptoms, scabies infection is also associated with physiological changes.Though the diseases seem to be less dangerous however, in neglected cases emaciation, weakness and anorexia causes significant morbidity and mortality in domestic and farm animals (Walton and Currie, 2007). A number of drugs viz., ivermectin (Thakuria *et al.*,2013)., doramectin (Kya *et al.*,2010); amitraz (Tarralo *et al.*,2009;Thakuria *et al.*,2013); had been used as miticides worldwide. The present study was conducted to evaluate the comparative efficacy of acaricides *viz*., keetguard (herbal acaricide), deltamethrin and amitraz in naturally infected *Sarcoptes scabei* mites in Black Bengal goats.

Materials and methods

A total of 32 confirmed clinical cases of scabies in Black Bengal goats presented to the department of Teaching Veterinary Clinical Complex, College of Veterinary Sciences & Animal Husbandry, R. K. Nagar, Agartala, Tripura were divided into 4 groups. Out of 4 groups; Group A as disease control were given weekly tap water bath for 3 consecutive weeks; Group B were given keetguard (herbal acaricide) liquid bath with water (1: 20 dil.) and was applied twice a week for 3 consecutive weeks; Group C goats were applied deltamethrin 12.5% @ 4 ml/liter of water at weekly interval for 3 times and Group D were applied with amitraz 12.5% @ 2 ml/liter of water at weekly interval for 3 consecutive weeks.

The goats were examined daily for the clinical improvements, and the skin scrapings, 1 cm^2 of skin area scraped from three different lesions on the body and were examined once in a week for the presence and density (the number of mites per ten microscopic fields) of the mites using skin scrapping examination (Kaya *et al.*,2010). Three most commonly observed clinical symptoms (intensity of pruritus, degree of crusts (hyperkeratitis) formation, and degree of alopecia) were assessed and rated on a scale from 0 (absent), present (1), low (2), medium (3), high (4) to severe (5). The three scores were added up and expressed as a Sarcoptes-induced skin lesions score (SSLS) that could have values between 0 and 15 (Kumar, *et al.*,2014). The clinical recovery was evaluated on the basis of improvement in SSLS and reduction in the mite density. The main efficacy criterion of the selected drug was the parasitological cure rate, which was calculated as the proportion of goats negative for sarcoptic mites on the basis of microscopic examination of deep skin scrapings

obtained on days 0, 7, 14, 21 and 28 post therapies.

Results and discussion

Parasitological and clinical examinations revealed presence of eggs, larva, nymph and adult stages of *Sarcoptes scabiei*. Clinical signs most commonly encountered were pruritis, alopecia, rough hair coat, hyperkeratitis and scaling however, erythrema and ulcerations noticed in only 4 naturally infected goats out of 32. Keetguard and amitraz-treated goats revealed both faster clinical (28 days) and parasitological (14 days) recovery however; deltamethrin-treated goats did not show complete clinical/parasitological recovery even after application of drug for 3 consecutive weeks (Table 1). Hence, the deltamethrin application was continued for further one more week.

Keetguard and amitraz-treated groups revealed non-significant difference for both parasitological and clinical improvement whereas all the treatment groups showed significant ($p< 0.05$) improvement from disease control group for both parasitological and clinical recovery (Table 1 and 2). The percent improvements in SSLS on days 7, 14, 21, and 28 were 32.13, 60.32, 82.97 and 100, respectively compared to day 0 for keetguard treated goats. The percent improvements in SSLS on days 7, 14, 21, and 28 were 16.13, 38.75, 51.61, and 72.65, respectively for deltamethrin-treated groups while 29.26, 60.03, 76.31 and 100, respectively for amitraz- treated groups compared to day 0 values. However, the percent increase in SSLS on days 7, 14, 21, and 28 were 17.25, 31.03, 46.62 and 54.21 respectively contrast to day 0 values for disease control goats. Keetguard and amitraz-treated goats also revealed faster reduction in the mite density, compared with the deltamethrin-treated groups (Table 2). However, clinical recovery was faster in keetguard treated group. Disease control goats (untreated goats) showed significant ($P<0.05$) aggravation in the SSLS and increase in the mite density at the respective day compared to treatment groups. Keetguard and amitraz-treated goats showed re-growth of hair on or after 21 days of treatment in naturally infested goats whereas re-growth of hairs were not noticed in deltamethrin-treated groups even after 28 days of treatment.

From the observations, the product Keetguard liquid showed faster parasitological recovery could be due to their high efficacy against oviposition/hatchability of eggs by female mites. Jumde *et al.*,(2013) noticed 95% efficacy against the oviposition/egg laying capacity of ticks whereas 100% efficacious against the hatchability of egg of treated females. Researcher noticed buffaloes and cattle treated five times at 6-day intervals (0, 6, 12, 18 and 24) with (AV/EPP/14) having similar constitutes of keetguard (containing the active ingredients: *Cedrus deodara*, *Pongamia glabra*, *P. pinnata*, *Azadirachta indica*, *Eucalyptus globulus* and *Acorus calamus*) resulted in elimination of 65.3, 87.6, 96.5, 99.6 and 100% of the ticks, respectively. Amitraz has also shown comparable efficacy to keetguard against Sarcoptes mites infections in goats as it is a product

Table 1: Comparison of the density of mites in goats treated with keetguard, deltamethrin and amitraz using skin scrapping examinations (mean±SD).

Groups	Day 0	Day7	Day 14	Day 21	Day 28
A	15.50±3.93	18.0±3.96^{c}	19.75±4.16^{b}	22.25±4.39^{b}	24±3.38^{b}
B	14.86±3.87	0.63±1.18^{a}	00±0.00	00±0.00	00±0.00
C	14.63±2.60	8.63±2.13^{b}	3.13±1.73^{a}	0.43±1.08^{a}	00±0.00
D	14.13±3.04	0.87±1.13^{a}	00±0.00	00±0.00	00±0.00

a, b, c Statistically significant difference ($P<0.05$) when compared with the values pertaining at the respective day of various groups

Table 2: Comparison of the Sarcoptes-induced skin lesions scores in goats treated with keetguard, deltamethrin and amitraz using skin scrapping examinations (mean±SD).

Groups	Day 0	Day7	Day 14	Day 21	Day 28
A	7.25±1.04	8.5±1.2^{c}	9.5±1.31^{c}	10.63±1.6^{c}	11.88±1.89^{b}
B	6.63±0.74	4.5±0.76^{a}	2.63±0.74^{a}	1.13±0.64^{a}	00±0.00
C	7.75±1.28	6.5±1.2^{b}	4.75±1.04^{b}	3.75±0.89^{b}	2.12±0.64^{a}
D	6.88±1.89	4.87±1.36^{a}	2.75±0.89^{a}	1.63±0.64^{a}	00±0.00

a, b, c Statistically significant difference ($P<0.05$) when compared with the values pertaining at the respective day of various groups

used intensively for demodicosis and sarcoptes mites infestation in dogs even though their use in livestock is still limited in India. Hence, the probabilities of development of resistant against amitraz to sarcoptes in these animals are very less. Amitraz belongs to the formamidine family and acts by inhibiting monoamine oxidase and prostaglandin synthesis and by stimulating the alfa 2 – adrenergic receptors of the arthropod nervous system (Tarralo *et al.*,2009). These goats also revealed significant (Pd"0.05) improvements in the SSLS and reduction in the mite density at the respective day post-therapy, compared to both deltamehrin-treated group and disease control group. Various authors have also demonstrated the effectiveness of amitraz against mange mites (*Sarcoptes scabiei var. cameli*) in camels (Lal *et al.*,1996) and mange in pigs (Griffiths, 1976).

Poor response of deltamethrin in present report point to resistant to ectoparasite population (Graf *et al.*,2004; Brito, *et al.*,2011). Comparative efficacy of deltamethrin is reported to be lesser than ivermectin against sarcoptes mite infections in pigs (Tome,1993).Conventional synthetic ectoparasitcides proved to be deterrent for causing environmental contamination, potential harmful residues in food, toxicity to workers and consumers (Graf *et al.*,2004).

In conclusion, the efficacy of keetguard (herbal acaricide) was found comparable to amitraz 12.5% against Sarcoptes mite infestation in goats. As amitraz is a synthetic parasiticide and its indiscriminate use may include harmful effects on environment, human being and on animals in addition to resistant to ectoparasites population over a due course of time. Authors suggests for the use of keetguard, a herbal acaricide contain almost insignificant side effects on environment and on living being.

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Biochemical and mineral profile of dromedary camels raised under different pastures conditions of Rajasthan

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Abstract

Blood biochemical and mineral profile of dromedary camels raised under extensive management in hot arid zone (Pokhran area) and semi arid zone (Udaipur) of Rajasthan state were evaluated. Serum protein, albumin, globulin and albumin: globulin ratio was statistically comparable, while lower albumin and higher globulin level was recorded in camels of Pokhran area. SGOT (AST) and SGPT (AST) values were also similar among the areas. Calcium and magnesium concentrations did not differ significantly. However, phosphorus level was lower than normal in Pokhran area, but it was significantly higher (Pd"0.01)in the Udaipur area. Presence of rock phosphate mines and soil rich in soluble phosphorus may be possible reason behind this observation.

Keywords*:* Blood biochemical profile, Camel, Mineral-profile, Semi- arid zone, Hot- arid zone

Camel are usually raised under extensive system of management in Pokaran area of Jaisalmer District. Soil is largely brown sandy to sandy loam type. Udaipur is located in the southern region of Rajasthan and is close to Gujarat. There are several rock phosphate mines in Udaipur and soil is also rich in soluble phosphorus.

Pastures of Pokhran area ischaracterized by higher population of bushes of *Zizyphusnum mularia* and *Prosopis juliflora*, lower population of *Prosopis cineraria* tree and grasses as *Lasiuruss indicus*, *Cenchrus* and *Octochocola* species. On the other hand Udaipur area has higher population of *Prosopis juliflora*, *Acacia tortalis, Acacia senegal*, *Inga dulcis*, apart from*Octothocola* species and mountain grasses. Plasma mineral concentration is reported to vary with soil, pasture condition and with grazing latitude in cattle (Dermauw*et al.*, 2013). However, there seems no report available on effect of pasture on blood biochemical and mineral profile in camel. The present study was carried out to analyze blood biochemical and mineral profiles of camel raised under semi arid and hot arid ecosystem or Rajasthan state.

Materials and methods

Study was conducted in the hot arid zone at Pokhran area (Lwan Sattasar, in Jodhpur; Khetolai, Lathi, Ganga Ram ki Dhani in Jaisalmer District) and the hilly terrain of semi arid zone at Udaipur area (Aadol in Jadol Tehsil of Udaipur) in the Rajasthan state. Blood samples of apparently healthy camels (n= 19 each)were collected using heparin as anticoagulant and were analyzed for total protein, albumin, SGOT, SGPT, creatinine, calcium, phosphorus and magnesium concentration by standard procedures. The data generated was analyzed by unpaired t-test using SPSS (1997).

Results and discussion

Blood biochemical and mineral profile of camels of Pokharan and Udaipur area is given in Table 1. Serum albumin concentration in camels of Udaipur area was significantly higher (Pd"0.01), whereas serum Globulin concentration was significantly lower (Pd"0.01) than animals of Pokhran area. Albumin: globulin ratio was higher in camel of Udaipur area. However, levels of total protein, albumin and globulin were within the normal range (Bogin 2000; Mohri *et al.*, 2008). Hence, it appears that difference in nutrient availability influence the protein profile of animals, but the body homeostasis in healthy animals some how maintain them within the normal range.Since the samples were drawn during the lean season, the values were marginally lower owing to fodder biomass availability and its maturity. SGOT and SGPT values did not differ significantly indicating normal liver function in animals from both the areas.Likewise, normal serum creatinine concentrations in both areas indicated normal kidney function in animals reared in two different pasture conditions.

Concentration of calcium in serum was similar among the two areas, however concentration of phosphorus was lower than normal at Pokhran which could be due to decline in availability of calcium and phosphorus during the lean season. Significantly higher (P<0.01) blood phosphorus level in Udaipur area could

Table 1: Blood biochemical and mineral profile of dromedary camels under range management

Parameter	Pokaran Area	Udaipur Area	SEM	P value
Number of animals	19	19		
Total protein (g/dl)	7.02	6.79	0.064	0.271
Albumin (g/dl)	4.09	4.67	0.033	0.000
Globulin (g/dl)	2.93	2.11	0.082	0.001
A:G ratio	2.57	3.31	0.059	0.010
SGOT (U/L)	68.50	77.92	45.163	0.095
SGPT (U/L)	8.74	8.26	8.298	0.835
Creatinine (mg/dl)	1.46	1.36	0.012	0.302
Calcium (mg/dl)	9.98	10.27	0.761	0.687
Phosphorus (mg/dl)	4.54	9.28	0.958	0.002
Ca:P ratio	0.75	1.20	0.259	0.000

be due to higher uptake of phosphorus from plants as well as soil and water as the area is rich in rock phosphate. Calcium: Phosphorus ratio in Udaipur animals was abnormal, perhaps due to excess phosphorus intake. Soil phosphorus concentration is reported to have significant effect on phosphorus concentration in pasture plants and blood of grazing cattle (Gizachew *et al.,* 2002). Ca:P ratios may affect intestinal absorption of minerals (Koshihara*et al.,* 2004). Mineral imbalances result in serious health problems. Diets insufficient in phosphorus negatively affect fertility, growth and milk production, while highphosphorus uptake is also harmful and predisposing formation of urinary calculi. Animals with improper Ca: P ratios may exhibit depraved appetites.

Primarily, camel browsesgreen leaves and pods of trees/ bushes, though grasses are consumed to a little extent due to its anatomical adaptations regarding its height. Grasses and herbaceous species are consumed to a lesser extent. Tree leaves that constitute 90% diet of camel are in general rich in Ca but poor in P (Shinde *et al.,* 2006). Biomass production has been found to range 1.2-2.1ton ha^{-1} in desert range lands of Jaisalmer. Availability of biomass is higher in the semi region of Udaipur compared to hot arid belt due to higher precipitation received in Udaipur area.

The results suggest need to rectify organic nutrient and the mineral imbalances through supplementationin camels raised under extensive system to check loss in productivity especially during the lean season.

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Comparative efficacy of cephelexin and amoxicillin-clavulanate in canine pyoderma

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Abstract

The present study was undertaken to evaluate the therapeutic efficacy of oral cephelexin and amoxicillin-clavulanate in canine pyoderma. It was conducted on 12 pyoderma affected dogs brought to TVCC, Pantnagar. Two groups were formed (n=6). Six healthy dogs were also included for comparison of the results. The clinical examination of the dogs was done every 7 days till complete cure and hemato-biochemical evaluation before and after treatment was performed. Amoxicillin-clavulanic acid and cephelexin were found in order of merit in case of bacterial dermatitis.

Keywords*:* Canine, pyoderma, cephelexin, amoxicillin-clavulanic acid, treatment

Mankind has a long and very complex history of association with animals. The history of human-dog relationship dates back at least fourteen thousand years (Clutton-Brock, 1995). During their service to the mankind, dogs suffer with so many ailments. Out of these ailments, skin ailments contribute a lot to their suffering. Skin diseases are among the commonly encountered problems in dogs. Pyoderma has an important role in these skin infections in canines. The skin diseases caused by bacteria are termed as pyoderma. The skin ailments become even more significant because of their zoonotic nature. Diagnosis of skin infections is complicated by the fact, that the skin has only a limited range of responses to a wide variety of cutaneous insults. The present study was planned to evaluate the therapeutic efficacy of two antibacterials in clinical cases.

Materials and Methods

During screening of dermatoses affected dogs, skin infections found positive for bacterial infection and negative for ectoparasites and fungal spores were grouped under bacterial dermatitis.

The antibiotics were selected based on antibiotic sensitivity test, in which cephelexin and amoxicillin-clavulanate combination showed the maximum zone of inhibition. Two therapeutic groups were formed with 6 dogs each along with a healthy control group (n=6).

Dogs of group 1 were treated with cephelexin[5] orally @ 30 mg/kg OD for at least 7 days while dogs of group 2 were treated with amoxicillin-clavulanate[6] orally @ 20 mg/kg bid for at least 7 days.

The supportive treatment included anti-histaminic (chlorpheniramine maleate at the rate of 2 mg/kg b.wt. i/m od), fatty acids supplement (Nutricoat Advance® at the rate of 5 ml orally bid for pups and 10 ml orally bid for adult dogs for 15-30 days).

The blood and serum samples of the dogs were collected first on day 0 and then after the cessation of therapy in various groups to evaluate the effect of the treatment. About 2 ml of blood sample was collected from cephalic vein of each dog, immediately after collection, blood was transferred to EDTA vials for the analysis of blood cellular components deploying standard laboratory procedures. Biochemical analysis for blood glucose, protein profiles, and enzymes from the serum samples were carried out and it was compared with that of healthy dogs.

Evaluation of therapy

The therapeutic efficacy of formulations was evaluated on the basis of significant variation in hemato-biochemical profiles, skin scraping examination and clinical recovery. Statistical analysis of the data were performed using software SPSS 16.0. t-test and ANOVA were used to find out any significant difference between groups (Snedecor and Cochran 1994).

Results and discussion

In first group where cephelexin was given to affected dogs, there was resolution of pruritis and vesicles by the day 7 of the therapy (Table I). All the dogs recovered by day 21 of initiation of therapy with the resolution of alopecia, erythema, papules, collarettes, seborrhoeic plaques and nodules. Initiation of recovery

Table 1: Therapeutic assessment of oral cephelexin (n=6)

Clinical examination of skin	0th day	7th day	14th day	21st day
Alopecia	++ (4)	+(2)	+(1)	-
Pruritis	++	-	-	-
Erythema	+++	++(2)	+(1)	-
Papules	+++	+(3)	+(2)	-
Pustules	++ (5)	++(2)	-	-
Vesicles	++(2)	-	-	-
Macules	++(4)	+(2)	-	-
Nodules	++(3)	+(1)	+(1)	-
Scales	++	++(2)	-	-
Epidermal collarettes	+++(5)	++(3)	+(2)	-
Rough hair coat	+++	++(2)	-	-
Eczema	++ (4)	+(1)	-	-
Seborrheic plaques	++(2)	+(1)	+(1)	-
Weeping lesions	++(1)	+(1)	-	-

Figures in parenthesis indicate the number of dogs affected

bacterial dermatitis *in vivo*. In the present study, the infections by mixed cultures took longer time to get cured in comparison to *Staphylococcus* infection which has also been reported by Hardman *et al.* (2001) that cephelexin has good activity against Gram positive bacteria and moderate activity against Gram negative microorganisms.

Evaluation of therapeutic efficacy of amoxicillin clavulanic acid combination against bacterial dermatitis in group 2 revealed marked improvement on day 7 when various lesions started disappearing including vesicles, macules and rough hair (Table 4). On day 14 of the institution of therapy, the alopecia, pruritis, pustules, scales, and eczema resolved completely. All the 6 dogs recovered on day 21 after treatment showing 100%

Table 2 : Effect (Mean±SE) of different treatments on hematological parameters in dogs

Parameters			Group K (cephelexin)		Group L (amoxicillin clavulanic acid)	
		Healthy (n=6)	BT (0th day)	AT(21stday)	BT (0th day)	AT(21stday)
Hb (g/dl)		15.034±0.39[a]	11.33±0.38 [b]	14.6±0.61 [a]	11.23±0.43 [b]	14.03±0.53 [a]
PCV (%)		45±1.73[a]	43.5±1.87 [a]	42.33±1.33 [a]	43.17±1.90 [a]	42±1.12 [a]
ESR (mm/h)		4.17±0.48[a]	8.67±0.42 [b]	4.33±0.49 [a]	8.17±0.48 [b]	4.5±0.43 [a]
TEC(10^6/cumm)		7.94 ±0.42[a]	5.35±0.29 [b]	6.87±0.33 [a]	5.51±0.30 [b]	7.07±0.29 [a]
TLC(10^3/cumm)		11.23±0.88[a]	18.38±0.48 [b]	12.48±0.54 [a]	18.24±0.29 [b]	12.97±0.52 [a]
DLC(%)	N (%)	70.17±2.69[a]	78.33±0.67 [b]	67.17±0.75 [a]	78.67±1.41 [b]	68.83±1.17 [a]
	E (%)	4.84±0.31[a]	3.5±0.62 [a]	3.17±0.48 [a]	3.5±0.67 [a]	4.33±0.42 [a]
	L (%)	20.5±2.78[a]	13.84±0.48 [b]	26.33±0.88 [a]	13.83±0.87 [b]	23.5±1.05 [a]
	B (%)	0.5±0.23[a]	0.5±0.22 [a]	0.17±0.17 [a]	0.33±0.21 [a]	0.17±0.17 [a]
	M (%)	4±0.25[a]	4±0.45 [a]	3.17±0.31 [a]	3.67±0.33 [a]	3.17±0.31 [a]

Figures having different superscripts across the columns are significantly different upto 5% level of significance.

Table 3: Effect (Mean±SE) of different treatments on biochemical parameters in dogs

Parameters		Group K (cephelexin)		Group L (amoxicillin clavulanic acid)	
	Healthy (n=6)	BT (0th day)	AT (21st day)	BT (0th day)	AT (21st day)
Glucose (mg/dl)	91.83±4.53[a]	116.17±3.89 [b]	92.17±2.60 [a]	115±8.56 [b]	87.17±4.40 [a]
Total Protein(g/l)	67.25±0.47[a]	68.64±0.42 [a]	67.94±1.05 [a]	68.34±0.73 [a]	69.99±1.14 [a]
Albumin (g/l)	33.64±0.59[a]	33.78±1.04 [a]	32.92±0.63 [a]	34.01±0.38 [a]	33.03±0.88 [a]
Globulin (g/l)	33.61±0.95[a]	34.85±1.12 [a]	35.02±1.59 [a]	34.34±0.55 [a]	36.96±1.69 [a]
A:G ratio	1.07±0.04[a]	0.97±0.06 [a]	0.95±0.05 [a]	0.99±0.02 [a]	0.90±0.05 [a]
ALT (IU/l)	58.66±5.25[a]	61.67±5.48 [a]	57.67±4.12 [a]	63.34±6.89 [a]	64.17±2.45 [a]
AST (IU/l)	47±2.62[a]	52.17±4.75 [a]	47.34±3.57 [a]	48.83±6.10 [a]	44.33±5.12 [a]

Figures having different superscripts across the columns are significantly different upto 5% level of significance.

was observed on day 7 post therapy when 4 dogs recovered completely. Thereafter 2 dogs recovered on day 21 showing 100% recovery. The hemato-biochemical parameters showed significant differences ($P<0.05$) towards normalcy in all the treated dogs and were similar to value recorded in healthy dogs (Table 2 and 3). Cephelexin was found highly effective against recovery. Hemato-biochemical parameters returned to normal post treatment with Amoxicillin-clavulanic acid (Table 2 and 3). Amoxicillin-clavulanic acid combination is bactericidal for both Gram positive and Gram negative organisms (Hardman *et al.*, 2001). Amoxicillin-clavulanic acid has a better oral absorption as it is stable in acid and produces more sustained blood

Table 4: Therapeutic assessment of amoxicillin-clavulanate (n=6)

Clinical examination of skin	0th day	7th day	14th day	21st day
Patches of alopecia	++ (5)	+(1)	-	-
Pruritis	++	+(1)	-	-
Erythema	++	+(1)	+(1)	-
Papules	+++(4)	+(2)	+(1)	-
Pustules	+++ (3)	+(1)	-	-
Vesicles	++(2)	-	-	-
Macules	++(1)	-	-	-
Nodules	++(2)	++(1)	+(1)	-
Scales	++	+(1)	-	-
Epidermal collarettes	+++	++ (3)	+(2)	-
Rough hair coat	+++	-	-	-
Eczema	++ (2)	+(1)	-	-
Seborrheic plaques	++(2)	+(1)	+(1)	-

Figures in parenthesis indicate the number of dogs affected

levels (Tripathi, 2003).

Although *in vitro* the sensitivity of amoxicillin associated with clavulanic acid was lesser than cephelexin against Gram positive bacteria but *in vivo* there was no specific difference observed between these two. Further, amoxicillin clavulanic acid combination responded better than cephelexin in case of infections with Gram negative bacteria. Coutinho and Cavalcanti (2005) found that the most effective antibacterial drugs against *Staphylococcus spp.* were amoxicillin associated with clavulanic acid and cefalexin.

On recovery basis, amoxicillin-clavulanic acid and cephelexin were comparable in therapeutic efficacy. However, clinical manifestations in some cases resolved faster by amoxicillin-clavulanic acid than cephelexin. Hemato-biochemical profiles returned to normal with both the drugs.

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Clinical Article

Contagious Ecthyma in a Singanoor sheep – A case report

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Abstract

Eight month old sheep was presented to Clinical Department of Veterinary Medicine, Veterinary college, Hassan, with history of Anorexia from 4 days. Clinical examination revealed Pyrexia -104°F, mucous membrane-congested, eczematous / scab lesions on muzzle, tongue with swelling, ruptured scab lesions, salivation. For confirmatory diagnosis, scab lesion of the mouth was collected in 50% glycerol Phosphate Buffered Saline. Sample was found positive for Contagious Ecthyma by PCR in Virology unit IVRI, Mukteshwar. Therapy of this case was completed with suitable antibiotic and supportive care.

Keywords: Contagious Ecthyma, PCR, Treatment

Contagious Ecthyma is a highly contagious, viral skin disease of sheep caused by Parapoxvirus. The morbidity rate is usually high in an unvaccinated flock (Housawi *et al.*,1991).

Eight month old sheep was presented to Clinical Department of Veterinary Medicine, Veterinary College Hassan, Karnataka with history of Anorexia from 4 days. Clinical examination revealed pyrexia (104°F), congested mucous membrane, eczematous, scab lesions on muzzle, commisures of the lips and tongue with swelling, pustules, cauliflower like growth, ulceration and salivation. Heart rate and respiratory rate were within the normal range. Based on clinical signs the disease was tentatively diagnosed as Contagious ecthyma.

Mouth scab lesion are removed carefully using a sterile scalpel and stored in a sealed plastic tube with 50% glycerol Phosphate Buffered Saline (PBS) and samples were sent to Division of Virology, Indian Veterinary Research Institute, Mukteshwar. Samples were found positive for Contagious Ecthyma.

The therapy was undertaken using a course of antibacterial agent Enrofloxacin @ 5 mg/kg BW i/m for 5 days to overcome secondary bacterial infection. For pyrexia, inj Meloxicam @0.3mg/kg BW i/m and twice a day for a week. Scab lesion dried off and complete recovery of animal was seen after 20 days.

Virus isolation is thought to be a gold standard but its time-consuming (Chan *et al.*, 2007). With the development of molecular biology, the PCR technique has become widely used to amplify the desired genomic fragments from tissue specimens and it has become a powerful tool in molecular diagnosis. The PCR is able to diagnose Contagious Ecthyma (CE) virus infection in specimens from affected animals (Inoshima *et al.*, 2000; Guo *et al.*, 2003).

In this study, the PCR is able to diagnose CE virus infection in specimens from affected animals. There is no treatment for orf and as the disease is caused by a virus, it cannot be treated using antibiotics. Most orf infections clear up on their own within a few weeks (Mckeever *et al.*, 1988) but the use of topical antibiotic paints, powders or aerosols can help prevent the establishment of secondary bacterial infections. In severe cases of secondary bacterial infection, the usage of a systemic antibiotic is recommended.

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Blood transfusion in a downer cow

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Abstract

Blood transfusion in cattle is relatively uncommon and performed on life threatening conditions. A pluriparous dairy cow weighing 320 kgs was brought with a history of lethargy, inappetance since two weeks. Clinical examination revealed severely blanched and icteric conjuctival and buccal mucous membrane, fever, swollen lymph nodes, tachycardia, aortic thudding at the right flank, severe panting and abdominal type of respiration. Hematology revealed anemia (7.6% Packed Cell Volume and Haemoglobin 2.5 g/dl). *Anaplasma marginale* (+++) was evidenced in the blood smear and then confirmed by Polymerase Chain Reaction. Treatment was initiated with intravenous Oxytetracycline @20mg/kg B.W diluted with Normal saline. On second day, the animal was brought in sternal recumbency with severe dyspnea and muscle fasciculations. After crossmatching 4 Ltrs was collected in blood bag from healthy cow and transfered to the recipient. The recipient cow showed progressive improvement including pink mucous membranes and mild appetite within 24 hours and subsequent recovery established within a week along with long acting oxytetracycline and iron injections every alternate day for 2 weeks. The PCV showed very little improvement on the first two days and then marginal increase on the 7th day post transfusion. There was no transfusion reactions and the cow resumed normalcy in three weeks,

Keywords*:* Anaplasmosis, Blood transfusion, Downer cow.

An adult dairy cow was presented with a history of lethargy, heaving, mild ataxia, standing in a position for a long time and inappetance for two weeks. Clinical examination revealed severely blanched and icteric conjuctival mucous membrane, icteric buccal and vaginal mucous membrane (Fig 1), fever 104.5^{0} F, swollen lymph nodes, tachycardia, aortic thudding, severe heaving and abdominal type of respiration. Ruminal atony, muscle fasciculations, ataxic gait were evidenced on general clinical examination. Pounding jugular pulse, severe dehydration more than 7%, poor capillary perfusion, dyspnea, rapid shallow respiratory patterns with open mouth respiration were recorded.

Materials and methods

Clinical examination revealed anaemic hypoxia and poor health status of the animal. Haematology revealed anemia of very low Hematocrit of 6.5% and Haemoglobin 2.0 g/dl, R.B.C 1.12 mm/cmm, W.B.C count 5000 cells/ cmm, serum total proteins 5.99 g/L, cytological picture of Hypochromasia, Anisocytosis, Codocytosis, negative saline agglutination tests indicating severe anaemia and pancytopenia. Liver function tests evidenced increased serum conjugated bilrubin of 1.33mg/dL and normal serum unconjugated bilrubin of 0.14mg/dL. Hypoproteinemia and Hypophosphatemia were recorded. *Anaplasma marginale* was evidenced in the blood smear and then confirmed by Polymerase Chain Reaction. Treatment was initiated with intravenous Oxytetracycline @20mg/kg B.W diluted with Normal saline (10 sachets).

On the subsequent day the cow deteriorated in condition with severe dyspnea and muscle fasciculations and was presented in sternal recumbency. The downer cow was stabilized with fluids and a whole blood transfusion was arranged.

A major and minor cross match between recipiant and healthy donor cow was performed. No incompatibility was evidenced and five litre (Veterinary blood bag *Jorvet*®) was used and 400 ml of CPDA solution was added (Fig 2). Using the 16 G needle with the adapter whole blood was collected from the jugular vein by aseptic measures. The collected whole blood was transfused at the rate of 1ml/kg/hr after evidencing no hemolytic reactions for the first 15 minutes. The entire transfusion was performed within two hours. No acute post transfusion reactions including urticaria, fever, enteritis were observed. The recipient cow however did not show much anticipated improvement within 24 hrs, the haematocrit was still 8.2 and the haemoglobin was 2.9 (Fig 2). After 72 hours progressive improvements including pink mucous membranes, rumination, mild appetite with elevated haematocrit of 24% and haemoglobin 8.5 g/dl, Total Protein 6.4 g/L was recorded. After four weeks, the cow resumed normalcy in clinical condition with haemoglobin 12.5g/dl and haematocrit of 35% and total protein 7.1 g/L.

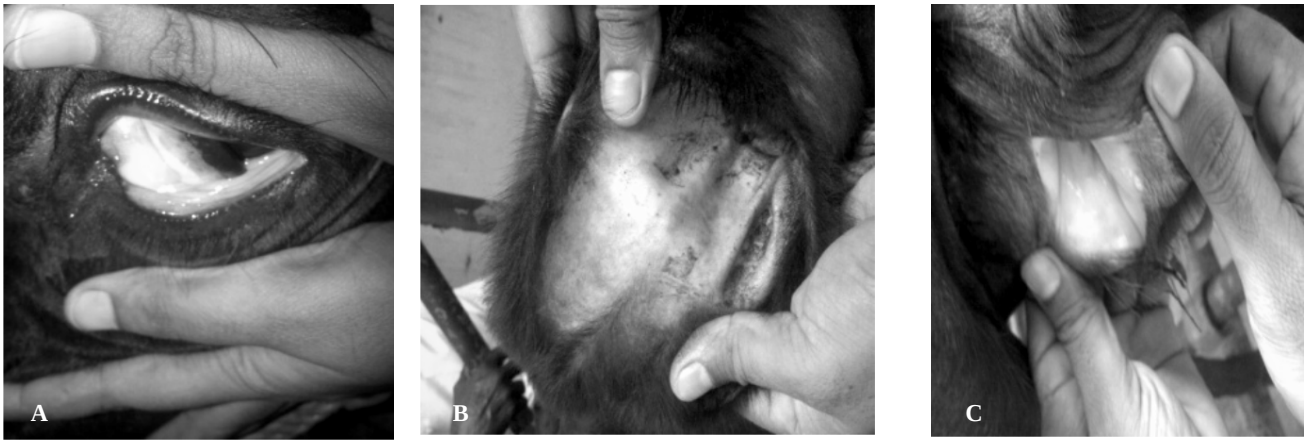

Fig 1. A- blanched icteric conjuctival, B-inner ear, C-vaginal mucous membranes

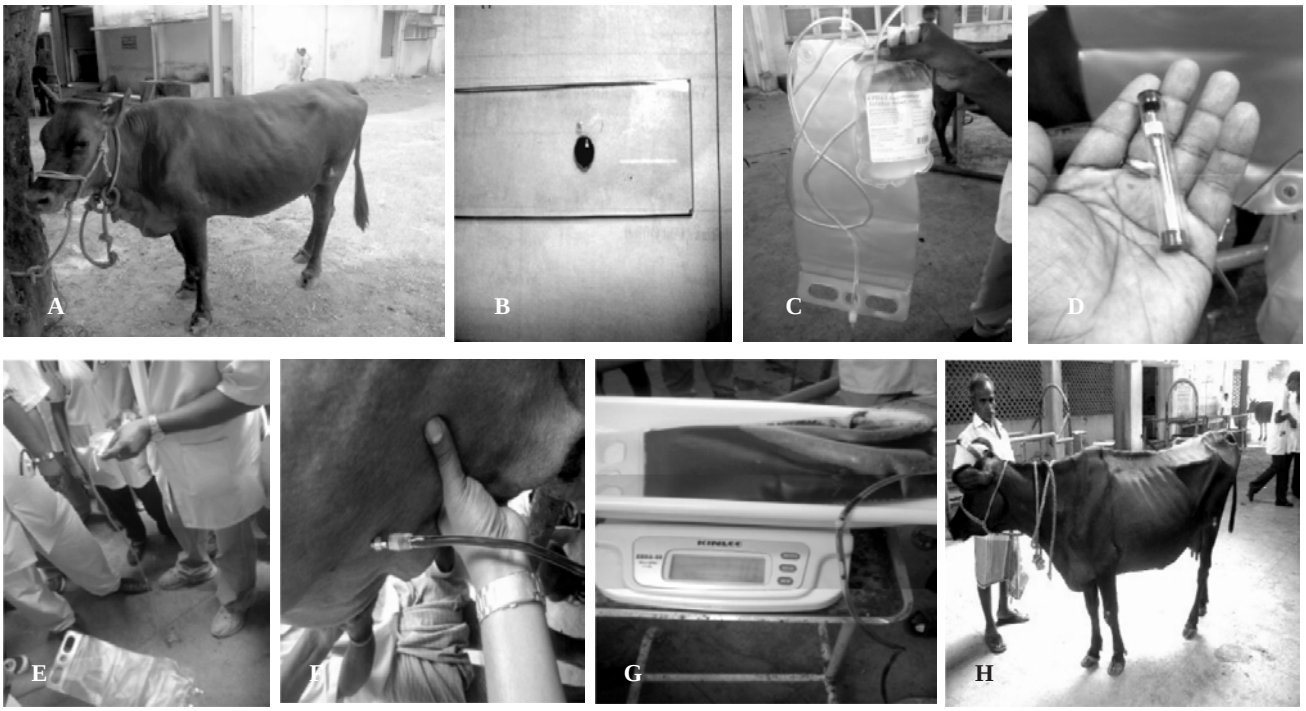

Fig 2. A-Donor cow, B- Blood cross match , C- Five liter blood bag ,CPDA ,D- Sterile 16 G needle, E –Mixing the CPDA, F-Jugular collection, G-Weighing the blood, H – Recipient post transfusion

*JORVET (J 520) 5 litre Blood bags available with separate CPDA 500 ml at Jorgensen Labs 1450 Van Buren Ave, Loveland, Colorado 80538, +1-Local: 970.669.2500,Toll Free: 1.800.525.5614,Fax: 970.663.5042

Results and discussion

Anaplasmosis, is an infectious parasitic disease of cattle caused by the microorganism *Anaplasma marginale*. This parasite infects the red blood cells and causes severe anemia. Adult cattle are more susceptible to infection than calves. (Richey E.J., 1992)

Lallemand, 2006 transfused blood to a heifer with eleven liters of blood, collected into 2 sterile plastic bottles from 2 blood donor cows. Sterile water (400 mL) with dextrose 50% (100 mL) and sodium citrate (16 g) as an anticoagulant was prepared and rate of perfusion was 1 mL/kg bodyweight (BW)/h during the first 15 min, while the heifer was closely observed to

detect any clinical sign of anaphylactic reaction.

Soldan, 1999 reported that the timely blood transfusion in cases of life-threatening anemia in cattle is relatively simple, less expensive to the farmer and clinically very rewarding. Braun, 1991 described blood transfusion in 35 cows with anaemia due to abomasal ulcers where in two animals died in spite of the treatment and three had to be slaughtered because of the deterioration in their condition. Bell, 2006 noted that some factors to be considered when selecting a blood donor viz., General health, Pregnancy, Conformation/ condition score, and Body weight. Blood volume is related to body weight (7-8%), hence animals weighing more than 375kgs can supply sufficient blood for most indications.

Adverse reactions are most commonly seen in very young animals or pregnant cattle. Signs of blood or plasma transfusion reaction include hiccoughing, tachycardia, tachypnea, sweating, muscle tremors, pruritus, salivation, cough, dyspnea, fever, lacrimation, hematuria, hemoglobinuria, collapse, apnea, and opisthotonos. Intravenous epinephrine HCl 1:1000 can be administered (0.2 to 0.5 mL) intravenously or (4 to 5 mL) intramuscularly (preferable) if clinical signs are severe (Hunt, 1999).

The total blood volume estimated in cattle is 80 mL/kg. From the donor animal up to 20-25% of total blood volume can be removed. Blood can be collected into bottles or bags using citrate anticoagulant (e.g CPDA-1) in equine transfusions. Further he quoted that in order to monitor transfusion reactions blood should first be transfused slowly an ruminant blood type discordance result in primarily complement-mediated hemolysis which encountered more often in patients with reduced vascular nitric oxide levels because of endothelial dysfunction.

This downer cow which showed signs of severe anemic hypoxia, recumbency responded very slowly to the transfused whole blood but progressively improved in 4 weeks of time concluding the importance of whole blood transfusion for haemoprotozoan diseases in cattle.

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Kennel cough in a Labrador Retreiver

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Abstract

A three year old male, Labrador dog was presented with the history of inappetance, retching, cough for the past one week and also showed reduced activity levels. The animal had paroxysmal coughing with intermittent sputum. Clinically the case was diagnosed as Kennel Cough. Thoracic Radiography, ECG and bronchoscopic assessment was performed and the case confirmed as Infectious Canine Trachea- Bronchitis (kennel cough) . The animal showed an uneventful recovery after 10 day treatment.

Keywords*:* Cough, dog, infectious tracheobronchitis,kennels.

Infectious tracheobronchitis (kennel cough) is a clinical syndrome of dogs characterized by acute onset of a dry, hacking cough (often followed by retching or gagging on mucous secretions) (Azetaka and Konishi, 1988). Kennel cough can be due to a single pathogen or to a complex of bacteria and/or viruses (Appel, 1981). The primary bacterium associated with kennel cough is *Bordetella bronchiseptica*. Signs of infection due *B. bronchiseptica* typically persist for 1–2 weeks, but organisms can be shed for 2–3 months following clinical recovery. Dogs can be resistant to re-infection for approximately 12 months following recovery (Bemis *et al.*, 1977). *Mycoplasma* spp., *Pastuerella multocida*, *Pseudomonas aeruginosa*, and *Escherichia coli* have been isolated from the respiratory tract of dogs and also might cause signs of kennel cough (Wagener *et al.*, 1984). Primary viral pathogens implicated in kennel cough include canine-adenovirus type 2 and canine-parainfluenza virus (Appel and Percy, 1970; Azetaka and Konishi, 1988). Other viruses suspected of causing kennel cough include canine-adenovirus type 1, canine-herpesvirus, canine-distemper virus, and canine-reovirus types 1–3 (Dhein and Gorham, 1986; Appel and Binn, 1987). Transmission of pathogens associated with kennel cough is both by direct contact and indirectly by aerosolized droplets from coughing or sneezing dogs and by fomites (such as dishes, and the clothing and hands of dog handlers) (Ford and Vaden, 1990). Treatment of ITB is symptomatic; however, due to the common occurrence of secondary infections with a broad spectrum of bacteria, antibiotic treatment is the first therapeu-tic approach. Antibiotics should be selected based on culture and sensitivity tests of airway specimens collected by tran-stracheal aspiration or bronchoscopy (Sumner *et al.*, 2011).

A three year old intact Labrador male dog was presented to the Small Animal Medical Clinic of Madras Veterinary College Teaching Hospital with the history of inappetance, retching and cough for the past one week. The working dog also showed reduced activity. Animal was alert and active. Physical examination revealed congested mucous membrane, temperature measuring around 39.6^0C, elevated pulse and heart rate (120 bpm), respiration rate dyspneic and lymph nodes were palpable and no other physical abnormalities were observed on palpation of abdomen. Paroxysmal coughing with intermittent sputum and cough test was positive. On thoracic auscultation, exaggerated breath sounds and wheezes were heard on both sides. Haematological parameters were within the normal range except for mild neutrophilia. Serum biochemistry was found to be within normal range. The animal was subjected to electrocardiography and echocardiography to rule out if there is any cardiac involvement. No abnormalities were observed in the above assessment. Therefore the cough was not due to any cardiac abnormalities. Thoracic radiography revealed pulmonary infiltration. On interpretation of the above physical and clinical examination, the case was tentatively diagnosed as canine infectious tracheobronchitis. Tracheo-bronchoscopic investigations revealed a severe tracheo-bronchitis, with hemorrhagic streaks and purulent secretions in the airway (Fig.1 & 2). Broncho-alveolar lavage was collected and subjected to culture and cytology. Microbiological examination confirmed the presence of *Bordetella bronchiseptica* organisms and they were found to be highly sensitive for Amoxicillin and Enrofloxacin, intermediately sensitive to azithromycin and resistant to gentamicin, cephalexin, cefotaxime and tetracycline. Clinical Management, the

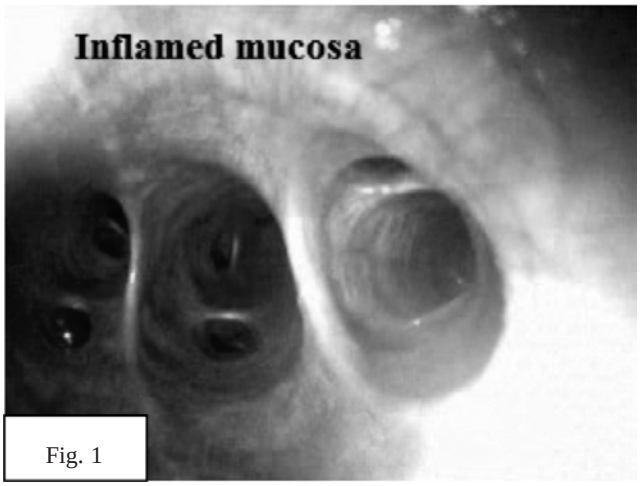

Fig. 1

Fig. 1

dog was given Tab. Bayrocin (Enrofloxacin) 150 mg orally s.i.d for first five days, Tab. Prednisolone 10 mg, s.i.d for a week. Animal showed marked improvement. Oral Enrofloxacin was continued for next five days. Animal showed clinical improvement by 10 days. After oral administration for 4 weeks with Tab. Enrofloxacin @ 5mg /kg b wt o d), the dog showed an uneventful clinical improvement. As the dog was housed in a kennel, the following advice was given to the caretaker to prevent the spread of the disease. Isolation of animals, until all clinical signs resolve, thorough cleaning and disinfection of kennel facilities with dilute (1:32) bleach solution and to report immediately if any other dogs in kennel show similar clinical signs.

Acknowledgement

The authors are thankful to Dr.S.R.Srinivasan, Director of Clinics, TANUVAS and the Dean, Madras Veterinary College for providing the necessary facilities.

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Osteodystrophia fibrosa in a non-descript buck-A case report

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Abstract

One year old buck was presented with the history of visible bilateral symmetrical swelling of the face. Inspection revealed abnormal gait and posture along with difficulty in eating and drinking. The buck had salivation and protruded tongue with inability to open mouth widely. Palpation of the head specifically mandible revealed soft rubbery consistency. The serum chemistry revealed elevated levels of alkaline phosphatase (431.9 IU/L) and Intact Parathyroid Hormone –PTH (434.60 pg/ml). The radiographic examination revealed enlargement and radiolucency of mandible along with displacement of teeth. ^{99m}Tc-MDP scintigraphy revealed higher radiopharmaceutical uptake at site of mandible and constitutes a valuable, non-invasive method for diagnosis of Osteodystrophia fibrosa in small ruminants.

Keywords*:* Buck, hyperparathyroidism, and ^{99m}Tc-MDP, Osteodystrophia fibrosa.

Osteodystrophia fibrosa (fibrous osteodystrophy) is a generalized bone disease caused by prolonged and excessive secretion of parathyroid hormone (PTH) (hyperparathyroidism). Primary hyperparathyroidism is a rare condition resulting from parathyroid adenoma. Secondary hyperparathyroidism, due to nutritional deficiencies or imbalances which result in lower serum-ionized calcium and increased synthesis and secretion of PTH, is more common in animals (Palmer, 1993; Woodard, 1997). Osteodystrophia fibrosa is characterized by marked bone resorption and fibrous tissue deposition. The affected bones are mainly the mandible and maxilla (Woodard, 1997). The occurrence of the disease and its etiopathogenesis in horses (Clark *et al.*, 1996) and goats is well described (Saha and Deb, 1973; Andrews *et al.*, 1983). Gamma scintigraphy is commonly used as an aid in the diagnosis of skeletal disorders in human and veterinary clinical practice. ^{99m}Technetium methylene diphosphonate (^{99m}Tc-MDP) bone scintigraphy is regarded as a highly sensitive and noninvasive method used for demonstration of functional changes on the bone before any anatomic pathology is apparent, and is used to investigate bone disease associated with renal failure resulting in fibrous osteodystrophy in human medicine (Karsenty et al 1986 and Nishida et al 2005). Present clinical case describes the imaging diagnosis of osteodystrophia fibrosa in a non-descript buck.

Case history and Clinical examination

One year old nondescript buck was presented to Department of Veterinary Nuclear Medicine, Bombay Veterinary College, Parel-Mumbai-12 with history of gradual enlargement of head especially at lower jaw. The above changes were noticed by the owner about one month before. Clinical examination of the goat showed poor body condition. The rectal temperature was normal (100.4^{0}F) and heart (84 beats/min) and respiratory rates (19 respirations/minute) were marginally altered. Buck showed symmetrical enlargement of the face and jaws and protrusion of tongue which prevent closing of mouth (Figure-1). The palpation of the mandible revealed pain and rubbery consistency.

Laboratory Examination

Serum calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) were estimated on semi biochemical auto analyzer (Auto-analyzer model: Erba-chem -7, India). PTH was measured by using the fully automated chemi luminescent immuno assay (CLIA).

Radiology and Bone Scintigraphy

The ventro-dorsol and lateral radiographs of skull were performed. ^{99m}Tc-MDP bone scintigraphy was carried out by using Millennium MPS gamma camera (GE, USA) attached with low energy general purpose (LEGP) collimator. 20 mCi of ^{99m}Tc-MDP was injected intravenously (by cephalous vein), while whole body images were recorded three hours post injection. The total injected dose of radiopharmaceutical was calculated by deducting the post-syringe activity from pre-syringe activity with decay correction. The right lateral images were selected for quantative radiotracer uptake at

Table 1: Biochemical finding in affected buck

Parameter	Observed values	Normal Range[a]
Ca (mg/dl)	7.31	8.90 to 11.70
P (mg/dl)	5.04	4.20 to 9.10
ALP (IU/L)	431.90	93.00 to 387.00
Intact PTH (pg/ml)	434.60	20.00

[a]From Kaneko (1994)

mandible. The manual region of interest (ROI) was drawn on mandible and percent radiotracer uptake was calculated in relation with injected dose.

Results and Discussion

In the present clinical case record, analogy of history and clinical examination showed gradual enlargement of head especially at lower jaw over a time period of about one month. These clinical findings were typical of Osteodystrophia fibrosa, as reported earlier in goats (Saha and Deb, 1973; Andrews *et al.*, 1983). The data of biochemical parameters is depicted in table -1. The concentrations of Calcium, phosphorus, Alkaline Phosphatase and PTH were 7.31mg/dl, 5.04 mg/dl, 431.9 IU/L and 434.60 pg/ml respectively. The recorded data suggests that the concentrations of Ca and P were normal, whereas the concentrations of ALP and PTH were elevated considerably when compared to normal reference range (Kenako, 1994). Smith and Sherman (1994) described that in clinical cases of Osteodystrophia fibrosa in goats, the serum chemistry

Fig.1: Front view of goat showing symmetrical enlargement of the face and jaws and protrusion of tongue.

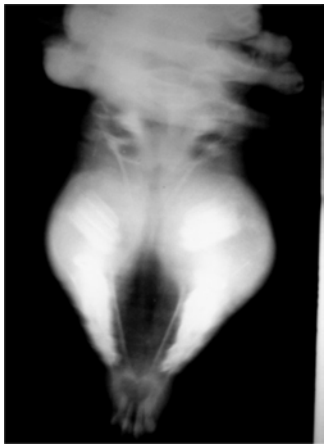

Fig.2: Radiograph showing enlargement and radiolucency of mandible bone.

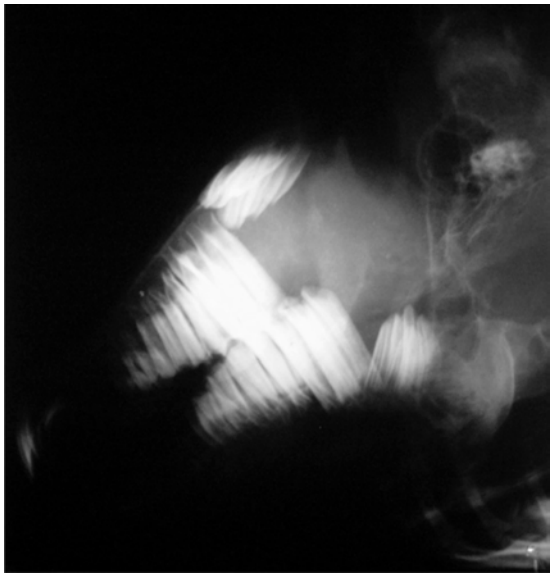

Fig.3: Radiograph showing displacement of teeth.

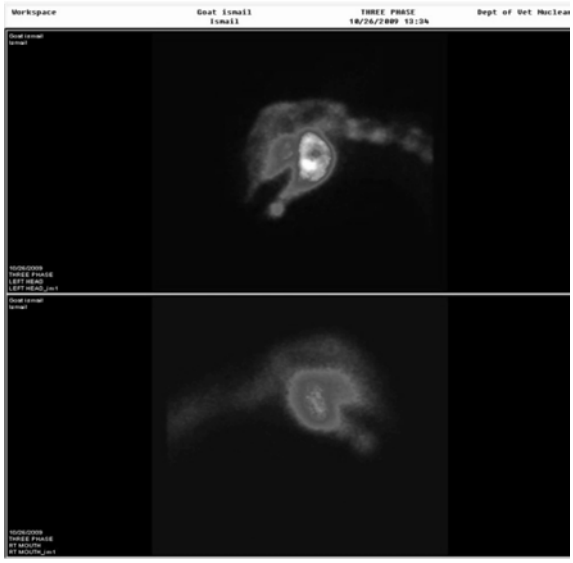

Fig.4: Bone scan showing radiopharmaceutical uptake at mandible

results are variable and elevation of serum alkaline phosphatase is the most consistent abnormality. They also described that hyperphophetemia and hypocalcaemia may also be noted but their absence does not rule out fibrous osteodystrophy. The radiographic examination revealed enlargement and radiolucency of mandible along with displacement of teeth (Figure-2 and 3). These findings are in agreement with findings of Aslani et al (2001). The delayed image quantative scintigraphic assessment of the bone scan showed 9.74 % (Figure -4) radiopharmaceutical uptake at mandible, which was appreciably high when compared with bone scan of healthy goat i.e. 1.75 %. This finding is in agreement with findings of Karsenty (1986), who recorded increased bone radiotracer uptake was in human patients suffering with osteodystrophy.

Gamma Scintigraphy, an uncommon technique in veterinary clinical practice, is an *in vivo* imaging tool with many disease diagnosis applications in companion animal practice. Present clinical case record is the first report of its own kind regarding application of gamma scintigraphy in diagnosis of Osteodystrophia fibrosa in small ruminants. The scintigraphy data generated in this clinical case record are accurate, detailed, comprehensive and clinically relevant resulting in greater confidence of clinician / physician in early diagnosis and deciding prognosis concern with of Osteodystrophia fibrosa in small ruminants. Thus, present clinical case concluded that the Gamma Scintigraphy as a valuable, non-invasive method to diagnose Osteodystrophia fibrosa in small ruminant patients.

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Tetanus in a crossbred cow : A case report

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Abstract

A case of tetanus with typical clinical signs in a crossbred cow following parturition is described in the present communication. History revealed postpartum complication handled under unhygienic conditions and clinical signs showed stiffness of all limbs, hyperaesthesia, erect ears, prolapsed third eye lid, lock jaw and severe bloat. The animal was treated with procaine penicillin G, diazepam and supportive therapy with due care and management but in spite of all efforts the cow succumbed next day.

Keywords: Cow, Management, Teatnus

Introduction

Tetanus is a neuro-muscular disease caused by an exotoxin (tetanospasmin) produced by *Clostridium tetani* growing under anaerobic conditions. Almost all mammals are susceptible to tetanus. But, there is considerable variation in susceptibility between the animal species, the horses being the most susceptible and cattle the least. Cows can get tetanus due to unhygienic practice following parturition due to contamination of genital tract by soil or faeces. In India, neonatal tetanus is well documented by many authors (Bhikane *et al.* 2005; Singh *et al.*, 2009; Das *et al.* 2011 and Upadhyay *et al.* 2013), but there is paucity of literatures on tetanus in adult cattle . Therefore, the present case report describes a clinical case of tetanus in a crossbred cow due to post-partum complications.

Case description

A three year old crossbred cow was presented at Large Animal Clinic of the University with complaints of anorexia, ruminal bloat and recumbency for past two days. History revealed recent parturition with retention of placenta, metritis, intrauterine manipulation and medication by a quack. The cow was not vaccinated against the tetanus. Clinical examination showed pyrexia (104^{o}F), normal heart rate (62 bpm), respiration rate (19/minute), lateral recumbency and generalised stiffness, opisthotonus and hyperaesthesia. There was retraction of the eye and prolapse of the third eyelid. Careful external examination of whole body did not reveal any wound. Haematology revealed neutrophilic leucocytosis with left shift (Hb-10.5gm%, TLC-21300/μL, DLC:-neutrophils-84%, lymphocytes-16%). Blood smear examination for haemoprotozoa was negative. The survey radiograph of reticular area revealed clear diaphragmatic line, no potential foreign body and multiple gas pockets in the reticular region.

Diagnosis

Recent history of retention of placenta and clinical signs were the primary means of making tentative diagnosis of tetanus in present case as the site of infection was unreachable for isolation of *Clostridium tetani* and many times unrewarding.

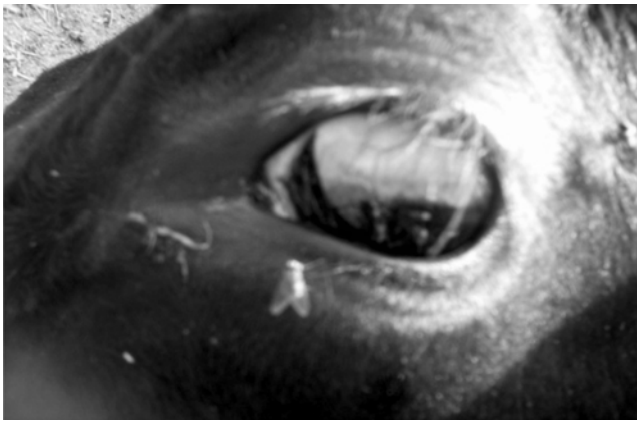
Treatment

The animal was treated with fluid (Normal Saline 10 litres I/V), Procaine Penicillin G (22000 IU/kg b.wt. I/M BID), Metronidazole (10 mg/kg b.wt. I/V BID), Diazepam (0.4 mg/kg b.wt. I/M BID) and multivitamin (10 ml I/M SID). Owner was advised to keep the animal in quiet and dark place with good nursing. But in spite of all efforts the cow succumbed next day.

Discussion

Tetanus has a worldwide distribution occurring in all types of livestock, but it is relatively rare in cattle. In present case, *C. tetani* might have been introduced via contaminated wound in reproductive tract due to postpartum complication. Introduction of infection to the genital tract at the time of parturition due to unsanitary practices is the usual portal of entry in cattle (Radostits *et al.* 2007). Clinical signs in the present case were observed 15 days after handling of post partum complications. Similarly, Smith (2009) also reported that incubation period of tetanus varies from two weeks to one month after bacterial inoculation. Clinical signs i.e. stiffness of all limbs, hyperaesthesia, erected ears, prolapsed third eye lid, lock jaw and severe bloat were

Fig. 1. Crossbred cow with opisthotonus and extended neck



suggestive of tetanus as described by other authors (Radostits *et al.* 2007, Smith 2009, Singh *et al.*, 2009; Das *et al.* 2011 and Upadhyay *et al.* 2013). Increased tonic muscular activity leads to pyrexia. Leukocytosis (21,300/μL) with neutrophilia (74%) and shift to left appeared to be due to genitalia trauma and infection.

Treatment was given as per the principles given by Radostits *et al.* (2007) i.e. elimination of causative bacteria, neutralization of residual toxin, control of muscle spasm and maintenance of hydration and nutrition. Broad spectrum antimicrobial therapy consisting of Penicillin for *C. tany* and Metronidazole for anaerobes were administered to control neutrophilic leucocytosis. Penicillin being drug of choice helps in the elimination of causative bacteria. Although parenteral and local administration of antitoxin is advocated to neutralize the residual effect of toxin but its effect after appearance of clinical signs is questionable (Upadhyay *et al* 2013). Diazepam was given in order to relieve tetany and spasm of muscles. Intravenous fluids with vitamins were the part of nutritional support particularly in cases where the animal is unable to eat and drink due to lock jaw. Despite of all these efforts, the death in this case might have been due to respiratory arrest/failure following spasm of the muscles of respiration.

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Therapeutic management of Transmissible Veneral Tumor in canine

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Abstract

Two labrador retriver (male and female) and a pomeranian (male) dogs were presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar with complaint of bleeding from genital organs. Detail clinical examination revealed serosanguineous exudate and haemorrhagic cauliflower shaped friable mass on external genitalia. Samples were taken from genitalia for exfoliative cytology using cotton swab and whole blood for complete blood cell count. Based on clinical and laboratory findings the cases were diagnosed as canine transmissible veneral tumor. Chemotherapy with vincristine sulphate at the dose rate of 0.025mg/kg intravenously once weekly for four weeks and supportive therapy with haemostats and multivitamines resulted in complete remission of the tumor mass in all three dogs.

Keywords*:* Sticker tumor,Transmissible Veneral Tumor, Vincristine sulphate

Introduction

Canine transmissible veneral tumor (TVT) is a benign reticuloendothelial neoplasm that primarily affects genitalia of sexually intact members of canine family. In India, TVT is known to be the most frequently reported neoplasm in dogs with an incidence rate of 23-43 % of the total number of tumors in canine population (Gandotra *et al.,* 1993). It affects both male and female population at same frequency. The disease is horizontally transmissible from male to female and vice versa during breeding among affected animals. The tumor is more common in sexually mature dogs of age between 2 to 5 years. The tumor mass may have a cauliflower like shape, and it can also be pedunculated, nodular or papillary. In males, lesions usually localize cranially on preputial mucosa, on the glans penis or on the bulbus glandis. Tumor masses often protrude from the prepuce (Higgins, 1966). In bitches the tumors are of similar gross appearance as in male dogs and can be localized in the vestibule and/or caudal vagina, protruding from the vulva and frequently causing deformation of the perineal region.

Differential diagnoses include other round-cell tumors like carcinomas and amelanotic melanomas, lymphoma, poorly differentiated mast cell tumors and histiocytomas (Ferreria *et al.,* 2000). Confirmatory diagnosis can be reached by histological and cytological findings. In contrast to the normal chromosome number of 78 of the canine, TVT cells contain an abnormal number of chromosomes ranging from 57 to 64 and averaging 59 and it is considered as an allograft (Theilen and Madewell, 1987).

Earlier surgical removal of tumor mass was adopted as treatment, but recurrence of the condition was a problem . In some cases the condition showed metastasis. Chemotherapy of TVT has been a suitable option in management of this condition in canine. However, untoward side effects of chemotherapy has been reported in some cases (Das and Das, 2000). In this clinical article we report sussessful management of transmissible veneral tumor in canine.

Case history and clinical examination

Three dogs (2½ year old female labrador retriever with 26kg body weight, 4 years male labrador retriever of 29 kg body weight and 2 year old male pomeranian of 10 kg bodyweight) were brought to Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar with the complaint of bleeding and nodular lesion on external genitalia. On anamnesis it was found that the bitch had a mating history with stray dog and other two dogs had no mating history and owner brought the dogs from another kennel.

Detailed physical examination findings are presented in Table 1. All three animals were appeared to be alert and active with reduced appetite. On clinical examination of the external genitalia of male dogs it was observed that there was presence of large hyperemic cauliflower shaped nodular masses at the caudal aspect of penis (Fig: 1). Vulvo-vaginal examination of the bitch revealed, accumulation of serosanguineous, foul smelling discharge at the ventral commissure of vulval

lips and a large haemorrhagic cauliflower shaped friable mass of 4-6cm diameter formed by the fusion of multiple nodules between the floor of the vestibule and caudal vagina (Fig: 2).

Whole blood was collected in EDTA for complete blood cell count. Samples were obtained from genitalia for exfoliative cytology using cotton swab and smear was stained using Giemsa stain. The haematological findings are presented in Table 2. The exfoliative cytology (Fig: 3) revealed presence of cells having round to oval shape with mild anisokaryosis and anisocytosis. Prominent mitotic figures *viz.* increased nuclear cytoplasmic ratio, round or oval nuclei with prominent nucleoli, scant cytoplasm and multiple clear cytoplasmic vacuoles could be found suggesting the diagnoses as canine transmissible veneral tumor.

Treatment

The animals were given chemotherapy using Vinca alkaloid *i.e.*, Vincristine sulphate intravenously slowly at the dose rate of 0.025mg/kg once weekly for four weeks diluted in distilled water. Due care was taken to prevent infiltration into perivascular space. In order to control bleeding, Botropase was given at the dose rate of 1CU (total dose) twice daily intramuscularly on the first day of presentation. Vitamin B complex supplementation was given intramuscularly in the form of Inj. Neurobion forte a total dose of 2ml once daily for 3days. The owner was advised to observe the animal for any untoward side effects. The animals were monitored daily for 3 days, thereafter weekly for four weeks. Per-genital examination on second visit revealed reduction in size of the masses of all three dogs and treatment with Vincristine sulphate was continued at one week interval for next three weeks.

On the forth visit, owner reported normal appetite and thirst for the animal. On clinical examination animal appeared to be apparently healthy with all vital signs in normal range (Table 1). On per genital examination with gloved hand, the tumor masses were found to be completely regressed (Fig: 4 and 5) and also no tumor cells could be detected in the EVC and CBC was found to be within the normal range (Table 2). The owners were advised to avoid dogs' misalliance with stray dogs.

Discussion

The ultimate goal of the treatment of transmissible venereal tumor (TVT) is complete cure, which may be achieved by surgical excision, radiotherapy, immunotherapy and/or chemotherapy. Surgical excision can be attempted if tumor is single and easily accessible. Radiation therapy can also be considered as an alternate method for treatment but have lot of side effects.

Antimitotic agents, such as vincristine sulphate is the preferred chemotherapeutic agents for treating this tumour (Boscos, 1988). Singh *et al.*, (1996) observed almost complete recovery using vinblastine intravenously at the rate of 0.1 mg/kg body weight on 4 to 6 occasions at weekly intervals. Transient side-effects, such as anorexia, vomiting or diarrhoea, were found after the start of the treatment. Das *et al.*, (1991), Maiti *et al.*, (1995) and Singh *et al.*, (1997) found that vincristine sulphate at the rate of 0.025 mg/kg body

Table 2: Complete blood count before and after chemotherapy

Blood	Before Treatment (Mean± SD)	After treatment (Mean± SD)
TLC (10^3/µL)	16.63 ± 1.02	16.5 ± 3.92
TEC (10^6/µL)	7.00 ± 1.64	6.90 ± 1.47
Haemoglobin (g/dL)	13.9 ± 4.55	14.7 ± 2.78
HCT (%)	43.8 ± 11.59	44.8 ± 7.93
MCV (fL)	62.23 ± 2.99	65.23± 2.42
MCH (pg)	19.5 ± 2.34	21.36 ± 0.59
MCHC (g/dL)	31.3 ± 2.43	32.76 ± 0.61
Platelet (10^3/µL)	314.67 ± 95.55	314.33 ± 45.01
Lymphocyte (10^3/µL)	3.33 ± 0.88	2.88 ± 0.45
Monocyte(10^3/µL)	0.73 ± 0.32	0.70 ± 0.10
Eosinophil(10^3/µL)	0.69 ±0.35	0.57 ± 0.38
Neutrophil (10^3/µL)	11.87 ±0.83	12.35 ± 3.03
PDW	17.13 ±1.27	15.77 ± 0.76
MPV	5.67 ±1.07	5.7 ± 0.1
RDW	14.77 ±1.27	14.77 ± 0.31

Table 1: Vital signs on first day of presentation

Clinical parameters	Labrador retriever female	Labrador retriever male	Pomeranian male
Rectal Temperature (ÚF)	101.6	102	100.8
Respiration rate (per min)	22	30	26
Heart rate (per min)	68	74	76
Mucous membrane	Slightly pale	Pink roseate	Pink roseate

weight intravenously at weekly intervals on 3 to 4 occasions is the most effective, safe and convenient chemotherapeutic agent, giving a better survival time even in TVT patients with extra-genital metastasis. Thus, it would appear that TVT can be considered as a neoplasm amenable to chemotherapy with vincristine sulphate alone. The better response reported for the single drug vincristine or vinblastine than with combined chemotherapy, may be due to there being less myelosuppression (Theilen and Madewell, 1987), no opposite synergy (Bender and Hamel, 1991) and non-development of resistance to the antineoplastic drug (Kanem and Winick, 1988).

CTVT is the most prevalent neoplasia of the external genitalia of the dog in tropical and sub-tropical areas. Diagnosis can be reached by typical physical and cytological findings. The tumor is primarily a sexually transmitted disease but it can be also spread by contact and licking in canine (Nelson and Kennedy, 1990; Kabuusu *et al.*, 2010). It is assumed that transmission is the result of exfoliated cells from the donor being "seeded" into the damaged genital mucosa of the recipient. Vincristine sulphate, as a single agent (IV once weekly) for four weeks is the most effective and common treatment protocol for TVT (Singh *et al.*, 1997).

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Fig 1: Cauliflower like TVT lesion (Male) before treatment

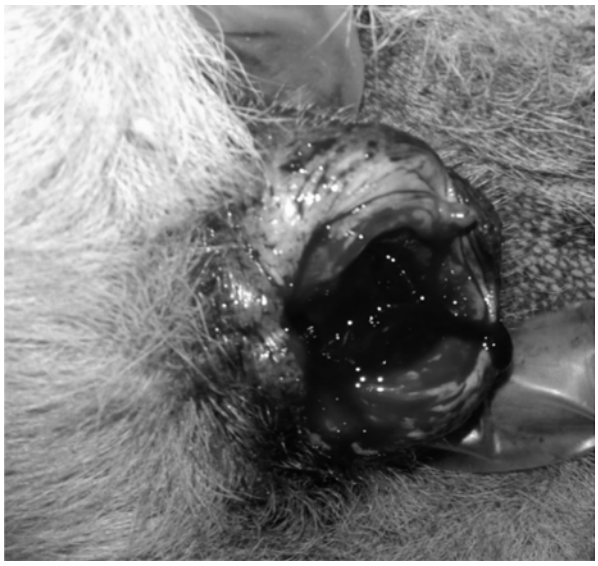

Fig 2: Cauliflower like TVT lesion (Female) before treatment

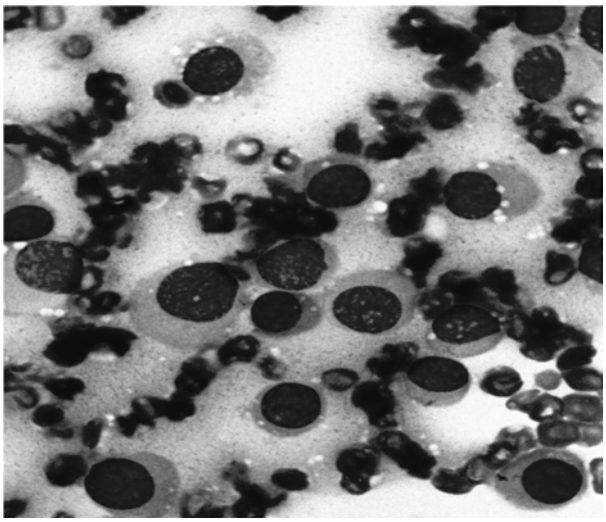

Fig 3: Exfoliative vaginal cytology (1000X) revealing TVT cells

Fig 4: Remissions of the lesions 4 weeks after treatment

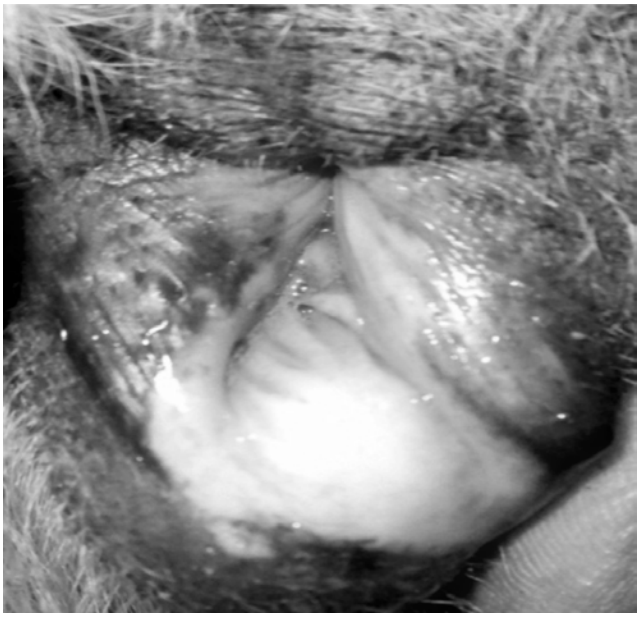

Fig 5: Complete remission of lesion and normal Vestibular floor after treatment

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ISVM Awards and Rules

The members of the ISVM are requested to send their applications in quadruplicate (4 copies) for the below mentioned awards with all relevant supportive documents including photocopy for proof of age, date of enrollment as life members etc., as required for specific award, through their controlling officer/Head of the department verifying their credentials/contributions.

General rules applicable to all the awards:-

1. Only members of ISVM are eligible to apply for any of the awards
2. Incumbent executive committee members of ISVM are not eligible for consideration of any of these awards, exception being FISVM.
3. Recipient of any ISVM award during last two years is not eligible to apply or to be considered for any of ISVM award
4. Persons applying for more than one award should indicate the preference order.
5. The minimum quorum for all the awards except FISVM is two.
6. The General Secretary, in consultation with the President, may reject application for any award for want of required quorum or incomplete application.
7. The General Secretary and President reserve all rights to accept/reject any application without assigning any reason for the same.
8. All the applicants will be required to submit half page write-up for the citation along with their original applications.
9. A sum of Rs. 250/- will be payable by the applicants as demand draft in favour of Indian Society for Veterinary Medicine, for each award applied as the processing fee to the ISVM.
10. The award may not be given for a year if an applicant does not get the qualifying minimum score.

1. SHRI RAM LAL AGRAWAL GOLD MEDAL

The Gold Medal has been instituted by M/s Indian Herbs Research and Supply Co. Saharanpur (Uttar Pradesh) in the memory of its Founder, Late Shri Ram Lal Agrawal. The award shall be conferred annually upon a scientist who is a life member of the Indian Society for Veterinary Medicine in recognition of his/her distinguished work/ contribution in the field of Veterinary Medicine. In every third year, the award shall be given to a scientist who has contributed significantly in the field of Indigenous Veterinary Medicine. The individual competing for this Gold Medal, should be of minimum 45 years of age as on 1st January of the year of evaluation.

2. INTAS YOUNG SCIENTIST AWARD

The ISVM award silver trophy to a young scientist (life member of ISVM) below 32 years of age on 1st of January of the year of presenting his/her own research findings at the time of convention of ISVM. Besides the award, citation will also be given. Entries in duplicate of full length research paper typed in double space on bond paper with zerox copy of the proof of age should be sent on or before the last date prescribed to the General Secretary, ISVM along with the certificate from the Head of the Department, verifying the place of research work and year during which work was done. The paper to be presented for this award shall not find a place as Abstract in the proceedings of the convention. Only the author's name will be given who will present the paper himself/ herself. The award in the form of trophy and a certificate to the selected young scientist shall be given at the Annual Convention in the same year. If the research work presented by the young scientist is the part of his/her post-graduate thesis, the same has to be mentioned clearly along with a certificate from the Advisor (Guide). The name of the guide will also figure in the award certificate. The executive committee may reject incomplete application/award for want of required quorum (minimum of 2 candidates). They also reserve all rights to accept/ reject the application without assigning any reasons for the same.

3. DR. D.C. BLOOD GOLD MEDAL

Dr. D.C. Blood Gold Medal will be awarded every year from the interest accruing on the corpus fund of Rs.

10,000/- deposited for the said purpose out of the savings of ISVM convention held at A.P.A.U., Hyderabad, 1990. The award will comprise a gold plated medal and a citation. The award is open for the life members of ISVM of the age above 32 and below 45 years as on 1st January of the year of evaluation.

4. SMT. P.Z. SHARMA GOLD MEDAL FOR CANINE MEDICINE

The Gold Medal will be awarded from the interest accrued on the corpus funds of Rs. 10,000/- deposited for the said purpose by Dr. S.Z. Sharma, Veterinary Clinic Sukhmani, TVPD Scheme, 10th Road, Mumbai-49 (Maharashtra).The medal will be awarded every alternate year from 1994 onwards (the date of commencement of award) to a life member of the ISVM in recognition to his/her outstanding contributions in the field of Canine Medicine during the last 5 years in the form of quality research and clinical publications, generating innovative ideas and development of newer techniques.

5. S.K. MYLSAMY GOUNDER GOLD MEDAL FOR POULTRY MEDICINE

The award is open for life members of ISVM. The applications indicating teaching, research and extension and other activities in the field of Poultry Medicine will be invited by the General Secretary every year and must be submitted by aspirants through respective Heads of Department/Office, verifying their credentials/contributions on or before the prescribed date.

6. DR. G.N. DUTTA MEMORIAL AWARD

This award will be conferred to an applicant who has completed 5 years as life member of ISVM and is of minimum 45 years of age as on 1st January of corresponding year. The award will be given in recognition of his/her meritorious research contributions during the last 10 years to the Veterinary Medicine especially in area of management of infectious diseases in animals.

7. P. K. DAS GOLD MEDAL

This award will be conferred to life member of ISVM in recognition of his/her outstanding contributions in the field of Clinical Toxicology during the last 5 years in the form of quality research and clinical publications, generating innovative ideas and development of newer techniques.

8. AWARD OF FELLOWSHIP OF ISVM (FISVM)

The life members of ISVM for the past 10 years are only eligible for the award of FISVM. ISVM Fellowship is awarded only to the members having master's degree in Veterinary Medicine/Preventive Medicine/Clinical Medicine and their designation should not be lesser than the rank of Professor. The fellowship is awarded to a person of high professional standing, who has rendered commendable service for the cause of Veterinary Medicine. The application for FISVM is required to be submitted along with comprehensive information about academic and professional achievements, distinguished published work/contributions in the field of Veterinary Medicine. A demand draft of Rs. 2000/- in favour of "Indian Society for Veterinary Medicine" must be enclosed with the application as the required fee for FISVM. The eligible ISVM members are requested to submit their application with detailed information under the following subheads: Academic and research achievements. Total particulars of employment; National and ISVM awards received;Total number of research and clinical publications (not popular articles) in peer reviewed Indian and Foreign Journals (enclose the list); List of published reviews in Journals and international proceedings/books; Books/Monographs published; Number of research and clinical publications relevant for specific award (enclose list) ;Contributions to the advancement of Veterinary Medicine; List of papers presented in international conferences/symposium held abroad and any other scientific achievements not covered in above cited information.

9. FIELD VETERINARIAN AWARD

i) The Field Veterinarian Award shall be given to a field veterinarian who is a life member of ISVM.

ii) The award shall be given in the form a plaque and certificate at ISVM convention based on the oral presentation by the applicant in National Symposium.

iii) The presentation shall be judged by a committee of three members nominated by the executive committee for

this purpose.

10. ISVM MERIT AWARD FOR POST GRADUATE RESEARCH:

There shall be two ISVM Merit Awards annually – one for a student pursuing PhD .degree in the discipline of Veterinary Medicine and one for a student pursuing MVSc. degree in the discipline of Veterinary Medicine (Veterinary Clinical / Preventive Medicine). The value of award for a Ph.D. and M.V.Sc student shall be Rs.5000/-andRs.4000/-respectively to be given once during the degree programme of the student. The applications for the award must reach the office of ISVM by (stipulated date) duly recommended by the guide and forwarded by the Head of the Department concerned.

11. BEST CLINCAL ARTICLE AWARD

It will be awarded to the best clinical article published in the Indian Journal of Veterinary Medicine during the year immediately preceding the year of annual conference of ISVM. The articles will be sent to three Judges by the Editor and based on the score; the best article will be selected and placed before the executive committee members for approval. The award will be in the form of a plaque and certificate to the first author, and certificates to co-authors to be given every year at the ensuing ISVM convention. 4. The criteria for scoring marks shall be as follows:Title 5 marks; Materials and Methods 10 marks; Results and Discussion 10 marks; Contribution to Science 5 marks

12. BEST RESEARCH ARTICLE AWARD

It will be awarded to the best full length research article published in the Indian Journal of Veterinary Medicine during the year immediately proceeding the year of annual conference of ISVM. The research articles will be sent to three Judges by the Editor and based on the score; the best research article will be selected and placed before the executive committee members for approval. The best research article award will be in the form of a plaque and certificate to the first author, and certificates to co-authors to be given every year at the ensuing ISVM convention. The criteria for scoring marks shall be as follows: Title 5 marks; Materials and Methods 10 marks; Results and Discussion 10 marks; Contribution to Science 5 marks

13. ISVM APPRECIATION AWARD

The ISVM Appreciation Award will be given to a life member of the Society. It will carry a citation and plaque of honour. The proposal for the award with justification will be made by the President, General Secretary, Organizing Secretary and Editor/Associate Editor during the executive committee meeting at the time of annual convention on the basis of services rendered by a life member for the betterment of ISVM/IJVM affairs. The executive committee will approve a maximum of 4 persons each year for this award from the names proposed as above.

Award Application procedure

The eligible members are requested to submit application for the awards/fellowship (SerialNo.1 to 10) with detailed information under the following subheads with detail description on specific need of the individual award: Academic and Research Achievements; Total particulars of employment; National / international and ISVM awards received Total number of research and clinical publications(not popular articles); in peer reviewed Indian and Foreign Journals(Enclose the list); List of published reviews in journals and international proceedings/ books; Books/monographs published; Number of research and clinical publications relevant for the specific award(Enclose the list); Contribution to the advancement of Veterinary Medicine; List of papers presented in the international Conference and Symposia

Remark Note:

(i) Members of the incumbent executive committee of ISVM are not eligible for consideration of any of these awards except for Fellow ISVM.

(ii) Recipient of any of the ISVM awards during last 2 years is not eligible to apply/or be considered, for example, any person getting the award in 2005 shall be eligible to apply in 2008 and so on.

(iii) Person applying for more than one award should indicate preference order.

(iv) Person competing for any of these awards cannot become member of any of the Judging Committee

GENERAL GUIDELINES FOR CONTRIBUTORS

The *Indian J. Vet. Med.* is published twice in a year, June and December. It contains review articles(guest), original/applied research articles, clinical observations, preliminary re-ports of scientific studies and short communications on Veterinary Medicine and Animal Health. In addition, the journal also publishes Letters to the Editor, Tips to Vets and other relevant information's. The manuscripts are accepted on the basis of scientific importance and suitability for publication on the understanding that they have not been published, submitted or accepted for publication elsewhere wholly or partly in any language. All authors are jointly and severally responsible to the various authorities for the contents of the articles. The Editorial board shall in any case not be held responsible in any manner whatsoever to the contents of the article and the views and interpretations expressed by the authors in the articles. In case the research work includes experimentation on animals, authors has to submit a certificate that the work carried out is with the approval of the Institutional Ethics Committee or as per the laws in force in the country in which it has been conducted. A certificate to this effect should be signed by corresponding author on behalf of all the authors. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

For an article to be published in IJVM it is mandatory that atleast one of the author should be a life member of the Indian Society for Veterinary Medicine. In case none of the author (s) is permanent member of the Indian Society for Veterinary Medicine he/she may apply for the permanent membership to "The General Secretary, Indian Society for Veterinary Medicine". The official language of journal is English. The articles should be addressed to "The Assistant Editor, Indian Journal of Veterinary Medicine, Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, U.P. (India)" and should be submitted by email only to **ijvmisvm@gmail.com.**

The manuscript should be typewritten in A4 size paper in Times New Roman, font size 12 on one side of the paper with wide margins (1.5 cm all around the page) and double spacing throughout the article except in abstracts, footnotes and references which should be in single spacing. It should be sent in duplicate. Each page of the manuscript should be numbered on the top corner including title page, references, tables, etc. All the pages should contain running title of the paper at the top.

Article once received will be allotted a registration number and will be send to reviews and on acceptance/ rejection will be send back to the corresponding author for modification if any. The author(s) should revise and modified the article in light of the recommendation of the reviewer and the editorial board and should adhere to the format of the journal (follow instruction to authors as given below). The revised article (one hard copy), soft copy in CD or as an attachment to email id ijvmisvm@gmail.com along with processing and publication fee of article should be submit to "The Assistant Editor, Indian Journal of Veterinary Medicine, Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, UP (India).

The modified articles should be submitted to editorial office within 15 days of receipt, failing which the publication of article may be delayed. A demand letter will be sent to the corresponding author for payments of processing and publication fee of the article. Only on receipt of full payments, the article will be taken up for publication and the author will be informed accordingly.

The manuscript should be organized in the following order in general:

Research Article

1. **Title Page:** Should be typed on separate page contain full title of the article, name of the author(s) along with their affiliation, name of the place (Department, College, University etc.) where work was done. Name of the corresponding author, complete postal address including Pin- code along with phone number and the email address at the bottom of the page.
2. **Manuscript:** In general should be arranged as follows: (Contributors should take care that name of the author(s), their affiliation and the institution name should not be included in this section and only be

mentioned in the title page only.)

A. Title: Title of the article should be clear, self descriptive in nature and should not contain abbreviation or symbols.

B. Abstract: Abstract should not exceed 300 words and should outline briefly the purpose of the study, important findings and conclusions. Repetition and generally known information should be avoided.

C. Key words: 4 to 5 Key word (Arrange in alphabetical order).

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G. Discussion: This should focus the interpretation of experimental findings along with reasoning. Do not repeat data presented in the introduction or information given in the result. References in this part should be cited as follows.....as observed by Kumar *et al.* (1984) or in parentheses..... were found (Dwivedi *et al.,* 1983; Singh and Singh, 1984). At last each article should have definite interpretation with research findings.

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***b.* For books:**

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. VIII edn., Iowa State University Press, Iowa, USA, pp. 287--192.

c. For chapter in a book:

Thomas, J.R. and Charles, C.C. 1997. Calcium regulating hormones and diseases of mineral metabolism. In: *Clinical Biochemistry of Domestic Animals. Kaneko*, J.J., Harvey, J.W. and Bruss, M.L. (eds.). V edn., Academic Press, London, pp. 619--702.

d. For thesis:

Singh, S.K. 1998. Studies on clinico-biochemical changes in Downer cow syndrome. M.V.Sc. thesis. Punjab Agricultural University, Ludhiana, India.

e. For proceedings of symposia/conference:

Shah, R.L., Kataria, J.M., Arya, S.C. and Verma, K.C. 1996. Study on inclusion body hepatitis in broiler chicks. *Proc. XX World Poult. Congress* held on Sept. 2-5, 1996, New Delhi, Vol. IV,pp.313--314.

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Body weight	b wt	Litre	I
Calory	cal	Meter	m
Centimeter	cm	Microlitre	µl
Counts per minute	cpm	Millligram	mg
Cubic centimeter	cm^3	Millilitre	ml
Degree centigrade	°C	Minute(s)	min
Degree Fahrenheit	°F	Once a day	o d
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	iq	Square centimeter	cm^2
Intravenous	iv	Subcutaneous	sc
Kilo calories	kcal	Thrice a day	tid
Kilogram	kg	Year(s)	yr
Twice a day	bid	Volts	v

All other abbreviations should be spelled out when first used in the text.

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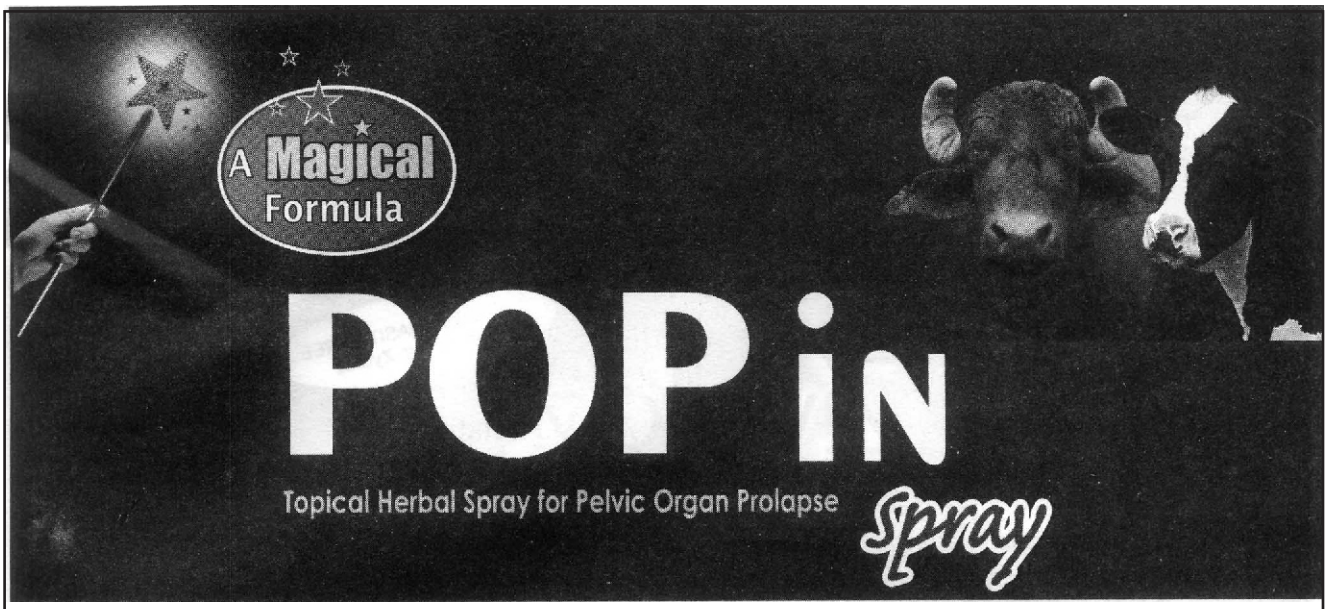


Features

- Reduces oedema
- Analgesic activity
- Topical antimicrobial
- Lubricant activity
- Modern drug delivery system (spray)
- Phytogenic in nature
- Economical

Benefits

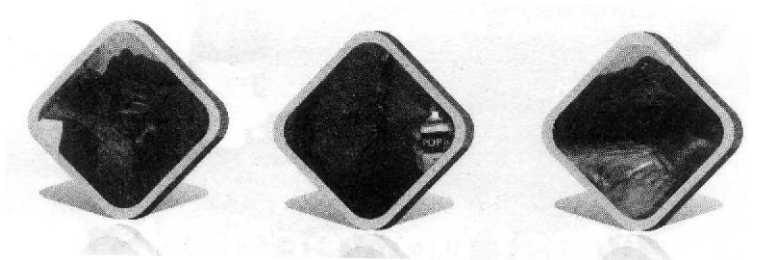
- Helps to reduce the size of prolapsed organ quickly. Thereby, facilitates repositioning of the, organ/s
- Helps to reduce pains
- Helps to maintain the area aseptic
- Facilitates easy repositioning of the organ/s
- Facilitates thorough application of the medicine aseptically
- Safe to animal as well as to the handler
- Saves money over conventional methods

Indications

- Vaginal Prolapse
- Cervico-Vaginal Prolapse
- Complete Uterine Prolapse

Presentation

150 ml aerosol spray can



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