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Contents

Contents

Indian J. Vet. Med. Vol. 34, No. 2, 2014 pp. 81-84

Research Article

Cutaneous punch biopsy: A diagnostic tool in dermatologic disorders

M.A. Kshama, S.Yathiraj and Suguna Rao
Department of Teaching Veterinary Clinical Complex
Veterinary College, Karnataka Veterinary, Animal and Fisheries Science University, Bangalore, Karnataka.

Abstract

Twenty nine dogs with severe dermatologic disorders which required a definitive diagnosis were selected as subjects of study. Punch biopsy was performed on the lesions using a 6 mm biopsy punch and the material collected was subjected to histopathology. It was possible to arrive at a definitive diagnosis in almost all the cases following histopathology thus proving that biopsy is a useful technique in the diagnosis as well as confirmation of diagnosis of dermatological disorders when the various other techniques are inconclusive.

Keywords: Dermatologic disorders, Histopathology, Punch biopsy

Dermatological disorders are one of the most common conditions encountered in dogs and yet one of the most frustrating problems to cope and treat. Though the type of dermatological disorders vary widely, the diagnosis is complicated by the fact that skin has only a limited range of responses to a wide variety of cutaneous insults thereby making the appearance of different diseases almost identical. Further, there may also be other concurrent conditions present resulting in an overlap of clinical signs. This makes it imperative to use appropriate and specific diagnostic techniques to accurately diagnose the condition and differentiate it from other similar disorders. Cytology is one such diagnostic technique which is not only simple and easy to perform Another very important technique that one needs to look at for the diagnosis of dermatological disorders is cutaneous punch biopsy. Though this may not be required in all the cases presented, whenever a diagnosis is in doubt or cytology does not provide sufficient information this is the technique to go for. Alguire and Mathes (1998) have stated that skin biopsy is an essential technique in the management of skin diseases and that it can help enhance the dermatologic care rendered by the practitioners. Pyoderma is one such condition which can be easily diagnosed by this technique, especially those cases where there is involvement of an underlying or concurrent condition, thereby making diagnosis difficult.

Materials and Methods

Dogs presented directly to Veterinary College Hospital, Bangalore or those referred from other clinics in and around Bangalore were used as subjects of study. Twenty nine animals showing either very severe inflammatory lesions, tumor like masses, recurring pyoderma or those animals in which no definite diagnosis was possible by skin scraping as well as cytology (impression smears /aspiration) were selected and subjected to cutaneous punch biopsy of the lesions after taking necessary aseptic precautions. The area in and around the lesion was cleaned with sterile cotton swab. Hair was clipped wherever required. Lignocaine Hydrochloride (2%) was injected subcutaneously all around the lesion. Six millimeter punch biopsy needle procured from M/s Basco, Chennai was used to punch out a small portion of the lesion along with normal skin. The skin was sutured with a single suture using 2.0 size suturing material and povidone iodine was applied on the site. The sample so collected was then placed on a thin piece of cardboard and flattened with the orientation directed in the direction of the hair growth. It was then transferred to a container with 10% Formaline and processed by routine paraffin embedding technique and the sections were cut and stained with Hemotoxylin and Eosin Stain. The histopathological slides were examined under the microscope.

Results and Discussion

Punch biopsy was done on 29 cases with dermatological disorders. The detailed results of the biopsy observation are illustrated in Table I & II and Fig 1 & 2. Infiltration of inflammatory cells onto the epidermis, dermis or hair follicles were found in 75.86% of the cases examined. This included 41.38% of lymphocytes, 31.03% of neutrophils, 27.59% of macrophages and13.79% of eosinophils. Epidermal and subepidermal hemorrhages and /or congestion was observed in 34.48% of cases. Acanthosis was observed in 31.03% of cases and dermal edema was observed in 27.59% of cases. Parakeratosis and hyperkeratosis was observed in 27.59% of the cases. Bacteria (cocci) was observed in 24.13% of the cases. Hyperplasia of hair

Table 1: Histopathological findings of skin biopsy in dermatological disorders in dogs during the study (n=29)

Observation	No of Cases	Per cent
Infiltration of inflammatory cells	22	75.86
1.Lymphocytes	12	41.38
2.Neutrophils	9	31.03
3.Macrophages	8	27.59
4.Eosinophils	4	13.79
Epidermal /subepidermal haemorrhages/ congestion	10	34.48
Acanthosis	9	31.03
Superficial dermal edema	8	27.59
Hyperkeratosis/Parakeratosis	8	27.59
Presence of bacteria	7	24.13
Thickened elongated hair follicles	4	13.79
Neoplasia like changes	3	10.34
Hyperplasia of sebaceous/sweat glands	2	6.90
Disruption of keratin layer	2	6.90
Presence of mites	1	3.44

follicles was observed in 13.79% of the cases. Changes suggestive of neoplasia such as lymphoma, as well as spindle cell sarcoma (fibrosarcoma) and trichoepithelioma, a benign tumor was found in 10.34% of the cases. Hyperplasia of sebaceous /sweat glands were seen in 6.90 % of cases. Disrupted keratin layer was seen in 6.90% of cases and presence of demodex mites was seen in 3.44% of cases

Thus based on the histopathological changes observed it was possible to diagnose these conditions which are listed in Table II. Superficial pyoderma / folliculitis was diagnosed in 27.59% of cases based on the presence of superficial perivascular or interstitial infiltration of inflammatory cells such as neutrophils, macrophages and lymphocytes (75.86% cases; Table II &Fig 3). An admixture of neutrophils, macrophages and lymphocytes, moderate dermal edema (27.59%) and presence of cocci (24.13%) (Table I) in epidermal layer and follicles were also observed which are in correlation with that reported by Gross *et al.* (2005)

Canine atopic dermatitis was diagnosed in 13.79% of cases. Changes suggestive of atopic dermatitis like severe acanthosis, hyperplastic changes of sebacious glands, congested dermal blood vessels with edema of dermis and presence of neutrophils and macrophages were found in these cases (Table I).This is similar to the observations reported by Olivry *et al*. (2001). However, Vaseem (2008) reported mild to moderate infiltration of inflammatory cells like mast cells, eosinophils, plasma cells and lymphocytes, acanthosis, hyperkeratosis, parakeratosis, spongiosis and sebaceous gland hyperplasia. Thus there is difference in the type of lesions observed in atopic dermatitis. This indicates that the histopathological findings should be corroborated with clinical signs as suggested by DeBoer and Hillier (2001) who stated that skin biopsy provides additional evidence to strengthen the case for definitive diagnosis but is not a foolproof test for diagnosing atopic dermatitis

Flea allergic dermatitis was diagnosed in 13.79% of cases (Table II). Changes such as acanthosis, patchy parakeratoses, infiltration of inflammatory cells like neutrophils, eosinophils and plasma cells, dermal edema and hyperplasia of sebacious glands. These changes were similar to that observed in Canine Atopic Dermatitis and there was no differentiating feature between CAD and FAD in the present study. However, Gross *et al.* (2005) indicated that presence of a large number of eosinophils in FAD may be a differentiating feature with reference to CAD. Changes suggestive of deep pyoderma / furunculosis was observed in 10.34% of the cases characterized by acantholytic and ulcerated epidermis with deep folliculitis characterized by neutrophilic inflammation inside the follicular wall with accumulation of neutrophils, eosinophils, and macrophages within and outside the follicles and dermal edema and hemorrhages / congestion similar to that described earlier by Gross *et al.* (loc.cit).

Acral lick dermatitis was diagnosed in 6.90% of the cases. Changes such as hyperkeratosis, parakeratosis, acanthosis of epidermal and superficial follicular epithelium with erosion and extensive ulceration and thickened and elongated hair follicles were observed in these cases. Similar findings along with

Table 2: Diagnosis based on skin biopsy of dermatological disorders in dogs

Diagnosis	No of Cases	Per cent
Superficial folliculitis	8	27.59
Atopy	4	13.79
Flea Allergy Dermatitis	4	13.79
Deep pyoderma/furunculosis& folliculitis	3	10.34
Acral lick dermatitis	2	6.90
Seborrhoeic dermatitis	2	6.90
Pyotraumatic dermatitis	1	3.44
Immune mediated (Pemphigus foliaceus)	1	3.44
Demodicosis	1	3.44
Cutaneous Neoplasia	3	10.34
a. Lymphoma	1	3.44
b. Trichoepithelioma	1	3.44
c. Spindle Cell Sarcoma/ fibrosarcoma	1	3.44

superficial dermal fibrosis with a vertical streaking pattern between intact hair follicles said to be characteristic of acral lick dermatitis has been reported by Gross *et al.* (loc.cit).

Seborrhoeic dermatitis was diagnosed in 6.90% of cases (Table II) with changes such as moderate to severe acanthosis and hyperkeratosis which was similar to changes such as hyperkeratosis, acanthosis, crusting and follicular keratosis said to be the distinctive feature of the condition as described by Gross *et al.* (2005).

One slide revealed the presence of demodex mites (3.44%) based on which a diagnosis of demodicosis was made for that case. Pyotraumatic dermatitis was diagnosed in 3.44% of the cases (Table II) and was characterized by severe ulceration and erosion of the epidermal layer along with disruption of the epidermis. Acanthosis, presence of neutrophils and cocci in epidermis was also observed (Table I). Similar findings of disruption of epidermis, moderate acanthosis, presence of neutrophils along with sharply demarcated borders of lesions from adjacent epidermis was reported by Reinke *et al.* (1987).

Changes suggestive of immune mediated condition, i.e. Pemphigus foliaceus was observed in 3.44% of cases. This was diagnosed mainly based on the presence of a large number of acantholytic keratinocytes and subcorneal pustules and corroborated based on clinical signs. This is in agreement with the report of Ihrke *et al.* (1985) and Gross *et al.* (2005).

Trichoepithelioma, a benign tumor of hair follicle was diagnosed in 3.44% of cases with characteristic changes such as the presence of well circumscribed but unencapsulated dermal nodule comprising of cystic structures of variable sizes and the horn cysts with abrupt keratinization as described by Gross *et al.* (loc.cit) .

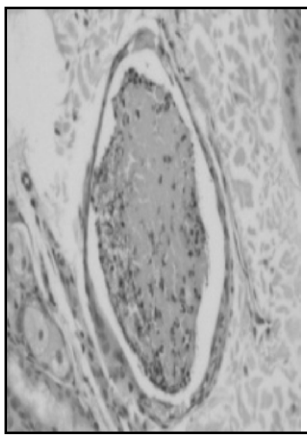

Fig 1. Cystic hair follicle with exudate consisting and necrotic material.

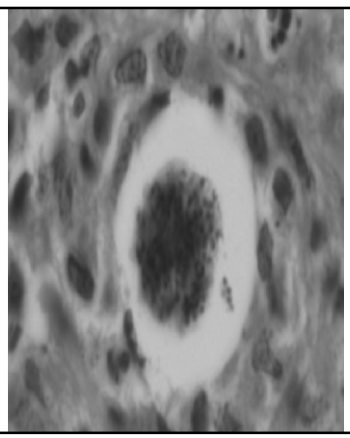

Fig 2. Skin showing a large number of cocci in a hair follicle.

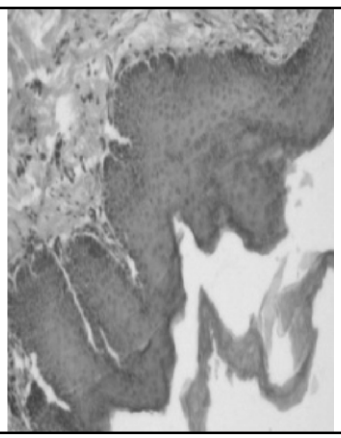

Fig 3. Severe acanthosis and hyperkeratosis.

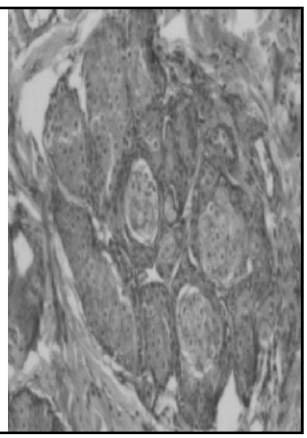

Fig 4. Hyperplasia of sebaceous glands.

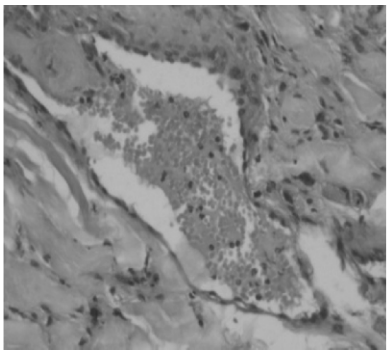

Fig 5 Punch biopsy section of skin of a dog with deep pyoderma showing congestion in the dermis.

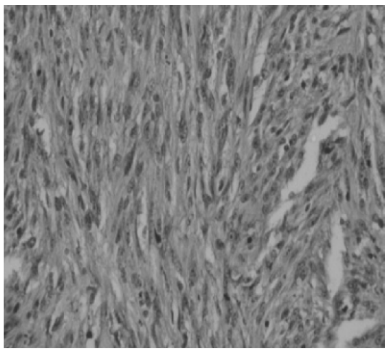

Fig 6. Interlacing bundles of fibrous connective tissue and spindle shaped cells suggestive of fibrosarcoma / Spindle cell sarcoma.

Neoplastic changes like Lymphoma was diagnosed in 3.44% of cases with histopathological changes such as pleomorphic cell structure having a mixture of a large number of reticuloendothelial cells, medium to large sized lymphoid cells with rounded nucleus and small nucleoli and many medium sized lymphoid cells with irregular nuclei as reported by De Bruijn *et al.* (2007). This was also in accordance with the findings of Fontaine *et al.* (2008) who reported in addition to these findings, moderate acanthosis, hyperkeratoses, spongiosis and mild to severe single cell necrosis of keratinocytes leading to epidermal necrosis and ulceration with the infiltrating cells being predominantly lymphocytes.

Spindle Cell Sarcoma (Fibrosarcoma) was diagnosed in 3.44% of cases with changes such as spindle cells arranged in interlacing bundles of varying size with the presence of immature proliferating fibroblasts (Table II & Fig 8). The cells had pale poorly defined scant cytoplasm and oval, fusiform or vesicular nuclei with one or more nucleoli. This is in agreement with the findings of Mukhopadhyay *et al.* (2012) who described the presence of interwoven bundles of immature fibroblasts and moderate numbers of collagenous fibres along with cells having hyperchromatic nuclei and disintergrated cytoplasmic area, hemorrhage and edema.

Cutaneous punch biopsy was found to be a useful technique for confirmation of diagnosis of dermatological disorders in dogs.

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Effect of "Rumilac Bolus" on rumen development and growth of dairy calves

M. Venkat Swamy, Dilip N. Muchlamb and K. Chandra Dash
Probiotics and Fermentation unit, Sanzyme Ltd Gagnpahad, Shamshabad, Hyderabad-501232, India

Abstract

A study was conducted to determine the effects of probiotics, yeast, enzymes and vitamins on rumen development and growth of neonatal Holstein dairy calves. Twenty-eight calves (Bull n=28) were assigned to five dietary treatments which included calf starter with no additive (C); *Saccharomyces boulardii,* (YC); mixture of *Bacillus coagulance, B. subtilis, B. licheniformis, Aspergillus nizer and Aspergillus oryza* (P); amylase, protease, lipase, alpha-galactosidase, beta-galactosidase, xylanase, cellulase, pectinase, glucanase and phytase (E); and a mixture of all the above (YCPEV). Calves were administered with treatments from day 2 to 56 in calf starter and from day 61 to 84 in calf grower diets. There was an increased consumption tendency ($P = 0.06$) for calves starter receiving YC than with no YC. Calves fed with starter containing P consume less water than all other calves ($P = 0.01$). Calves fed with YCPEV showed an increased body weight compared to other calves at week 6 and 8 ($P < 0.05$). There were no differences among treatment groups for hip and wither height ($P > 0.1$). Calves consuming YC had higher fecal scores than those with no YC in their starter ($P < 0.05$). However, all fecal scores were well within normal ranges typically seen in healthy calves. There were no differences among treatment groups on pH, ammonia, BHBA, butyrate, and propionate ($P > 0.1$). Incorporating YCPEV into starter may result in an increased growth. However, this effect is continued after weaning.

Keywords: *Aspergillus spp, Bacillus spp,* Enzyme supplements, *Saccharomyces boulardii*; Probiotics, Vitamin supplements.

Calves are very important for a farmer basically the dairy heifers are the future of the dairy industry. In order for a dairy farmer raising his own heifers to have a successful operation, he or she must strive for these heifers to reach breeding size as quickly as possible. An obtainable goal is to have heifers reach an optimum breeding weight of 550-770 lb. at approximately 13 months of age (Smith, 2007). The sooner the heifers calve, the sooner they become profitable to the herd. Calf producers must find a cost effective method to have these heifers reach their breeding weight as soon as possible. A proper solid feed is required to stimulate rumen development. A neonatal calf's digestion functions as a mono gastric animal, with the abomasum being the primary compartment for digestion. As the calf ingests solid feed, the rumen begins to take over as the primary compartment. Studies show that concentrates promote an increased rate of rumen development over roughages (Beharka *et al*., 1998; Heinrichs and Lesmeister, 2000).

Rumen development is primarily stimulated by dietary change and is characterized by change in physical size, wall thickness and papillae formation. Papillae the main surface area of the rumen, allows greater absorption and metabolism of volatile fatty acids (VFA) and others. A combination of liquid and solid feed in the diet is extremely important to rumen growth and papillae development. Concentrates promote an increased rate of rumen development when compared to forage. Due to the increased concern with antibiotics and other growth stimulants in the animal feed industry, research of other feed additives, such as direct-fed microbial (DFM) that contain viable and naturally occurring microbes, has increased. An interest in the effects of DFM on animal health and performance has heightened (Krehbiel *et al*., 2003). The United States Food and Drug Administration (USFDA) require feed manufacturers to use the term "direct-fed microbial" (Krehbiel *et al*., 2003) and mandates the declaration of details of viable microbes. Direct-fed microbials have been frequently added to milk replacer for gastrointestinal health benefits, as well as improving average daily gain, daily feed intake, and feed conversion. Use of these supplements in calves as a preventative practice has increased from 13.1% to 20% from 1996 to 2007 (USDA, 2008).

A probiotic is defined as a live microbial feed supplement that improves the intestinal microbial balance of the host animal (Cruywagen *et al*., 1995). Combination of yeast and Probiotics have many benefits when added to a diet. They stimulate desirable microbial growth in the rumen and stabilize rumen pH, fermentation and end product formation. Increase in nutrient flow post ruminally, nutrient digestibility, and the alleviation of stress through enhanced immune response are other benefits of DFM (Yoon and Stern, 1995). Bacteria typical to the intestine (e.g., Lactobacilli) have shown an increased response in growth and health when compared to other bacteria (Abe *et al*., 1995).Yeast cultures have also been shown to

improve growth performance and health of calves when supplemented in the milk replacer. The culture is a yeast-fermented product that contains live and dead yeast cells, the media the cells were grown on, and the metabolic by-products produced by the yeast during fermentation (Linn and Raeth-Knight, 2006). When fed to cattle, yeast cultures have been shown to stimulate cellulolytic bacteria in the rumen, improve fiber digestion, and stabilize rumen pH (Rossi *et al*., 2006). Despite their complex mechanisms of action and variable animal response, Exogenous Non-starch Polysaccharidases (ENP) has received much research interest in ruminant nutrition (Beauch emin*et al., 2004*). Apparently, no or little inclusion of forage-fiber in pre-weaning calf diets has attracted much less interest to the use of ENP for young calves. From rumen development and health perspectives, however, little dietary forage would not explain overlooking the desperate need for greater utilization of non-forage polysaccharides by young dairy calves (Baldwin *et al., 2004).*

Pre-ruminant calves possess negligible activity of the enzymes degrading starch and cell-wall polysaccharides (Van Soest, 1994). Meanwhile, adequate supply of volatile fatty acids (VFA) from microbial fermentation is crucial for the proliferating expansion of rumen epithelia and effective hepatic metabolism of VFA (Baldwin *et al.,* 2004). Early establishment of fibrolytic capacity appears determining for such early development in the rumen and hepatic metabolism to occur (Van Soest, 1994). Universally, dairy calves are usually weaned between 5 to 12 weeks of age, depending on growth rate, body size and milk price. The milk price, nonetheless, may not play a mojor role at the expense of a desirable calf growth. Accordingly, early utilization of starter polysaccharides by the calf could hasten the reticulo rumen maturity, lower the weaning age, save milk and reduce labor costs (NRC, 2001). Dairy Industries with rather long commercial weaning age (>5-7 weeks) and a growing consumer demand for dairy products would benefit the most from such optimized nutrient utilization by dairy calves.

Based on the current knowledge of probiotics and yeast culture and the limited information on the supplemental use in calf starter and effects on rumen development, the objective of this study was to determine the effects of probiotics and yeast culture in calf starter on rumen development and growth parameters in neonatal dairy calves.

Materials and Method

Animals and dietary treatments

Twenty-eight Holstein calves (n=28) were utilized in a sixteen week experiment to determine the effects of dietary inclusion of yeast culture, probiotics with enzymes and vitamins on growth and rumen development. Randomly, the claves were selected from the farm (Mr. Jayapal Reddy Dairy Farms, Mahaboob Nagar, Andhra pradesh, India) and the calves were housed at standard farming conditions. Calves were separated from their dams at birth, weighed, and individually housed in 2.5-m^2 calf hutches with a 2.8-m^2 wire enclosure on rock bedding until day 56. Calves were received 4 quarts of colostrum from their dams and should be orally vaccinated against Rotavirus and Coronavirus (Calf Guard, Pfizer Animal Health, Lenexa, KS). Day 2 and 3 of life, calves received transition milk from their dams in bottles. On day 4 of life, calves were offered MR(milk replacer) containing decoquinate (20% protein, 20% fat; Nutra Blend LLC, Neosho, MO) at 10% of their birth weight and bucket trained. Refusal of MR, if any, was weighed and discarded. Calves were then randomly assigned to one of five dietary treatments in blocks of five according to birth date as follows: ^{0}control calves (C) receiving 0 P, 0 YC, 0 E or 0 YCPEV; ^{1}calves receiving at a dose of 10gm/day/animal of the supplement of yeast culture of *Saccharomyces boulardii*, (YC) as a percentage of feed as fed (UB-Biosac 'Unique-28', Unique Biotech Ltd., India); ^{2}calves receiving a minimum total CFU count of 4.0 x 10^9 per gram of *B. coagulance, B. subtilis, B. licheniformis, A. oryza and A. niger* (P) at a dose of 10gm/day/animal, (Customized Preparation, Sanzyme Ltd., India); ^{3}calves receiving at a dose of 10gm/day/animal of the supplement of enzymes amylase, protease, lipase, alpha-galactosidase, beta-galactosidase, xylanase, cellulase, pectinase, glucanase and phytase (E) (Peezyme FS Vet, PVS Laboratories Ltd., India); and ^{4}calves receiving the supplement of *S. boulardii; B. licheniformis* and *B. subtilis, A. oryza, A. niger*; amylase, protease, phytase, cellulase with vitamin-B1, Vitamin-B6 & Vitamin-C (YCPEV) at a dose of 1bolus/day/animal (Sanzyme Ltd., India). Calves were offered MR once daily at 10% of birth weight at AM feedings from day 4 until abrupt weaning at day 42. Calves were fed their respective treatments in an

18% crude protein (CP) calf starter until eight weeks of age at ad libitum levels. Starter (Table 1) is offered at 0630 and 1600 hours, with starter intake recorded for each calf. Calves were initially offered 227 g of starter, and remaining feed is weighed at each delivery time. Starter increased at 227 g increments when calves refused less than 36 g of feed. Water was offered adlibitum beginning on day 4. Calves were initially offered 3.859 kg of water, and remaining water was weighed at each delivery time. Additional water was offered (3.859 kg) when the calf consumed all water. On day 57, calves were moved to pens with access to ryegrass pasture and free choice grass hay. Calves were offered a grower diet (Table 2) containing their respective treatments at a level of 2270 g/ calf/ day. Calves were offered water and hay at adlibitum levels. At 12 wk, calves are removed from treatment and placed on a control grower until the end of the experiment at 16 wk.

Sample collection

Calves were observed and fecal scores recorded according to Larson *et al*. (1977). Scoring was as follows: for fecal fluidity, 1 = normal, 2 = soft, 3 = runny and 4 = watery. Body weights were recorded beginning at birth and again at 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 112 d of age. Wither height and hip height were measured at 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 112 d of age. Rumen fluid was collected via stomach tube for analysis of VFA and NH3 at 14, 28, 42, and 56 d of age 4 hr post-feeding and 70, 84, and 112 d of age pre-feeding.

Rumen fluid was analyzed for pH immediately, after which 1 mL of phosphoric acid (20% w/v) was added and stored at 4 ^{O}C till analysis. At 28, 42, and 56 d of age post-feeding and 84 and 112 d of age pre-feeding, blood was collected via jugular venipuncture for analysis of ²-Hydroxybutyrate (BHBA). Blood collected or BHBA analysis was collected in 10 mL collection tubes containing sodium heparin, centrifuged for twenty minutes at 600 x g, and plasma separated and stored frozen (-20°C) protected from light until analysis.

Analytical procedure

Plasma was analyzed for BHBA using commercial spectrophotometric kits (²-Hydroxybutyrate Liquicolor® Kit; Stanbio Laboratory, Boerne, TX). Acidified ruminal fluid was thawed at room temperature and clarified by centrifuging at 30,000 x g for 20 min. The clarified supernatants were then decanted and analyzed for NH4+ using a modified phenol-hypochlorite reaction adapted from Broderick and Kang (1980).

VFA was analyzed as follows; A 4 mL sample of ruminal fluid was mixed with 1 mL of 25% (wt/wt) meta-phosphoric acid containing 10 g/L 2-ethylbutyric acid, an internal standard for VFA quantification. The mixture of ruminal fluid and meta-phosphoric acid was then centrifuged at 30,000 x g for 25 min. Concentrations of individual VFA were measured by GC using a NUCON GC 5765 equipped with a 15-m EC-1000 column with an internal diameter of 0.53 mm and a film thickness of 1.2 ¼m (Nucon, New Delhi, India). The reagent preparation procedure and temperature gradient for VFA analysis was adapted from Grigsby *et al*. (1992) and Bateman *et al*. (2002), respectively.

Statistical analysis

Data were analyzed using the mixed procedure of SAS (Littell *et al*., 1998). Values reported are least square means. Significance was declared at *P d"* 0.05, and a trend was reported if 0.05 <*P* 0.10.

Results and Discussion

Performance data

Least squares means for average daily starter intake calves fed C,YC, P, E or YCPEV were presented in Table. 1. Least squares mean for overall average daily starter intake for calves fed C, YC, P, E or YCPEV are presented in Table 3. Overall mean of average daily starter intake was not significantly affected (P > 0.1) by the addition of YC or P in the feedstuffs. Overall means for starter intake are presented in Table 3. Calves receiving calf starter containing YC tended to consume more than other calves (P = 0.068). However, Quigley *et al*. (1992) found no significant effect of YC on intake of starter. As expected, there was a significant week effect (P < 0.0001). As calves aged, starter intake increased among all treatments. Least squares means for water intake are presented in Table 3. As expected, water intake increased (P < 0.0001) with age regardless of treatment. Calves consuming starter containing P drank less overall than other treatment groups (P = 0.017).

Table 1: Calf Starter Composition

Ingredients,% as Fed	C	YC	P	E	YCPEV
Rolled Corn	35	33.25	35	34.5	33.25
Kentwood Custom Heifer-R[1]	1.5	1.5	1.5	1.5	1.5
Pro-Lak	2.5	2.5	2.5	2.5	2.5
Stock Pellets 16%	10	10	10	10	10
Country Acres H&M	10	10	10	10	10
Rumensin/Vitamin E Premix[2]	1	1	—	1	1
Cargill Pellet Milk +	2.5	2.5	2.5	2.5	2.5
Dried Distillers Grain	4	4	4	4	4
Soybean Meal 48	3.5	3.25	3.5	3.5	3.25
Sweet Stuff	7.5	7.5	7.5	7.5	7.5
Protein Pellets (SBM)	10	10	10	10	10
Crimped Oats	10	10	10	10	10
Molasses	2.5	2.5	2.5	2	2.5
Rumensin/Vitamin E/Probiotic premix[3]	—	—	1	1	—
Yeast Culture[4]	—	2	—	—	2

C=Control, YC=Yeast culture, P=Probiotic, E= Enzyme, YCPEV= Yeast culture, Probiotic with Enzyme and Vitamin.
[1]Kentwood Custom Heifer-R contains Monensin 2,400 g/ton, Calcium(Min) 15.00%, Calcium(Max) 18.00%, Phosphorus(Min) 5.75%, Salt(Min) 18.00%, Salt(Max) 21.00%, Magnesium(Min) 2.60%, Potassium(Min) 0.90%, Sulfur(Min) 1.00%, Cobalt(Min) 25 ppm, Copper(Min) 800 ppm, Iodine(Min) 80 ppm, Manganese(Min) 2,700 ppm, Selenium(Min) 20 ppm, Zinc(Min) 2,750 ppm, Vitamin A(Min) 200,000 IU/lb, Vitamin D-3(Min) 45,000 IU/lb, itamin E(Min) 1,000 IU/lb, [2]Rumensin/Vitamin E Premix contained 94.5% dried distiller's grain, 0.5% Rumensin, and 5% Vitamin E [3]Rumensin/Vitamin E/Probiotic Premix contained 89.5% dried distillers grain, 0.5% Rumensin, 5% BioPlus 2B, Chris Hansen Biosystems, and 5% Vitamin E, [4]Diamond V XP Yeast Culture, Diamond V Mills, Inc.

Table 2: Calf Grower Composition

Ingredients,% as Fed	C	YC	P	E	YCPEV
Rolled Corn	37.5	35.8	37.5	35.7	35.7
Kentwood Custom Heifer-R[1]	2	2	2	2	2
Dried Distillers Grain	10	10	10	10	4
Soybean Meal 48	15	14.8	15	10	14.8
Whole Cottonseed	5	5	5	5	5
Cottonseed Hulls	2.5	2.5	2.5	2.5	2.5
By-Product Mix	25	25	25	25	25
Molasses	2.5	2.5	2.5	2.5	2.5
Rumensin/$CaCO_3$ Premix[2]	0.5	0.5	—	—	—
Rumensin/$CaCO_3$/Probiotic premix [3]	—	—	0.55	0.55	0.55
Yeast Culture[4]	—	2	—	2	2

C=Control, YC=Yeast culture, P=Probiotic, EV= Enzyme, YCPEV= Yeast culture, Probiotic with Enzyme and Vitamin.
[1]Kentwood Custom Heifer-R contains Monensin 2,400 g/ton, Calcium(Min) 15.00%, Calcium(Max) 18.00%, Phosphorus(Min) 5.75%, Salt(Min) 18.00%, Salt(Max) 21.00%, Magnesium(Min) 2.60%, Potassium(Min) 0.90%, Sulfur(Min) 1.00%, Cobalt(Min) 25 ppm, Copper(Min) 800 ppm, Iodine(Min) 80 ppm, Manganese(Min) 2,700 ppm, Selenium(Min) 20 ppm, Zinc(Min) 2,750 ppm, Vitamin A(Min) 200,000 IU/lb, Vitamin D-3(Min) 45,000 IU/lb, Vitamin E(Min) 1,000 IU/lb, [2]Rumensin/$CaCO_3$ Premix contained 94.5% dried distiller's grain, 0.5% Rumensin, and 5% Vitamin E [3]Rumensin/CaC)3/Probiotic Premix contained 89.5% dried distillers grain, 0.5% Rumensin, 5% BioPlus 2B, Chris Hansen Biosystems, and 5% Vitamin E [4]Diamond V XP Yeast Culture, Diamond V Mills, Inc

Table 3: Average daily starter intake and water intake for calves till 56 days

	Treatment					P Value				
	C	YC	P	E	YCPEV	SEM	YC	P	E	YCPEV
Starter (g/Day)	623.9	695	483	677	685	69.10	0.06	0.23	0.40	0.50
Water (L/Day)	4.21	4.15	2.96	3.54	3.10	0.47	0.40	0.01	0.45	0.48

SME= Standard Error of the Mean

Table 4: Effect on average body weight (Kg) at different experimental period

	Treatment					P-Value				
	C	YC	P	E	YCPEV	SEM	YC	P	E	YCPEV
Birth, d 0	36.3	38	38.2	38.3	38.5	1.75	0.78	0.71	0.38	0.72
Weaning, d 42	53.9	55.4	52.3	56.9	57.2	1.43	0.02	0.99	0.26	0.35
Remove from hutch, d 56	65.5	68	62.4	68.2	68.5	1.97	0.04	0.47	0.4	0.39
End of trt. d 84	89.9	91.3	88.5	90	91.8	3.35	0.56	0.56	0.97	0.96
End of trial d 112	111	117	113	117	117.9	3.79	0.16	0.77	0.83	0.83

SEM=Standard Error of the Mean, Fecal Score Scale: 1=normal, 2=soft,3=runny,4=water

Table 5: Least squares means for hip height and wither height through 112 days of age and fecal scores through 56 days of age of experimental calves

	Treatment					P Value				
	C	YC	P	E	YCPEV	SEM	YC	P	E	YCPEV
Hip (cm)	87	88.84	87.56	87.33	88.65	1.04	0.55	0.76	0.63	0.68
Wither (cm)	83.9	85.48	84.98	84.63	86.66	1.03	0.66	0.72	0.69	0.85
Fecal Score	2.4	2.48	2.42	2.41	2.49	0.65	0.02	0.46	0.42	0.82

SME= Standard Error of the Mean; There were no treatment effects ($P > 0.05$) for hip and wither heights. Treatment effects were observed for fecal score ($P < 0.05$).

Table 6: Least squares means of ruminal pH, NH3, and VFA and plasma BHBA for calves fed diets containing no additives (C), yeast culture (YC), probiotics (P), enzymes (E) or yeast culture, probiotics and enzyme with vitamins (YCPEV) through 112 days of age. There were no significant effects of treatment ($P>0.05$).

	Treatment					P Value				
	C	YC	P	E	YCPEV	SEM	YC	P	E	YCPEV
pH	6.47	6.42	6.26	6.4	6.39	0.07	0.82	0.15	0.14	0.2
NH3,mg/dL	6	5.7	5.56	6	6.58	0.75	0.62	0.73	0.73	0.35
Acetate,mmol/L	29.8	30	31.86	29.6	31.78	3.25	0.88	0.24	0.25	0.82
Butyrate,mmol/L	4.31	4.58	4.5	4.3	4.56	0.56	0.55	0.88	0.89	0.79
Propionate,mmol/L	20.43	22.16	23.86	20.4	23.43	2.36	0.6	0.21	0.22	0.59
Total VFA,mmol/L	58.29	58.14	62.57	58.1	62.86	5.28	0.97	0.39	0.39	0.96
BHBA, mmol/L	0.25	0.27	0.26	0.26	0.26	0.01	0.65	0.84	0.85	0.56

SEM=Standard Error of the Mean

Least squares mean for body weight of calves at 0 d, 42 d, 84d, and 112 d are presented in Table 4. Calves consuming YC showed higher body weights at d 42 and d 56 when compared to calves not consuming YC ($P<0.05$), but the effect of YC was not significant overall ($P > 0.1$). As expected, there was a significant week effect on body weight ($P < 0.0001$). Quigley *et al*. (1992) found no significant effects of YC on ADG or intake of starter. Higginbotham and Bath (1992) reported no significant effects of P on ADG when added to waste milk. However, Lesmeister *et al*. (2004) reported improvement in average daily gain when 2% supplemental YC was added to a calf starter diet. Least squares mean for wither and hip height of calves fed C, YC, P, E or YCPEV are presented in Table 5. No treatment effect was observed, but, as expected, there was a significant effect of time for wither and hip height for all treatments ($P <0.0001$). In contrast to the current experiment, Lesmeister *et al*. (2004) reported increased hip height when 2% supplemental YC was added to a calf starter diet. Least squares means for fecal scores for calves fed C, YC, P, E or YCPEV are presented in Table 5. Calves consuming YCPEV had higher fecal scores than those with no YCPEV in their starter ($P= 0.0253$).

However, all fecal scores were well within ranges typically seen in healthy calves, so the biological significance of this effect is minimal. Magalhaes *et al*. (2008) found that the addition of YCPEV to calf starter significantly improved fecal scores, along with decreasing mortality rates in calves experiencing high incidence of diarrhea. Timmerman *et al*. (2005) also observed a suppression of diarrhea in calves fed MR supplemented with P. Cruywagen *et al*. (1995) observed no effect of the addition of P in MR on the occurrence of diarrhea. These additives may improve intestinal

health when calves are experiencing problems. But there were no health problems observed in the calves in the current study that may explain the lack of treatment effect on fecal scores.

Rumen development

Least squares means of rumen fermentation parameters for calves fed C, YC, P, E and YCPEV are presented in Table 6 Ruminal pH was influenced by age of the calf ($P < 0.05$). These results agree with Beharka *et al*. (1998) who reported a quadratic change in the relationship between pH and age of the calf. Ghorbani *et al*. (2002) observed no effect on rumen pH in feedlot steers when fed a diet containing P. Quigley *et al*. (1992) also observed no significant response in rumen pH to YC in calf starter. Least squares means of NH3 for calves fed C, YC, P, E and YCPEV are presented in Table 6. No treatment effect was observed, but there was a significant week effect on NH_3 concentrations ($P < 0.05$). This result agrees with Vazquez-Anon *et al*. (1993) who reported a significant age effect of rumen NH_3 concentrations with concentrations higher at 4 wk after weaning. However, Anderson *et al*. (1987) observed higher NH_3 concentrations in un weaned calves than in weaned calves. Quigley *et al*. (1992) reported that the addition of *S. boulardii* to calf starter had no effect on NH_3 concentrations. Newbold *et al*. (1996) found that NH_3 concentrations increased when mature sheep were fed *S. boulardii*, but this result could be attributed to differences in species and age. Least squares mean of plasma BHBA for calves fed C, YC, P, E or YCPEV are presented in Table 6. No treatment effect was observed, but BHBA levels of calves for all treatments increased over time ($P < 0.0001$). Others have agreed with this increase in BHBA with age (Coverdale *et al*., 2004; Quigley *et al*., 1991). Similarly, Lesmeister *et al*. (2004) did not observe treatment effects in BHBA when calves were fed YC in calf starter. However, Quigley *et al*. (1992) observed an increase in BHBA when calves were fed YC in calf starter coinciding with an increase in the concentration of butyrate reported. Least squares mean of acetate, butyrate, propionate, and total VFA are presented in Table 6. There was a significant effect of time for all VFA concentrations ($P < 0.0001$). Other dairy calf studies have also observed this increase in VFA concentrations with age (Beharka *et al*., 1998; Coverdale *et al*., 2004). Similarly, Ghorbani *et al*. (2002) observed no influence of YC on propionate and total VFA, but found that incorporation of P in feed increased the concentration of acetate in feedlot steers. Other studies revealed that YC caused an increase in acetate, butyrate, and total VFA in beef steers (Martin and Nisbet, 1990) and in dairy calves (Quigley *et al*., 1992).

The non-linear response in DMI might indicate a more pronounced effect of ENP on less digestible, forage-based diets that in turn causes a faster ruminal outflow rate and greater feed intake response, as pointed out by Beauchemin *et al*. (2003). After all, the result of enzymes using in the starter of calves resulted better performance on growth than the control group.

Calves consuming calf starter containing YCPEV showed an increase in growth and starter intake at 42 d of age and 56 d of age, but once calves were put into a group feeding situation and given access to forage, this difference was not seen. The fecal scores for calves consuming YCPEV were slightly higher; it is possible that the addition of YCPEV to calf diets may decrease the incidence of diarrhea during times of stress. Further studies are needed to determine the effects on growth and incidence of diarrhea during times of stress. However, 36 rumen developments remains unaffected overall by the addition of YC, P, E and YCPEV to grain diets. Proper utilization of feedstuffs remains essential for proper rumen development in the young dairy calf.

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Clinicopathological changes in lower respiratory tract infections of canine

Latesh Bhagat, J.L.Singh[1], S.K.Rastogi[2], Arup Kr. Das[3], R. Houzha[4] & D.B.Mondal[5]
Department of Veterinary Medicine , College of Veterinary and Animal Sciences,
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar-263 145, U.S.Nagar, Uttarakhand

Abstract

The present study was aimed to identify the clinicopathological profile of canine lower respiratory tract infections. Abnormal breathing pattern, dyspnoea, coughing, tachypnoea, open mouth breathing, cyanotic mucous membrane, anxiety, glazed look, extended head and neck, paradoxical respiration, abnormal lungs sound etc. were the characteristic clinical signs of lower respiratory tract infections. Study of hematological profile of affected dogs revealed significant increase in the values of Hb, ESR, TLC and neutrophils and decreased in the value of TEC and lymphocytes. Arterial blood gas analysis showed significant decrease in blood pH, pO_2, sodium, potassium and bicarbonate, while significant increase was found in pCO_2 in affected dogs. The pulse oximetery of dogs revealed decreased oxygen saturation of blood and increased pulse rate of the affected dogs. The spirometric study also revealed decreased tidal volume and increased respiration rate in affected dogs. Thoracic radiographic investigation of the cases revealed pulmonary congestion, bronchitis, pneumonia, pleurisy and diffuse consolidation of lungs.

Keywords: Arterial blood gas analysis, Canine, Lower respiratory tract infections, Spirometric study

Prompt recognition of the underlying respiratory disease and complete familiarity with emergency diagnostic and therapeutic procedure including oxygen therapy, patent airway opening and use of various chemotherapeutic agents can lead to the successful management of many emergency respiratory patients. Main clinical signs of respiratory distress include tachypnea, open-mouth breathing, cyanotic mucous membranes, loud breathing, restlessness, anxiety, glazed look, extended head and neck, paradoxical respiration (abdominal wall and chest wall moving opposite to one another during respiration). Tachypnea is the most consistent sign. The severity of respiratory compromise is not always manifested clinically, although patients that are exhibiting any of the above signs are most likely to be in severe distress (Macintire *et al*., 2006). In this study, various diagnostic methods have been used for the proper and accurate diagnosis of lower respiratory tract infections in canines.

Materials and Methods

Canine patients (n=24) clinically suffering from lower respiratory tract infections were included in this study. The inclusive criteria for animals was apparent respiratory problems like abnormal breathing pattern, dyspnoea, coughing, tachypnoea, open mouth breathing, cyanotic mucous membrane, restlessness, anxiety, glazed look, extended head and neck and paradoxical respiration.Eight (n=8) apparently healthy dogs of either sex, irrespective of breed or age were selected for control group on the basis of the clinical, haematogical , coprological examinations, thoracic radiography, GSH estimation, spirometry, pulse oximetry and arterial blood gas analysis.

Screening of dogs for respiratory diseases was based on clinical and stethoscopic examination of respiratory system, cytological examination of blood and thoracic radiography. Out of the total 39 screened dogs for respiratory diseases, 24 dogs with respiratory diseases were randomly selected and divided into 3 groups namely A, B and C. Basis of groups A,B and C Was on the basis of therapeutic regimen. . These groups of dogs were further subjected to confirmatory diagnostic techniques and each group was treated with a suitable of antibiotics recommended for inflammatory and other affections of lower respiratory system. In addition to affected dogs, a group of 8 apparently healthy dogs[1] from the local area, were taken as control and designated as group D.

About 4 ml of blood was collected from cephalic vein of each dog. Immediately after collection, 2 ml was transferred to EDTA vials for the analysis of blood cellular components and remaining 2 ml was allowed to clot and serum samples was deproteinized with the help of 10% meta phosphoric acid and stored at -20°C for GSH estimation. About 200µl of arterial blood was collected from femoral artery using a Becton Dickinson one ml syringe, that has been supplied with lyophilized lithium heparin as an anticoagulant for analysis of partial

[1] Corresponding author -Email- drjlsingh@rediffmail.com [2] Prof & Head Deptt of Physiology & Biochemistry,[3] Assoc Prof Deptt of Surgery & Radiology,[4] Asstt Prof Deptt of Physiology & Biochemistry,[5] P.S. , Division of Medicine, IVRI. Izzatnagar, Bareilly

pressure of oxygen, carbon dioxide and other parameters. The blood samples were collected before and after medication of each dog of various groups.

The blood samples were analyzed within two hours of blood collection for complete haematology as per the procedures described by Jain (1986). Coprological study was conducted by direct smear method and Baremann technique (Soulsby, 1982). Glutathione enzyme estimation in stored serum samples was done by method provided by Ellman (1959) with DTNB (5,5-dithiobis, 2, nitrobenzoic acid).

Thoracic radiographs from different views was taken for evaluation of localizing disease process, narrowing and prioritizing the differential diagnosis, determining the extent of disease involvement, and monitoring the progression of disease and response to treatment. Right lateral and ventro-dorsal view were preferred as described by Thrall (2002). X-rays were obtained using 60 mA mobile X-ray machine[2].

Both lateral and ventro-dorsal of the thoracic region of the animal's radiographs were taken. For lateral view the sternum and spine were in the same horizontal plane and the front legs were pulled as far as cranially as possible. For ventro-dorsal views, dogs were carefully positioned to assure precise superimposition of the sternum and vertebrae. Exposure time and kilo voltage were adjusted according to the technique chart.

Tidal Volume of lungs was recorded using spirometer and student physiograph (Biodevice Ambala cantt) in twenty four clinical cases of dogs (grouped as A, B and C) and eight healthy dogs (grouped as D) in the Department of Veterinary Physiology, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar. Spirometer was set at sensitivity of 10 μV and paper speed at 2.5 mm/S. A mask made by plastic bottle properly padded with the cotton and adhesive tape to avoid injury was used as a mouth piece. To obtain accurate results, the animal was allowed to relax properly and then mask of spirometer was placed over the mouth of dog completely sealing it. Tidal volume can be recorded by observing the displacement of drum and comparing it with the marking on the spirometer. These movements of drum were also recorded over a graph paper on physiograph attached to the spirometer with the help of isotonic coupler.

Analysis of arterial blood gases and electrolyte was performed with the help of Electrolyte and Blood Gas Analyzer[3]. Evaluation of pO_2, pCO_2, blood pH, HCO_3^-, Na^+, K^+ and Cl^- parameters in the arterial blood samples were undertaken.

Arterial blood samples were collected from femoral artery using a Becton Dickinson one ml syringe supplied with lyophilized lithium heparin chloride. The animal was placed in lateral recumbency, the right rear limb was being held perpendicular to the table to expose the left inguinal area. The pulse was palpated in the femoral triangle between two fingers to accurately locate the artery. The needle was laid directly on the top of the artery, then stabbing into it with a short, jabbing motion. Blood specimen collected, was analyzed immediately within 10-15 min.

Pulse oximetery (%HbO_2 saturation and pulse rate) was recorded by BPL make pulse oximeter (Modal cleo) in all affected dogs (grouped as A, B and C) and eight healthy dogs (grouped as D) in the Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Pantnagar. To obtain accurate results, animal was allowed to relax properly and then probe of pulse oximeter was placed at the ear flap of dog after cleaning it properly with spirit swab.

Results and Discussion

During the course of clinical screening of dogs with respiratory tract infections at Veterinary Teaching Hospital, C.V.A.Sc., G.B.P.U.A. &T. Pantnagar, a total of 39 cases were found positive, among them randomly 24 cases were selected and subjected for detail study of clinical abnormality presents.

The most striking clinical finding was dyspnoea, in 75.00 % clinical cases. The second most striking clinical finding was coughing, in 70.83% clinical cases. The third most striking clinical finding was abnormal lungs sound (66.67%) followed by paradoxical respiration and dehydration, in 62.50% clinical cases. The others important clinical sign was high rise in temperature (104-106°F), in 58.33% clinical cases. Extended head and neck and exercise intolerance were also important clinical findings, observed in 50.00% clinical cases. Polypnoea and restlessness/ anxiety were also observed in 45.83% clinical cases. Nasal discharge, open mouth breathing and excessive panting were recorded in 41.66% clinical cases. Glazed look, cyanotic mucous membrane and tachypnoea were observed in

25.00% clinical cases. Sitting on haunches was observed in 16.67% clinical cases. The detailed overt clinical examinations of dogs suffering with lower respiratory tract infections were carried out and results recorded(Table 3).

All these finding were typical finding of inflammatory condition of pulmonary system and are in agreement with the signs and symptoms described by (Clercx *et al.,* 2000; Garcia *et al.,* 2001; Macintire *et al*, 2006; Radhakrishnan *et al.,* 2007; Smith and Byers, 2009).

Dyspnoea, nasal discharge, inappetance and rise in body temperature in chronic bronchopneumonia also has been reported earlier (Amrute *et al.* 2009). Moist rales on auscultation of lungs supports the observation of Payungporn *et al.* (2008). Increased rectal temperature, respiration and heart rate in aspiration pneumonia also has been reported earlier (Kogan *et al.* 2008) with increased/ decreased or adventitious lung sounds. Decreased and muffled lung sound has been reported by Nelson 2005. Corcoran *et al.* (1999) also described the clinical features of chronic coughing, dyspnoea, tachypnoea, inspiratory crackles, wheezes and rhonchi audible on chest auscultation, and deteriorated progressively over months to year in chronic respiratory condition of 29 West Highland white terriers. Drobatz *et al.* (1999) reported stupor or coma, coughing or gagging, and respiratory difficulty in dogs that exposed to smoke in residential fires.

Mean ± S.E values of saturation of oxygen in blood (sO_2) and pulse rate (PR) of all the group of dogs on 0 and 10th day of post medication has been detailed in Table 4.

Prior-medication values of sO_2 in groups A, B and C were significantly ($p<0.01$) lower as compared to group D suggesting impaired oxygen uptake in blood from diseased lungs. However on 10 days post treatment, significantly ($p<0.01$) increased values of sO_2 of groups A, B and C were observed in comparison to their pre-treatment values. The improvement in sO_2 level indicated positive effect of various treatments adopted for the dogs.

Prior-treatment values of Pulse rate in groups A, B and C were significantly ($p<0.01$) higher as compared to group D. After, 10 days of treatment, a significant ($p<0.01$) decrease was recorded in values of pulse rate of groups A, B and C in comparison to their pre-treatment values. However, post-treatment values of group A and B were significantly ($p<0.05$) higher as compared to group D suggesting a little poorer response of the treatment in these dogs. Similar findings with decrease in sO_2 with pulse oximeter was recorded by Radhakrishnan *et al.* (2007); Zeugswetter *et al.* (2007).

Mean ± S.E values of haemoglobin (Hb), erythrocyte sedimentation rate (ESR), total erythrocytes count (TEC) and total leucocytes count (TLC) of all the groups of dogs on 0 and 10th day of observation has been given in Table 5.

Pre-treatment (0 day) values of Hb in groups A, B, and C were significantly ($p<0.05$) higher as compared to group D. Even post-treatment (10th day)

Table 3: Distribution of respiratory deficits in dogs.

S.N.	Nature/ Type of clinical Signs	No. of affected animals	FOD of clinical signs (in %)
1	High rise of temperature (104-106^{0}F)	14	58.33
2	Coughing	17	70.83
3	Restlessness/Anxiety	11	45.83
4	Dyspnoea	18	75.00
5	Nasal Discharge	10	41.66
6	Glazed look	6	25.00
7	Open mouth breathing	10	41.66
8	Extended head and neck	12	50.00
9	Sitting on Haunches	4	16.67
10	Excessive Panting	10	41.66
11	Cyanotic mucus membranes	6	25.00
12	Exercise intolerance	12	50.00
13	Polypnoea	11	45.83
14	Tachypnoea	6	25.00
15	Paradoxical respiration	15	62.50
16	Abnormal Lungs sound on auscultation	16	66.67
17	Dehydration (mild to moderate)	15	62.50

Hb values in these groups were significantly ($p<0.05$) higher as compared to group D. Pre-treatment values of ESR in group A were significantly ($p<0.01$) higher as compared to other three groups. However, after 10 days of treatment, significant ($p<0.01$) decrease in values of ESR of groups A and C was recorded in comparison to pre-treatment. Group B dogs also showed significant ($p<0.05$) decrease in values of ESR at post 10 days of treatment. Pre-treatment values of TEC in group A were significantly ($p<0.05$) lower as compared to group D whereas other two groups registered non significant decline. However, after 10 days of treatment, significant ($p<0.01$) increase in values of TEC of groups A and C were recorded in comparison to their pre-treatment values. Pre-treatment values of TLC in groups A, B and C were significantly ($p<0.01$) higher as compared to group D. However, after 10 days of treatment, significant ($p<0.01$) decrease in values of TLC of groups A, B and C was recorded in comparison to their pre-treatment values.

Differential Leucocytes Count is presented in Table 6 .Prior-treatment values of neutrophils in groups A, B and C were significantly ($p<0.01$) higher as compared to group D. However, after 10 days of treatment, significant ($p<0.01$) decreased values of neutrophils of groups A, B and C was recorded in comparison to their pre-treatment values which were closed to the control group D. Pre-medication values of lymphocytes in groups A, B and C were significantly ($p<0.01$) lower as compared to group D. However, after 10 days of treatment, significant ($p<0.01$) increase in values of lymphocytes of groups A, B and C was noticed in comparison to their pre-treatment values which were closed to the control group D. Pre-treatment and post-treatment values of monocytes and eosinophil in groups A, B, and C did not show any significant changes as compared to group D. Haemoprotozoan examination in wet blood smear and JSB (Jashwant Singh Bhattacharya) staining of blood smear did not show presence of any haemoprotozoan parasite.

Similar changes in the hematological parameters of patients suffering from respiratory diseases were observed by Nelson and Couto (2003). Authors stated that the complete blood count of patient in cases of respiratory diseases may show the anemia of inflammatory disease, white blood cell response characteristic of an inflammatory process of the lungs, neutrophilic leukocytosis and left shift. Eosinophilia is commonly encountered as a result of hypersensitivity or parasitic diseases involving organs other than lungs.

Amrute *et al.* (2009) observed decreased RBC count and neutrophilia (85%) with shift to left in a case of chronic bronchopneumonia in a Great Dane pups on hematological examination.

Infiltration of neutrophils was reported by Payungporn *et al.* (2008) on histological investigation of pulmonary system of dogs died due to Canine Influenza virus (H3N8) infection in Florida. Neutrophilia with left shift was reported by Kogan *et al.* (2008) in 88 dogs with aspiration pneumonia.

Radhakrishnan *et al.* (2007) studied 65 cases of dogs during the period of 1993-2002, considered to have community acquired pneumonia, where 57% of dogs had evidence of leukocytosis, neutrophilia and band neutrophilia.

Lymphopenia, elevated band neutrophils, segmented neutrophils and monocytosis was observed by Chvala *et al.* (2007) in a dog with pneumonia due to simultaneous Canine Distemper virus, Canine Adeno virus Type 2, and *Mycoplasma cynos* infection.

Leucocytosis has been reported in 10 Beagle dogs having interstitial pneumonia experimentally induced by intravenous administration of Bleomycin

Table 4: Pulse oximetery values of dogs with lower respiratory tract infections.

Groups	Saturation of Haemoglobin with oxygen HbO_2 (%)		Pulse Rate(per min)	
	0 day	10^{th} day	0 day	10^{th} day
A	$86.40^{ap} \pm 1.86$	$96.64^{**} \pm 0.59$	$146.25^{bq} \pm 4.27$	$106.88^{**b} \pm 5.34$
B	$91.47^{bp} \pm 1.47$	$98.23^{**} \pm 0.23$	$138.00^{bq} \pm 6.07$	$103.62^{**b} \pm 5.67$
C	$91.96^{bp} \pm 1.78$	$97.46^{**} \pm 1.12$	$135.25^{bq} \pm 6.22$	$94.37^{**ab} \pm 2.9$
D	$98.10^{cq} \pm 0.32$	98.52 ± 0.18	$89.00^{ap} \pm 2.09$	$85.00^{a} \pm 1.33$

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals.Values with different superscript 'a,b,c,d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

Table 5. Hematological parameters of dogs with lower respiratory tract infections

Groups	Hemoglobin (g/dl)		ESR(mm/hour)		TEC (x 10^6/ µl)		TLC (x 10^3/ µl)	
	0 day	10th day	0 day	10th day	0 day	10th day	0 day	10th day
A	$16.00^{b} \pm 1.05$	$13.76^{b} \pm 0.15$	$7.25^{bq} \pm 1.44$	$1.50^{**} \pm 0.38$	$5.50^{a} \pm 0.38$	$6.60^{**a} \pm 0.15$	$18.02^{bq} \pm 2.95$	$9.07^{**} \pm 0.39$
B	$15.67^{b} \pm 1.58$	$13.30^{b} \pm 0.36$	$3.62^{apq} \pm 1.10$	$0.62^{*} \pm 0.32$	$6.61^{b} \pm 0.44$	$7.25^{b} \pm 0.25$	$17.01^{bq} \pm 1.32$	$9.01^{**} \pm 0.22$
C	$16.57^{b} \pm 1.42$	$13.40^{b} \pm 0.38$	$3.87^{apq} \pm 0.61$	$1.62^{**} \pm 0.37$	$5.85^{b} \pm 0.17$	$6.77^{**ab} \pm 0.11$	$17.87^{bq} \pm 1.45$	$9.60^{**} \pm 0.32$
D	$11.50^{a} \pm 0.46$	$12.12^{a} \pm 0.35$	$1.37^{ap} \pm 0.37$	0.62 ± 0.26	$6.72^{b} \pm 0.12$	$6.65^{a} \pm 0.11$	$8.31^{ap} \pm 0.33$	8.85 ± 0.52

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals. Values with different superscript 'a,b,c,d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

(Fleishman *et al.,* 1971).

Simultaneous reduction and then increase in lymphocytes was observed in the present study which was also of secondary nature due to neutrophilia.

These alterations of blood cellular picture in this study may be due to some inflammatory reactions in the body, irrespective of infectious agents. Finding of these pictures is suggestive of infectious lower respiratory tract diseases which may be due to either bacterial/viral/fungal/verminous infections or primary viral and overwhelming bacterial infections.

On coprological examination of dogs of all groups, revealed presence of *Ancylostoma* spp. eggs in five clinical cases. These cases were further treated with the combination of the anthelmintic drugs (Praziquantal, Pyrantel pamoate and Fenbendazole[4]) along with main therapeutic agent . On post-medication coprological examination, no case revealed presence of any pathogenic parasitic egg and intestinal protozoan cyst.

Soulsby (1982) reported pulmonary damage caused by larvae of *Ancylostoma* spp. due to their migration in lung alveoli during life-cycle. Haemorrhagic pneumonitis is produced by larvae as they leave pulmonary circulation to enter the alveoli. Watson, *et al*. (2006) also reported pneumonia in nine Cavalier King Charles Spaniels caused by *Pneumocystis* spp. Jeanfaivre *et al*. (1996) described a case of woman presented with bilateral pleural effusions caused by *Toxocara canis*.

Thoracic radiography was performed in 24 dogs of all the groups (i. e. groups A, B and C). Radiological examination revealed marked abnormalities in 18 dogs. The X-ray of normal lungs appeared black (negative contrast) in the thoracic cavity, and absence of any bronchial or alveolar pattern. Out of 18 abnormal radiographs, four (22.22%) radiographs showed pulmonary congestion with the presence by engorged pulmonary arteries and veins. Three (16.66%) radiographs showed bronchitis as per bronchial pattern of X-ray, whereas three (16.66%) radiographs showed bronchitis along with severe pulmonary congestion. Five (27.77%) radiographs revealed presence of pneumonia, identified by presence of alveolar pattern of X-ray and three (16.66%) cases revealed consolidation of lungs or pleurisy.

Similar pattern of X-rays were reported by various workers (Clercx *et al.,* 2000; Zeugswetter *et al.,* 2007; Kogan *et al.,* 2008; Amrute *et al.,* 2009). Radiographic abnormalities were classified as interstitial, bronchial, broncho-interstitial, or alveolar (Norris *et al.,* 2001; Norris *et al.,* 2002).

Garcia *et al.* (2001) reported a clinical case of canine mycotic pneumonia in a male Schnauzer dog in which, thoracic radiography revealed a mixed pattern of increased alveolar, interstitial and bronchial densities, compatible with a bronchopneumonia. Drobatz *et al.,*

Table 6: Differential leucocytes count of dogs with lower respiratory tract infections

Groups	Neutrophils(%)		Lymphocytes(%)		Monocytes(%)		Eosinophils (%)	
	0 day	10th day	0 day	10th day	0 day	10th day	0 day	10th day
A	$91.75^{bq} \pm 1.09$	$76.50^{**} \pm 1.28$	$4.75^{ap} \pm 0.79$	$21.00^{**} \pm 1.25$	1.25 ± 0.49	1.37 ± 0.37	2.25 ± 0.90	0.75 ± 0.25
B	$88.87^{bq} \pm 1.51$	$75.25^{**} \pm 1.44$	$6.50^{ap} \pm 0.86$	$22.00^{**} \pm 1.92$	0.87 ± 0.35	1.62 ± 0.18	3.75 ± 1.47	1.12 ± 0.35
C	$89.62^{bq} \pm 1.61$	$77.00^{**} \pm 2.02$	$7.62^{ap} \pm 1.42$	$21.25^{**} \pm 1.75$	0.75 ± 0.25	1.25 ± 0.41	2.00 ± 1.45	0.62 ± 0.41
D	$76.87^{ap} \pm 1.58$	76.87 ± 1.31	$22.25^{bq} \pm 1.31$	21.37 ± 1.34	1.75 ± 0.36	1.12 ± 0.35	1.12 ± 0.35	0.75 ± 0.25

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals. Values with different superscript 'a,b,c,d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

(1999) performed retrospective study of 27 dogs exposed to smoke in residential fires from 1988-1997. Most common thoracic radiographic findings were an alveolar or an interstitial pattern.

Mean ± S.E values of serum glutathione (GSH) concentration of all groups of dogs at 0 and 10th day of observation presented in Table 7. Pre-treatment values of GSH in groups A, B and C were significantly ($p<0.01$) lower as compared to group D. However, 10 days post treatment, significant ($p<0.01$) increase in values of GSH of groups A, B and C were observed in comparison to their pre-treatment values.

The finding of this study on serum glutathione estimation was also supported by various workers (Duthie *et al.,* 1991; Rahman and MacNee, 2000; Drost *et al*., 2005). Kluchova *et al.* (2007) reported the similar findings with glutathione in humans lower respiratory system affections.

Mean ± S.E values of blood pH, partial pressure of Oxygen (pO_2) and partial pressure of carbon dioxide (pCO_2) of all the groups of dogs at 0 and 10th day of observation depicted in Table 8.

Prior medication values of blood pH in groups A, B and C were significantly ($p<0.05$) lower as compared to group D. However, on day10 post treatment, significantly ($p<0.05$) improvement in value of blood pH of groups A, B and C were observed in comparison to their pre-treatment values.

Prior treatment values of pO_2 in groups A, B and C were significantly ($p<0.05$) lower as compared to group D. However, on 10 days post treatment, significantly ($p<0.01$) improvement in values of pO_2 of groups A, B and C were observed in comparison to their pre-treatment values. Significantly ($p<0.05$) lower values of pO_2 in groups B and C was noticed as compared to group D in post 10 days of treatment.

Pre-treatment values of pCO_2 in groups A and B were significantly ($p<0.05$) higher as compared to group D. Significant ($p<0.05$) decrease in values of pCO_2 of groups A and B observed on 10 days post treatment, whereas non significance decline in group C were observed in comparison to their pre-treatment values.

Mean ± S.E values of sodium, potassium, chloride and bicarbonate concentration of all the groups of dogs at 0 and 10th day of observation depicted in Table 9.

Pre-treatment values of sodium (Na^+) in groups A and C were significantly ($p<0.05$) lower as compared to group D. No significant change in the values of sodium was observed even after 10 days post-treatment in all the groups, in comparison to their pre-treatment values.

Pre-treatment values of potassium (K^+) in groups A and C were significantly ($p<0.05$) lower as compared to group D. After, 10 days post treatment, no significant changes in the values of potassium was observed in all the groups, in comparison to their pre-treatment values.

No significant changes in the values of chloride

Table 7: Serum GSH (Glutathione) values of dogs with lower respiratory tract infections

Groups	GSH (mMol/L)	
	0 day	10th day
A	$1.54^{ap} \pm 0.12$	$1.97^{**} \pm 0.05$
B	$1.63^{ap} \pm 0.05$	$2.09^{**} \pm 0.05$
C	$1.57^{ap} \pm 0.10$	$1.98^{**} \pm 0.05$
D	$2.03^{bq} \pm 0.08$	2.07 ± 0.06

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals. Values with different superscript 'a,b,c,d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

Table 8: Arterial blood gas analytes of dogs with lower respiratory tract infections

Groups	Blood pH		pO2(mm Hg)		pCO2(mm Hg)	
	0 day	10th day	0 day	10th day	0 day	10th day
A	7.35 ap± 0.01	7.39 *± 0.01	88.62 ap± 1.91	96.00 **ab± 0.46	45.62 cq± 1.03	38.12 **± 0.61
B	7.38 bpq± 0.001	7.40 *± 0.001	92.12 apq± 0.81	94.6 **a± 0.46	41.75 bp± 1.16	38.25 *± 0.45
C	7.37 abp± 0.001	7.39 *± 0.001	90.75 ap± 1.01	95.00 **a± 0.82	40.75abp± 0.77	38.00 a± 0.86
D	7.40 cq± 0.001	7.40± 0.002	96.50 bq± 0.26	96.87b± 0.35	38.75 ap± 0.75	39.25± 0.75

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals.
Values with different superscript 'a,b,c,d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

Table 9: Blood electrolytes concentration of dogs with lower respiratory tract infections

Groups	Sodium(mMol/L)		Potassium (mMol/L)		Chloride(mMol/L)		Bicarbonate (mEq/L)	
	0 day	10th day	0 day	10th day	0 day	10th day	0 day	10th day
A	145.12a± 3.05	148.20 ab± 1.14	4.07 a±0.23	4.15 a± 0.08	106.62± 3.39	109.88± 1.83	21.66 ap ± 0.39	23.81**± 0.20
B	148.00 ab± 3.00	148.12 ab± 1.44	4.46 ab± 0.57	4.20 a± 0.06	110.88± 2.96	110.75± 1.11	21.45 ap ± 0.58	23.45**± 0.22
C	145.19 a± 1.83	144.70 a± 2.45	4.11 a± 0.12	4.37 a± 0.15	108.02± 1.00	107.74± 2.72	22.01 ap ± 0.36	23.77 ** ± 0.26
D	153.75 b± 1.50	153.25 b± 1.49	5.10 b± 0.11	5.06 b± 0.08	112.25± 2.02	111.88± 1.25	23.94 bq± 0.14	24.07± 0.04

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals.
Values with different superscript 'a,b,c,d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

(Cl^-) in all the groups were noticed when compared among groups as well as pre-treatment and post-treatment values within a group

Pre-treatment values of bicarbonate (HCO_3^-) in groups A, B and C were significantly ($p<0.01$) lower as compared to group D. However on 10 days post treatment, significant ($p<0.01$) increase in values of bicarbonate of groups A, B and C were observed in comparison to their pre-treatment values.

Decrease in partial pressure of oxygen and increase in partial pressure of carbon dioxide during lower respiratory infections was also reported by various workers (Radhakrishnan *et al.,* 2007; Kogan *et al.*, 2008; Syrja *et al.,* 2009). Radhakrishnan *et al.* (2007) in their study on pneumonic dogs had also recorded decreased in blood pH. Haskins (2004) has also emphasized the measurement of partial pressure of oxygen and carbon dioxide in arterial blood specimens to assess pulmonary functions.

Light *et al.* (1981) reported hypoxemia and hypercapnia in dogs with pneumonia. They concluded that hypoxemia in pneumonia was due to both increased shunt and venous admixture in the infected regions, and that local hypoxic vasoconstriction was in most instances ineffective in directing blood flow away from the consolidated lobe. Kluchova *et al.* (2007) had also reported the same findings in humans with COPD.

Mean ± S.E values of tidal volume (TV) and respiration rate (RR) of all the groups of dogs at 0 and 10th day of observation depicted in Table 10.

Prior-treatment values of tidal volume (TV) in groups A, B and C were significantly ($p<0.01$) lower as compared to group D. However on 10 days post treatment, significantly ($p<0.05$) increased values of tidal volume of groups A, B and C were observed in comparison to their pre-treatment values.

Pre-treatment values of respiration rate (RR) in groups A, B and C were significantly ($p<0.01$) higher as compared to group D. However on 10 days post treatment, significantly ($p<0.01$) decreased values of RR of groups A, B and C were observed in comparison to their pre-treatment values. At post 10 days of treatment, significantly ($p<0.05$) higher value of respiration rate in groups A, B and C was observed as compared to group D.

Similar findings of decreased tidal volume and increased respiration rate were also reported by Silverstein (2004).

In the present investigation the causes of hypoxemia and hypercapnia may be probably due to defective oxygenation of blood in affected pulmonary tissue and retention of carbon dioxide in venous system. In the majority of the cases suffering from bronchitis, bronchopneumonia, pneumonia and pleurisy, the extent of pulmonary tissue involvement was moderate to severe in nature, even those cases where having the cardinal signs of respiratory distress and respiratory failure like open mouth breathing/gasping, dyspnoea, cyanosis of mucus membrane, abnormal lungs sound, coughing, paradoxical respiration and moderate to severe febrile reaction. It is therefore, concluded that the diagnosis of lower respiratory system affection can be made by carrying out the detailed clinical examination of respiratory system and conducting of pulmonary function test. In this context, a set series of tests comprises of auscultation, X-ray, Spirometery, Blood gas analyses, Pulse oximetery may be exploited as routine diagnostic tests for the diagnosis of lower respiratory tract infection.

Table 10: Spirometric values of dogs with lower respiratory tract infections

Groups	Tidal Volume(ml)		Respiration Rate(per min)	
	0 day	10th day	0 day	10th day
A	$173.75^{ap} \pm 36.49$	$371.88^{*} \pm 83.24$	$69.87^{dr} \pm 4.53$	$37.00^{**b} \pm 1.69$
B	$156.25^{ap} \pm 36.10$	$303.12^{*} \pm 42.89$	$48.25^{bq} \pm 2.91$	$35.50^{**b} \pm 1.05$
C	$173.75^{ap} \pm 33.48$	$293.12^{*} \pm 41.00$	$59.25^{cqr} \pm 4.40$	$37.12^{**b} \pm 1.79$
D	$403.60^{bq} \pm 19.15$	400.00 ± 18.29	$25.37^{ap} \pm 1.11$	$26.125^{a} \pm 1.15$

Values with superscript '*' ($p<0.05$) and '**' ($p<0.01$) differ significantly when compared in a row at different time intervals. Values with different superscript 'a, b, c, d' ($p<0.05$) and 'p,q,r,s' ($p<0.01$) differ significantly when compared among different groups.

Abnormal breathing pattern, dyspnoea, coughing, tachypnoea, open mouth breathing, cyanotic mucus membrane, anxiety, glazed look, extended head and neck, paradoxical respiration, abnormal lungs sound, high rise of temperature (104-106^{0}F), dehydration and abnormal posture were the important clinical signs suggestive of lower respiratory tract infection. Hematological study revealed significant increase in Hb, ESR, TLC and neutrophils, while significant decrease was found in TEC and lymphocytes counts in affected dogs prior to treatment. Hb, ESR, TEC, TLC, neutrophils and lymphocytes were restored to normalcy after 10 days of treatment.

Among the various diagnostic procedures used in the present study, arterial blood gas analysis proved to be most valuable method of diagnosing lower respiratory tract infections. Blood pH, pO_2, sodium, potassium and bicarbonate were found decreased significantly, while significant increase was found in pCO_2 before institution of treatment in the present study, which was restored to normalcy after treatment. Pulse oximetry also showed normalcy in saturation of hemoglobin with in blood and pulse rate of affected dogs after 10 days of medication. The spirometric study also revealed decreased tidal volume and increased respiration rate in infected dogs which were restored to normalcy after 10 days of medication. Thoracic radiography was also proved to be one of the very effective diagnostic tools in finding out of the actual position and extent of the lesions present in the pulmonary system.

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Isolation and identification of Canine Parvo Virus (CPV-2) from the faecal samples of dogs in Bhubaneswar, India

A.K. Nayak, P. Das, S. Ranabijuli, N. N. Mohanty, K.K. Sardar and H. K. Panda*
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry,
Orissa University of Agriculture and Technology Bhubaneswar – 751003, Odisha

Abstract

The present study was undertaken to detect the presence of canine parvo virus (CPV) in the susceptible population around the city of Bhubaneswar, India. Haemagglutination Inhibition (HI) test was employed for primary screening of the faecal samples which was followed by Fluorescent Antibody Test (FAT) and Polymerase chain reaction (PCR) for confirmatory diagnosis. A total of 24 faecal and serum samples were screened from the dogs suffering from acute vomition and diarrhoea with or without blood. MDBK (Madin Darby Bovine Kidney) cell line was utilized to isolate the virus and PCR of VP1/VP2 gene confirmed the presence of virus in cell culture supernatant. Seroprevalence study of 24 serum samples employing HI test revealed 14 (58.33%) samples to be positive while FAT sensitivity was found to be on the lower side (12.5%).

Keywords: Canine Parvo Virus – 2, Madin Darby Bovine Kidney, Haemagglutination Inhibition, Fluorescent Antibody Test, Polymerase Chain Reaction

Parvo viral hemorrhagic gastroenteritis is one of the highly fatal infectious diseases of dogs caused by two distinct forms of parvo virus namely canine parvo virus-2(CPV-2) which is the pathogenic form and canine parvo virus-1(CPV-1) or the minute virus of canine (MVC). The first report of canine parvovirus enteritis was from USA (Eugster and Nain, 1977), while the identification and documentation of the causative agent was done in Canada by Appel *et al.* (1979). CPV-2 has spread globally to be established as an endemic infection in domestic and wild canids (Parrish *et al*., 1988. Like many other viruses, genetic evolution of CPV-2 has given rise to new antigenic types (Parrish *et al.,* 1991). Shortly after the emergence of CPV-2, two variant strains CPV-2a & CPV-2b have now completely replaced CPV-2 globally (Truyen *et al.,* 1996) and with the emergence of CPV-2c thereafter have compelled researchers for continuous monitoring of the dynamics of virus.

After the first report of the disease in Madras city (Balu *et al*., 1981) incidences of it has been found increasing during recent past in the Indian subcontinent (Sherikar and Paranjape, 1985; Meerarani *et al*., 1996; Phukan *et al*., 2004; Kumar *et al*., 2010). In Odisha Banja *et al*. (2002) reported the presence of the virus by an epizootiological study of canine parvoviral enteritis in and around Bhubaneswar city. Although a large number of unvaccinated canine species vulnerable to the disease suffers with varied degree of severity and gastroenteritis of unidentified cause, no calculated approach was ever made for screening the population for estimating the status of the virus. The above laid facts prompted the present study among dog population in and around the city of Bhubaneswar, Odisha, India to provide an effective and accessible diagnostic assay for the disease detection and implementing timely control and prophylactic measures for preserving the life of our companion canines.

Materials and methods:

A total of 24 faecal and serum samples were collected from dog showing clinical of parvo viral infection. Dogs of Rectal faecal samples were collected and processed as per the method described by Nandi *et al*. (2009) for preparation of antigen for haemagglutination test, fluorescent antibody test and isolation of CPV in the MDBK cell line. Inactivated serum samples (56°C for 30 minutes) from the 24 suspected dogs along with positive (known vaccinated) and negative (known unvaccinated) reference serum were collected.

Isolation of CPV virus

Syringe filtered (milipore, 0.45µm) faecal supernatants subjected to log dilution at 10{ 'MM were infected to MDBK (Madin Darby Bovine Kidney) cells obtained from NSCC (National Centre for Cell Science, Pune, India). Three blind passages were carried out following which three more passages were carried out for the adaptation of the virus in the cell line. Daily observation was carried out at 48h, 72 h, 96h and 120h for development of CPE. For detailed study of microscopic changes in the cells Haematoxylin Eosin

(HE) method of staining was utilized on infected cells grown on Leighton's tube with cover slip (Nandi *et al*., 2009). The Reed and Muench (1938) method was utilized to determine the tissue culture infective dose (TCID… €) of the sixth passage virus in MDBK cell line.

Haemagglutination Inhibition Test

The Haemagglutination test was performed using 1% pig RBC as per the method of Azetaka *et al*. (1981) in 96 well U-bottom micro titre plates to obtained the 4HA unit, which then followed by Haemagglutination inhibition test. A two fold dilution of 50µl of positive reference serum was made, starting from an initial 1:2 dilution in ice cold PBS in the 96 well U-bottom micro titre plate. An equal quantity of 4HA unit antigen was added to all the wells. The micro titre plate was kept in incubator at 37°C for about 1 hour. 50 µl 1% PRBC (Porcine RBC) suspension was added in 50µl to each well. Control wells were included with positive control antigen, positive control serum and a cell control in a separate row of micro titre plate. The plate was incubated at 4°C for 2-4 hours. The result was read after the pig RBC as control was settled completely. Highest dilution of the serum that inhibits the haemagglutination of porcine RBC was considered as the end point titre and was recorded.

Fluorescent Antibody Test (FAT)

Direct fluorescent antibody test was performed for screening of fecal smears and cover slip preparations of infected MDBK cell line (Yang *et al*., 2009).

Molecular characterization of antigen

The PCR amplification of VP1/VP2 (3025 to 3706) gene of purified CPV was done using QIAGEN DNA extraction kit and forward primer (5' GAA GAG TGG TTG TAA ATA ATT 3') and reverse primer (5' CCT ATA TAA CCA AAG TTA GTA C 3') (Imperial Biomedics) as per the method of Pereira *et al*. (2000).

Results

The adaptation of the CPV in MDBK cell line showed characteristic CPE like rounded cells, development of granulation of cells, syncytia formation and lyses of cells at 72 h and 96 h post infection (Fig 1, 2, 3). HE staining of the 72h, 96h infected cells revealed characteristic intranuclear inclusion bodies (Fig. 4). Further identification by HI & FAT confirmed the fact of adaptation of CPV-2 isolates on MDBK cell. This fact was strengthen by PCR assay of all the isolates from the cell culture supernatant, which amplified a part of VP1/VP2 (3025 to 3706) gene of CPV to yield a product of 681 bp (Fig. 5), thereby confirming all the isolates to be CPV-2. The tissue culture infective dose (TCID… €) of the sixth passaged virus in MDBK cell line was found to be $10^{5.6}$/ 0.1 ml.

Sero-prevalence study of 24 serum samples employing HI test revealed 14 (58.33%), samples to be positive. Out of the 14 positive samples in HI test 3 (21.43%), 01 (7.14%), 05 (35.71%), 01(7.14%), 03(21.43%), 01(7.14%) samples showed the HI titre of 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 respectively. Further, the screening of the faecal samples

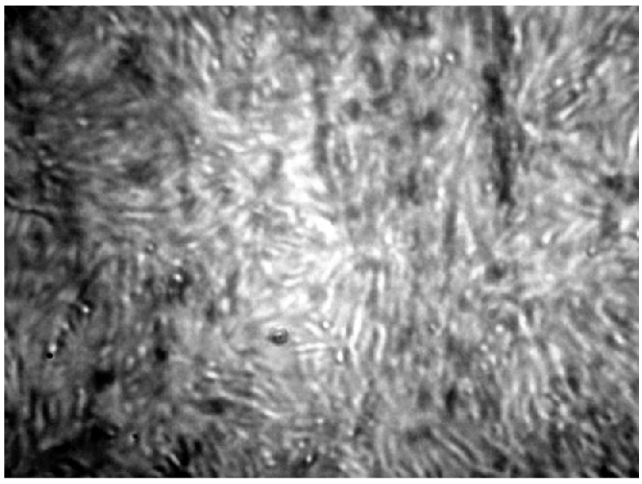

Fig. 1: Control MDBK cell (Unstained) (10X)

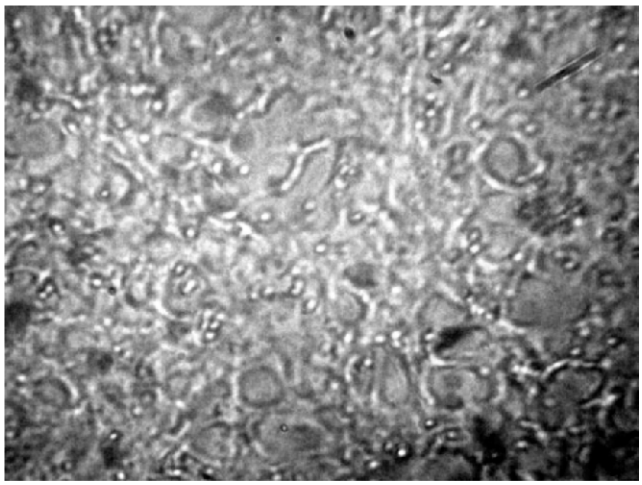

Fig. 2: MDBK cell infected with CPV -2 at 3rd passage level showing CPE (rounding & syncitia) at 72 hours post infection (unstained) (10X)

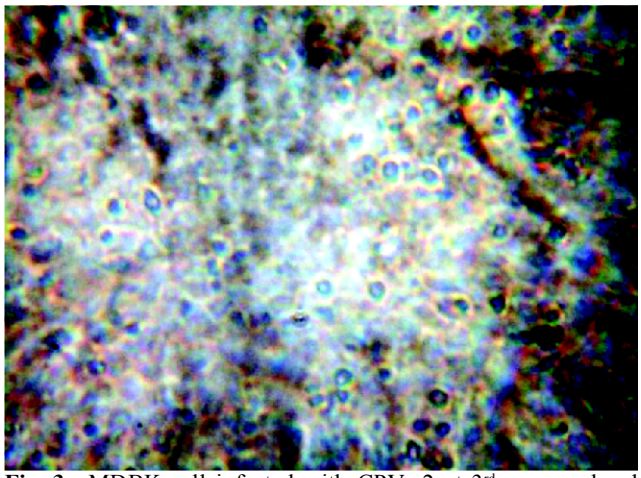

Fig. 3: MDBK cell infected with CPV -2 at 3rd passage level showing intense CPE at 96 hours post infection (unstained) (10X)

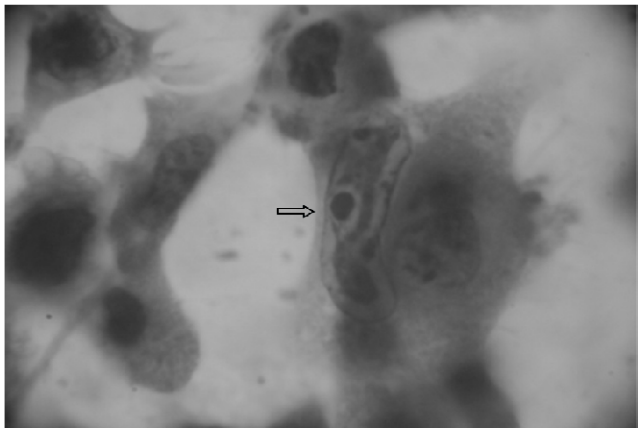

Fig. 4: MDBK cell infected with CPV-2 at third passage level stained with H&E stain showing characteristic intranuclear inclusion bodies at 72 hours post infection (100X)

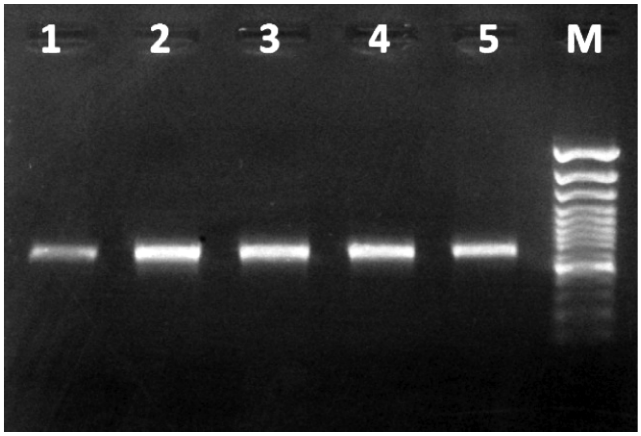

Fig. 5: PCR analysis of CPV -2 showing characteristic band
Lane M: 100 bp molecular size ladder
Lane 1 to 4: 681 bp PCR amplicon of VP 1/ VP 2 region
Lane 5: Positive control (Vaccine virus)

by FAT revealed only 3 numbers (12.5%) to be positive while all the cell culture isolates found positive under the same process.

Discussion

Adaptation of the CPV -2 virus by MDBK cell line and development of characteristic CPE as observed were also reported by Mochizuki *et al.* (1993), Appel *et al.* (1979), Kumar *et al.* (2010) while infecting in MDCK cell line. Although previous isolation of CPV was recorded with MDCK (Madin Darby Canine Kidney), CrFK (Crandell Reese Feline Kidney) and A72 is there (Appel *et al.*, 1979; Spibey *et al*., 2008; Nandi *et al*., 2009; Yang *et al.*, 2010) but use of MDBK cell line is believed to be the first of its kind. Further the similar tyoe of tissue culture infective dose (TCID … € = $10^{5.6}$/ 0.1 ml) of the sixth passage virus in MDBK cell line has been recorded previously by Carman and Povey (1982).

HI test used for preliminary screening of the infection has also been reported by Azetaka *et al.* (1981) and Teramoto *et al*. (1984), Kumar *et al.* (2003) as one of the standard diagnostic aid. The observed titre of HI (1:64 to 2048) is in accordance with many previous findings (Carman & povey, 1984; Ok *et al*., 2000; Yang *et al*., 2010). Fluorescent assay test of the faecal samples showed a lower degree of sensitivity (12.5%) as compared to other diagnostic methods. Use of FAT for diagnosis of the CPV from tongue epithelium and cell culture supernatant was previously suggested by Mc Knight *et al*. (2007) and Yang *et al*. (2009).

The PCR assay of all the isolates from the cell culture supernatant showed the amplification of VP1/ VP2 (3025 to 3706) gene of CPV to yield a product of 681 bp thereby confirming all the isolates to be CPV-2 (Pereira *et al*., 2000).

The present study revealed the circulation of CPV – 2 in the canine population of Bhubaneswar. HI and FAT can be considered as quick diagnostic aids for preliminary screening of the suspected animals which can be further strengthened by isolation of virion by cell culture and PCR for more confirmatory diagnosis.

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Seroprevalence of *Trypanosoma evansi* in cattle of Karnataka

N. R. Sudhakar, A. K. Tewari, B. R. Maharana[1], M. Sobha Rani [2], M. D.Venkatesha [3], V. Sreevatsava[2], S. Ranganatha[2] Ramesh. V. Jagapur[3], Shivani Sahu[4], Karuna Irungbam[5], B. C. Saravanan and H. V. Manjunathachar

Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, Bareilly Uttar Pradesh 243122.

Abstract

Trypanosoma evansi is widely prevalent in bovids in the tropics. Incidence is more common during monsoon coinciding with the increased population of Tabaniid and other haematophagus flies. In bovines, surra manifests as a chronic disease associated with anaemia leading to severe production losses. In absence of pathognomonic signs accurate diagnosis of disease in ruminants is difficult. Though direct microscopic observation of the haemoflagellate in circulation may be the diagnostic gold standard for surra, the method is insensitive and impractical for population prevalence studies. Therefore, serodiagnosis is the preferred approach for predicting the presence of the pathogen in a susceptible livestock population. In the absence of systematic studies on seroprevalence of *T. evansi* in Karnataka the present study was undertaken. A diagnostic micro ELISA, laboratory standardized with whole cell lysate antigen of *T. evansi*, was applied to determine the seroprevalence of surra in cattle in three major agro-climatic zones of Karnataka. A total of 1352 cattle were surveyed during April 2013 to June 2014, out of these, 202 serum samples were found positive for presence of antibodies specific to *T. evansi* with an overall seroprevalence of 14.94%. The result indicates a wide prevalence of surra in cattle of Karnataka.

Key words: Cattle, Karnataka, Seroprevalence, *T. evansi*.

Trypanosoma evansi is the most important haemoprotozan parasite of domestic and wild animals. *T. evansi* has the widest distribution among all the pathogenic animal trypanosomes and is responsible for causing a fatal wasting disease called surra (OIE, 2012). The disease is a severe burden to the already impoverished economy of the developing world due to high morbidity and mortality, decreased milk yield and work efficiency of several infected livestock species. The most significant economic impact of surra in the tropics come from the chronic form of the disease in cattle, where abortion, infertility, reduced milk yield and weight loss affects their yield and drought capabilities (Kurup and Tewari, 2012).

Under field conditions, the disease is diagnosed on the basis of clinical signs which are not sufficiently pathognomonic and generally confused with other chronic wasting diseases, notably helminthosis and malnutrition. Emphasis has recently been laid on the development of more sensitive and specific serological and DNA based tests for the diagnosis of surra in domestic animals including ELISA (Reid and Copeman, 2002; Desquesnes *et al.,* 2009).

Surra is widely prevalent in India among different economically important livestock species (Batra *et al.,* 1994; Pathak *et al.,* 1997; Sinha *et al.,* 2006; Nair *et al.,* 2011; Kundu *et al.,* 2013, Kumar *et al.,* 2013). Occurrence of the disease has been reported from Rajasthan, Haryana, Gujarat, Punjab, Uttar Pradesh and Kerala. Reports on the prevalence of *T. evansi* infection in bovines of Karnataka (Muraleedharan *et al.,* 1991 and 2005: Harish *et al.,* 2006), were based on blood smear examination whereas Krishnappa *et al.* (2006): Malakondaiah *et al.* (2013) conducted a study on seroprevalence of surra in bovines using Passive haemagglutination tests and ELISA respectively. Though the disease has not been documented from some states of India, absence of report does not preclude its occurrence given that the agro-climatic conditions of India favour predisposition of the infection in various important livestock and wild species across the country. The occurrence of disease is comparatively less in Madhya Pradesh, Tamil Nadu and Maharashtra. (Pathak *et al.,* 1993; Kumar *et al.,* 2013; Veer Singh and Tewari, 2012). In the absence of systematic studies on seroprevalence of *T. evansi* in cattle from Karnataka

[1] Assistant.Profesor Dept. of Parasitology,College of Veterinary Science & Animal Husbandry J.A.U., Junagadh, Gujarat 362001.
[2] Institute of Animal health and Veterinary Biologicals, Hebbal, Bangalore, Karnataka 560024.
[3]Chief Veterinary Officer, Polyclinic, Department of Animal Husbandry and Veterinary Sciences, Bagalkot, Karnataka 587101.
[4]Assistant Professor, Dept. of Parasitology, College of Veterinary and Animal Sciences. SVPUAT Meerut 250110.
[5]Scientist, Animal Biochemistry section, I.V.R.I. Izatnagar, Bareilly 243122.

the present study was undertaken.

Materials and Methods

Parasite

Cattle isolate of *T. evansi*, maintained as cryostock, in the laboratory were used in the present study following revival in mice.

Study protocol

Sera samples were collected from cattle during April 2013 to June 2014. A total of 1352 cattle sera samples were collected and tested in the present study. Blood samples were collected from cattle of either sex, reared either in intensive cattle farms or semi-intensive/ free range habitats from five different parts of Karnataka, *viz.* Bagalkot (n = 165), Belgaum (n = 265), Davanagere (n=129), Dharwad (n=166) and central cattle breeding farms at Hesaraghatta, Bangalore (owned by state department, n = 627). The survey areas were spread across three different agro-climatic zones of northern, central and southern parts of Karnataka. Jugular blood samples of cattle, collected under sterile conditions in dry tubes, were allowed to clot for 24 h at 4°C. The separated sera samples were collected and aliquoted in 1.5 ml labeled microtubes and were sent to laboratory maintaining the cold chain to store at -80°C. Previous history of surra in the area as well as details of the animals screened, viz. age, sex and breed, were recorded.

T. evansi whole cell lysate (WCL) antigen

T. evansi whole cell lysate antigen (Te-WCL) was prepared using purified trypanosomes as described earlier (Tewari, 2003). Briefly, disruption of cells was performed using ultrasonic cell disruptor ((Soniprep 150, UK) at 20 KHz using with 4 disruptions of 15 s each with 30 s interval in ice-bath at 20% duty cycle. The sonicated whole cell lysate was centrifuged at 10,000×g for 15min at 4°C and supernatant was collected. The protein concentration of WCL antigen was determined using protocol described by Bradford. (1976) and adjusted to 1mg/ml. The antigen was stored at -80°C till further use.

Reference sera for ELISA

Reference positive and negative sera of bovine trypanosomosis maintained in the Protozoology Laboratory, Division of Parasitology, were used in the study. Reference sera for bovine Babesiosis and Theileriosis, available in the Protozoology Laboratory, IVRI, were used for cross reactive studies.

Enzyme linked immuno sorbent assay

ELISA was performed by slightly modifying the protocol originally standardized by Luckins (1977). Flat bottom polystyrene ELISA plates (Nunc, USA) were coated overnight with 100 µl Te-WCL antigens (concn. 5 µg ml^{-1}), blocked with 5% skim milk powder in PBS and 100 µl of 1: 100 diluted sera samples loaded in duplicate. The plates were incubated at 37°C for 1 h, followed by the addition of the secondary, goat anti-bovine IgG-peroxidase conjugate (Santa Cruz Biotechnology, USA) at a dilution of 1:5,000. The plates were incubated further at 37°C for 60 min, washed and developed with freshly prepared O-phenylenediamine (Amresco, USA). The plates were read at 492 nm by an ELISA reader (Biorad, USA) after stopping the reaction with 50 µl of 3M HCl. The cut-off value for designating a sample sero-positive or to be from a reactor was determined by adding four standard deviations to the mean O.D. values of the known negative sera O.D values.

Results

A total of 202 cattle sera samples out of 1352 samples were tested positive for *T. evansi* specific antibodies giving an overall prevalence of surra in 14.94% cattle of Karnataka which is briefly summarized in Table 1. Highest prevalence of *T. evansi* was observed in Bagalkot district of Karnataka followed by Belgaum, Cattle Breeding Farms at Hesaraghatta, Bangalore and Dharwad and Davanagere.

Trypanosoma evansi infection was more prevalent in cattle during the monsoon season spanning June to September followed by post monsoon months of October to December. The occurrence was least during summer season from March to May (Table 2).

Occurrence of *T. evansi* was recorded most in cattle aged 3 to 6 years followed by 6 to 8 years and 1 to 3 years and the number of seropoitives was lowest in cattle aged more than 8 years. No positive animal below the age of one year could be detected *T. evansi* seropositive (Table 3). No animal below the age of one year could be detected seropositive for *T. evansi*.

Table 1: Overall Seroprevalence of surra in cattle at different parts of Karnataka

Place of collection	No. of sera samples collected.	No. of positive sera	Percentage prevalence (%)
Bagalkot	165	27	16.36
Belgaum	265	43	16.22
Davanagere	129	14	10.85
Dharwad	166	21	12.650
Central and state cattle breeding farms, Hesaraghatta, Bangalore.	627	97	15.470
Total	1352	202	14.94

Table 2: Season-wise prevalence of surra in cattle of Karnataka

Type of season	No. of samples examined	No. of positive samples	Percentage prevalence (%)
Winter season (January- February)	469	67	14.28
Summer season (March- May)	346	35	10.11
Monsoon(June- September)	285	58	20.35
Post monsoon(October-December)	252	42	16.66
Total	1352	202	14.94

Table 3: Age-wise prevalence surra in cattle of Karnataka

Age of the animals	No. of samples examined	No. of positive samples	Percentage prevalence (%)
< 1 year	125	0	0
1-3 years	274	46	16.78
3-6 years	359	67	18.66
6-8 years	307	53	17.26
>8 years	287	36	12.54
Total	1352	202	14.94

Table 4: Sex-wise prevalence of surra in cattle of Karnataka

Sex of the animal	No. of samples examined	No. of positive samples	Percentage
Male	535	47	8.78
Female	817	155	18.97
Total	1352	202	14.94

Seroprevalence of *T. evansi* was more in adult animals than in the young ones.

Female cattle (18.97%) were more prone to *T. evansi* infection than the male animals (8.78%) (Table 4).

Discussion

Trypanosomosis or surra is a huge burden to the agro-based economy of the tropical countries including India. Growth and productivity is directly affected in the infected lot. Delayed reproductive maturity and abortion in infected cattle is common. A state-wise prevalence of the pathogen is one of the major pre-requisites to assess the quantum of economic loss assessment associated with the disease. Direct demonstration of the unicellular haemoflagellate in the blood and tissue fluid is considered as 'gold standard.' The parasitological techniques associated with the demonstration of the parasites in the biological samples, termed as standard Trypanosome detection methods' (STDM) are still used widely, however, the methods largely lacks the diagnostic sensitivity. Serological tests confer the benefit of large scale screening of sera samples and thereby making it feasible to cover a population for prevalence studies. However, a rigorous standardization of the test is prerequisite to achieve the desired level of diagnostic sensitivity.

To achieve the goal of analysis of large number of samples to detect for trypanosomes, an ELISA was laboratory standardized for high throughput assay of sera samples procured from field conditions. The ELISA results revealed more prevalence of *T. evansi* in cattle (14.94%) than what were speculated from Karnataka. The study showed that trypanosomosis was more prevalent in northern parts of Karnataka. The prevalence of disease during the monsoon months was maximum,

which could be well correlated with the increased vector concentration. Though it is difficult to make a correlation of the disease to age and sex, the present study revealed adult animals were more prone to infection in comparison to their younger counterparts and females were more prone to infection as compared to male animals. The findings are comparable to the earlier reports on prevalence of *T. evansi* in cattle of Karnataka and other parts of India. The reasonss female sexual biasness is ascribed to stress factors like lactation and pregnancy which predisposed females more to infection (Mallick and Dwivedi, 1981).

So far as the age-wise prevalence of *T. evansi* infection in bovines is concerned, the adult animals are more prone to infection due to increased demands for production and reproduction including immunological and nutritional status of the animals which play a vital role and could be attributed to the old age susceptibility because of the hypofunction of the immune system (Krishnappa *et al.,* 2002; Roy *et al.* 2004; Muraleedharan *et al.,* 2005).

Previously prevalence of surra was reported in 0.04% to 27.98% of cattle from Karnataka employing different diagnostic parameters, *viz*. clinical symptoms exhibited, response to therapy and laboratory examinations. The different laboratory examination techniques include microscopical examination of Giemsa stained blood smear and application of some serological tools, *viz*. passive haemagglutination test, ELISA and enzyme immuno transfer blot (EITB) analysis (Krishnappa *et al.,* 2002; Muraleedharan *et al.,* 2005; Malakondaiah, 2007).

In the absence of specific pathognomonic sign diagnosis of trypanosomosis in ruminants is extremely difficult.

In the present study, it was concluded that the prevalence of *T. evansi* infections in the Karnataka, Indian subcontinent in different seasons and increase in its prevalence in the monsoon season and reaching its peak in October and November could be due to the Tabanid fly breeding at the highest level and the incidence was lowest in April and May when water resources are dried up and fly breeding reduced to large extent. However, *T. evansi* infection was recorded throughout the year (Jindal *et al.,* 2005). The variations in the prevalence pattern of *T. evansi* infections in bovines could be due to the vector population increase in considerable number during rainy and post-rainy seasons than the winter and summer seasons in a year. Inclement weather such as hot and humid climate in the months of monsoon and thereafter has also been incriminated to depress the body defense mechanism thereby resulting in the exacerbation of *T. evansi* infection in bovines.

The present findings of ours are in agreement with that of previous studies on the prevalence of surra with little variation which could be due to the stage of the infection while collecting the blood because parasites could be observed in peripheral circulation only in acute infection (Gill, 1991) and the district-wise variation could be due to variation in the exposure to the vector during grazing and geographical locations like forest area and rainfall and nutritional status and susceptible host population. On the other hand, the present study revealed much higher percentage of infection in cattle which may due to random sampling and larger sampling size the geoclimatic factors in the south, north and central zone of the state could also be one of the important factors.

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Short Communication

Comparative efficacy of antibacterial in subclinical mastitis

Brejesh Singh, M.L.V. Rao, D. K. Gupta and P. C. Shukla
Department of Veterinary Medicine
College of Veterinary Science and Animal Husbandry Rewa – 486001, M.P., India

Abstract

The study was carried out to compare the efficacy of three antibacterial agents administered through intramuscular and intramammary route for treatment of subclinical mastitis (SCM) in lactating cows. Thirty six cows with SCM were randomly divided into three groups with twelve animals each and administered Ceftriaxone, Ceftizoxime and Amoxicilin plus Sulbactum respectively. Each group was further divided into two subgroups with six animals each. In first group, Ceftriaxone was given by intramuscular and intramammary route respectively to its subgroups and the same routes was followed in group second and third for Ceftizoxime and Amoxicillin plus Sulbactum. The efficacy was assessed by clinical examination of animals and different laboratory tests (i.e., pH, Electrical Conductivity and Somatic Cell Count). It was concluded that intramuscular route was superior to the intramammary route in the treatment of SCM.

Keywords: Subclinical Mastitis (SCM), Intramuscular, Intramammary.

Subclinical mastitis in cows is characterized not only by presence of pathogens but also by presence of biochemical changes in the milk. It causes greater economic losses than clinical mastitis (Joshi and Gokhale, 2006). Treatment of subclinical mastitis on early diagnosis may reduce these losses. Once the disease develops clinically, it becomes difficult to be cured properly. This work compares the therapeutic efficacy of three antibiotics i.e. Ceftriaxone, Ceftizoxime and Amoxicillin plus Sulbactum using intramuscular and intramammary routes in subclinical bovine mastitis.

Materials and Methods

On the basis of Modified California Mastitis Test (MCMT), pH, electrical conductivity and somatic cell count, 36 cows with subclinical mastitis were selected. These cows were divided into three equal groups of 12 cows, each group consisting of two subgroups of six cows. Six apparently healthy cows were taken as control group.

Antibacterial Ceftriaxone was given in subgroup R-1 @ 5mg/Kg body weight intramuscularly for 5 days and in subgroup R-2 @ 250mg/quarter intramammary for 5 days. In subgroup 3 and 4 Ceftizoxime was given@ 5mg/kg body weight single shot and @ 250mg/quarter intramammary single shot respectively. In subgroups 5 and 6 Amoxicillin plus Sulbactum were respectively given @ 12mg/Kg body weight intramuscularly for 5 days and @ 300 mg/ quarter intramammary for 5 days. The data obtained from clinical trial was statistically analysed using RBD and student 't' test (Snedecor and Cochran, 1994).

Results and Discussion

In respect of findings of electrical conductivity (EC), on comparison with the healthy control value i.e., 4.72±0.06. It was found that in group I, subgroup R-1(intramuscular), the pre treatment value was 6.63±0.06 while the post treatment value was noticed to be 4.73±0.11 which showed drastic and greatly significant. On the contrary in the subgroup R-2, no significant changes were noticed. Similar observation were recorded in subgroup R-3 and R-4.

As has been depicted in table-1, it is evident that in group-1, subgroup R-1 (Intramuscular) the pre treatment somatic cell count (SCC) value was 14.83±1.16 and the post treatment value were noticed to be 4.33±0.42 which show significant changes as compared to control value i.e. 4.16±0.47 cell/ml whereas in subgroup R-2 (intramammary) very little change i.e. from 14.50±1.69 to 11.80±1.45 was observed.

We also analyzed the same in group II and found that no significant changes were noticed in the values of pre and post treatment neither in R-3 (intramuscular) (14.33±1.69 to 11.80±1.45) nor in R-4 (intramammary) (14.66±1.36 to 13.33±0.92) when compared to the control. But in group III, subgroup R-5 (intramuscular) significant changes were observed in the values which changes from 13.66±1.38 to 4.16±0.47, whereas in subgroup R-6 (intramammary) very little changes that i.e. from 14.16±1.64 to 11.50±1.28 was recorded.

In different groups percent wise animal cure rate and quarter cure rate were 100% & 100% (Ceftriaxone, intramuscularly), 33.33% and 65%

Table: Biochemical alteration in milk pre and post treatment of antibacterial agents.

S. No.	Parameters	Healthy control (n=6)	Group- I (Ceftriaxone)				Group- II (Ceftizoxime)				Group- III (Amoxicillin + Sulbactum)			
			R-1 (Intramuscular) (n=6)		R- 2 (Intramammary) (n=6)		R-3 (Intramuscular) (n=6)		R- 4 (Intramammary) (n=6)		R-5 (Intramuscular) (n=6)		R- 6 (Intramammary) (n=6)	
			Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment	Pre treatment	Post treatment
1.	pH	6.42±0.02	7.58±0.05	6.26±0.01**	7.34±0.08	7.10±0.66	6.96±0.03	6.82±0.02	6.98±0.05	6.63±0.11	7.02±0.06	6.42±0.01**	7.22±0.11	6.98±0.17
2.	Electrical conductivity (ms/cm)	4.72±0.06	6.63±0.06	4.73±0.11**	6.43±0.09	6.03±0.11	6.50±0.09	6.16±0.15	6.58±0.12	6.14±0.36	6.53±0.08	4.66±0.05**	6.51±0.08	6.41±0.13
3.	Somatic cell count (10^3cells/ml)	4.16±0.47	14.83±1.16	4.33±0.42**	14.50±1.69	11.80±1.45	14.33±0.55	12.16±0.47	14.66±1.36	13.33±0.92	13.66±1.38	4.16±0.47**	14.16±1.64	11.50±1.28

** -Differ significantly ($P < 0.05$)

(Ceftriaxone, intramammary), 16.66% and 47.05% (Ceftizoxime, intramuscularly), 16.66% and 47.05% (Ceftizoxime, intramammary), 83.33% and 94.73% (Amoxicillin + Sulbactum, intramuscularly) and 50% and 63.15% (Amoxicillin + Sulbactum, intramammary) respectively.

The highest therapeutic efficacy after intramuscular administration was seen with Ceftriaxone (100% and 100%) followed by Amoxicillin + Sulbactum (83.33% and 94.73%) and Ceftizoxime (16.66% and 47.05%) when animal cure rate and quarter cure rate was taken into consideration, respectively.

The study showed better therapeutic efficacy of all the drugs through intramuscular route over the intramammary route. Similar observation was also reported by Ekman and Astrom (1994). An uneven distribution of many substances throughout the udder (Ehinger and Kietzmann, 2000) and risk for contamination increases when infusing the product via the teat canal (Erskine, 2003) and possible irritation of the mammary tissue caused by the preparation. Earlier *in vitro* studies showed that antimicrobials may disturb phagocytosis when given intramammary (Nickerson and Pappe, 1986).

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Efficacy of area specific mineral mixture on production performance in cows

S.P. Waghmare, D.B. Sarode, A.Y. Kolte, N.P. Dakshinkar, V. P. Pathak, and S.G. Mode
Department of Veterinary Clinical Medicine
Post Graduate Institute of Veterinary and Animal Sciences, Akola -444104, Maharashtra.

Abstract

The efficacy of formulated area specific mineral mixture was carried out in lactating cows of saline affected area of Purna river valley of district Akola showing reduced milk yield. Total 49 lactating cows of 2 to 3rd lactation and in 2-4 month of lactation period were selected and divided into two groups. One group (21 cows) was kept as a control without supplementation of mineral mixture (Group I) and second group (28 cows) was supplemented with formulated mineral mixture @ 25 g / animal / day along with normal diet for 60 days (Group II). The study revealed improvement in milk yield on day 60 (4.97 l) of post supplementation and recorded 26.04 % increase in milk yield on day 60 in mineral supplemented group (Group II), whereas further decrease in milk yield was recorded (3.76 %) on day 60 in control group. In Group II significant improvement in level of Ca, Zn, Fe, Mn and apparent improvement in level of serum P, Cu and Mg level was observed in lactating cows on day 60 of treatment than control group. Supplementation of formulated mineral mixture improved the milk yield and serum mineral profile, thus proved to be effective in lactating cows to compensate the requirement for achieving milk production.

Keywords: Dairy cow, Mineral mixture, Milk yield, Mineral profile

Minerals play major role in health, production and reproductive potential of dairy animals. Because of the intrinsic involvement of the micro nutrients in various physiological process, imbalance of these nutrients impairs performance of animals. Dairy animals are more prone to mineral imbalance due to lactation. The deficiency of these minerals occurs either due to improper supplementation or due to feeding of pasture raised on mineral deficient soil (Waghmare, 2009). In most cases it differs from one region to another due to different soil composition, intensity of cropping, precipitation pattern, salinity of soil and soil erosion pattern etc. Most practical and cost effective method of supplementing the deficient minerals is through strategic measures using area specific mineral mixture by assessing the mineral status in soil, feeds / fodder and in serum of animals in different agro-climatic zones. In the present investigation the efficacy of formulated area specific mineral mixture was assessed on the basis of improvement in milk production and serum micro-macro mineral profile in dairy cow herd maintained in saline tract area of Purna river valley of Akola district.

A total of 49 lactating cows of 2 to 3rd lactation from saline affected area of Akola district showing reduced milk yield were selected and divided into two groups. One group (Group I) of 21 lactating cows was kept as a control without supplementation of mineral mixture. Second group (Group II) of 28 lactating cows was supplemented with formulated mineral mixture @ 25 g / animal / day along with normal diet for 60 days.

The milk yield was recorded on '0' day (before supplementation) and on 15, 30 and 60 day post supplementation. The serum macro and micro minerals concentration were estimated on '0' day and on 60th day post supplementation. Macro minerals viz. Calcium (OCPC method), Phosphorus (UV-molybdate method) and Magnesium were estimated by using Autospan diagnostic kit (SPAN diagnostic Ltd, Surat) on Serum Autoanalyser (Autochem- 2011). Micro-minerals viz., Cu, Zn, Fe and Mn were estimated on Atomic Absorption Spectrophotometer (AAS) model 1202 (Varian) after digesting the samples.

The mineral mixture was formulated as per the interpretation of data of mineral content of feed/fodder and serum and requirements of the animal maintained in saline tract area of Akola district (Waghmare, 2009) as per the guidelines by Gowda *et al,* (2004). The composition of formulated mineral mixture was Di-calcium phosphate 40 parts, Calcium carbonate 36 parts, Di-ammonium phosphate 10 parts, Copper sulfate 1.7 parts, Zinc oxide 2.0 parts, Manganese sulfate 2.0 parts and Common salt (base) 8.3 parts. The formulated mineral mixture contained Ca: 20-21%, P: 9-9.5%, Cu: 0.41%, Zn: 1.4% and Mn: 1.6%.

Data were analyzed statistically by factorial completely randomized design (FCRD) and completely randomized design (CRD) as per the method described by Snedecor and Cochran (1994).

The average milk yield (l) recorded was 3.88 ±

0.22 and 3.67 ± 0.21 in group I and group II on '0' day of treatment, respectively. The lower milk yield in deficient animals observed on day '0' might be due to imbalance of minerals and disturbed metabolic pathway (Underwood and Suttel, 1999). As such no improvement was observed in milk yield on different intervals in respect of group I, which had received no mineral supplementation. However, in group II (mineral supplemented) the average milk yield showed increasing trend at different intervals and there was improvement in milk yield (26.04 %) on day 60 (4.973) of post supplementation, whereas decrease in milk yield (3.76 %) was recorded on day of 60 in control group (group I). The above findings revealed that the animals received formulated mineral mixture comprised of various minerals had shown an improvement in milk yield and indicated effectiveness of formulated mineral mixture in restoring milk production. This could be achieved by supplementation of formulated mineral mixture containing adequate calcium and phosphorus source to lactating cows of saline affected area of Purna valley of Akola district. These findings are in agreement with Samanta *et al.* (1995), who also reported increase in milk yield in mineral deficient cattle supplemented with formulated mineral mixture.

Mean serum Ca, P, Mg, Cu, Zn, Fe and Mn level in lactating cows before supplementation of mineral mixture ('0' day) were 8.89 ± 0.61, 4.18 ± 0.16, 2.40 ± 0.11, 0.82 ± 0.26, 0.67 ± 0.06, 1.76 ± 0.1 and 0.38 ± 0.01, respectively, indicating moderately low level of serum Ca, P, Cu, Zn, and Mn in lactating cows. The mean serum Ca level improved significantly on day 60 post supplementation as compared to control group, whereas serum Mg and P level improved apparently on day 60 post supplementation in mineral supplemented group (Group II) as compared to non supplemented group (Group I). The improvement in the level of these minerals might be due to supplementation of formulated mineral mixture containing source of these minerals received during lactation phase of cows. Similar improvement in the level of serum macro minerals were also reported by Hussain Kafil (2006) after supplementation of mineral mixture during lactation in cows. Significant improvement in level of serum Zn, Fe, Mn was observed in lactating cows on day 60 of treatment as against group I, where no significant alteration was noticed in the level of micro mineral profile. The improvement in level of serum Cu, Zn, Fe and Mn could be due to the effect of supplementation of mineral mixture which has brought the spectacular result to compensate the inadequacy of these minerals in the animal body. Similar findings were also reported by Hussain Kafil (2006) on supplementation of mineral mixture to lactating cows.

The overall study concluded that the specially formulated area specific mineral mixture for lactating cows of Purna river valley of saline tract area of Akola district improved milk yield and serum macro and micro mineral profile. Thus, proved to be better in lactating animals to compensate the requirement for achieving milk production and may be recommended for lactating cows of saline affected region to meet the requirement for optimum milk production.

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Haematological changes in dogs suffering from babesiosis

Raihan Alam, A. Phukan, D. Kalita, B. C. Baishya and P. R. Dutta
Department of Clinical Medicine, Ethics and Jurisprudence
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam

Abstract

An experiment was carried out to study the haemato-biochemical changes in babesia infected dogs treated with Diminazine aceturate, Clindamycin phosphate and Imidocarb dipropionate. Supportive therapy to all the affected dogs were rendered with chlorpheniramine meleate,5% dextrose normal saline, dexamethasone sodium,ranitidine hydrochloride, multivitamin and iron dextran inj. Haematological and biochemical values from corresponding groups during pre-treatment and post-treatment days were estimated as per the standard methods. There was increased in the values of Hb (g %), PCV (%), TEC (10^6/cumm) and lymphocyte in the treated groups between "0" and "7th" day of treatment. The TLC and neutrophil declined between "0" and "7th" day of treatment. However, lymphocytosis persisted after 7th post treatment day. The level of eosinophil, monocyte and basophil count did not show significant variation.

Keywords: Babesiosis, Dog, Haematological changes.

Canine babesiosis is a tick borne haemoprotozoan disease caused by parasites of the genus Babesia. Determination of haemato-biochemical changes before and after treatment with different antibabesial drugs is important to determine the efficacy of treatment. The present study was undertaken to evaluate the effect of various antibabesial treatments on haemato-biochemical values in dog suffering from babesiosis.

Materials and Methods

Sixty six dogs diagnosed positive for babesiosis were equally divided into 3groups irrespective of age, sex and breed. Positivity to babesiosis was detected by examining the Giemsa stained blood smear as per the method described by Thompson and Hunt (1966). In group A, B and C, Diminazine aceturate @5mg/kg b.wt. i.m. -two doses at 48 hours interval, Clindamycin phosphate @ 25 mg/kg body weight i.m. twice daily for 5 days and Imidocarb dipropionate @ 5mg/kg body weight i.m. single dose were used, respectively. Supportive treatment was given simultaneously to all the animals consisting of- pheniramine maleate(Avil-Hoechst Pharma.,Mumbai) @1ml/dog i.m., dextrose normal saline @10-15ml/kg body weight i.v., dexamethasone sodium @0.5-2mg/kg body weight i.m., ranitidine hydrochloride @0.5mg/kg body weight i.m., multivitamin @1ml/dog i.m. and iron dextran @1ml/dog i.m.. The supportive treatment was repeated at every 24hours intervals up to 3days.

Blood sample (2ml) from each affected dog was collected for 4 occasions –on 0th, 3rd ,5th and 7th day post treatment by aseptic puncturing of either cephalic or saphenous vein. About 2 ml of blood was taken to a vial containing 2 drops of 10% EDTA for haematological examination. Haematological examination was done as per the method of Schalm *et al*. (1975).

Statistical analyses of the experimental data were carried out by using the methods described by Snedecor and Cochran (1994).

Results and Discussion

Changes in haematological and biochemical parameters of babesia infected dogs before and after treatment are presented in Table 1.

There was decreased values of haemoglobin (Hb), PCV, TEC and lymphocyte in Group-A, B and C in babesia infected dogs and increased between "0" and "7th" day of treatment. Carlos *et al*. (1989) recorded similar results. The probable cause of decreased in Hb, PCV and TEC might be due to intravascular haemolysis and a profound hypotension which resulted from stimulation of production of Kallikrein and vasoactive amines.

The total leucocytic count in the present study declined gradually in Group-A, B and C between "0" and "7th" day of treatment. The present finding was in agreement with Kalra and Singh (1984) who also had made similar observation.

There was significant increased of neutrophil values before treatment and decreased in between "0" and "7th" day of treatment. However, lymphocytosis

Table 1: Pre and post treatment haematological values of babesia infected dogs

Parameters	Group	'0' day	3rd day	5th day	7th day
Haemoglobin (g %)	Gr-A	8.46_{B}±0.30	8.86_{A}±0.31	9.00_{A}±0.30	9.23_{A}±0.27
	Gr-B	8.59_{B}±0.32	8.78_{A}±0.34	8.83_{A}±0.35	9.20_{A}±0.30
	Gr-C	8.50_{ABC}±0.21	8.70_{AB}±0.22	8.80_{A}±0.23	9.25_{A}±0.22
PCV (%)	Gr-A	32.36_{ABC}±0.82	32.86_{AB}±0.48	33.5_{A}±0.48	34.22_{A}±0.34
	Gr-B	31.36_{D}±0.56	32.54_{C}±0.55	33.5_{B}±0.59	34.04_{A}±0.29
	Gr-C	32.77_{ABC}±0.86	32.95_{AB}±0.49	33.59_{A}±0.45	34.22_{A}±0.39
TEC(million/mm^3)	Gr-A	3.38^{a}_{D}±0.09	3.52^{a}_{C}±0.09	3.70^{ab}_{B}±0.07	4.16^{a}_{A}±0.09
	Gr-B	3.42^{b}_{D}±0.11	3.60^{b}_{C}±0.10	3.82^{b}_{B}±0.08	4.12^{a}_{A}±0.07
	Gr-C	3.69^{b}_{C} ±0.12	3.75^{b}_{C}±0.12	3.95^{b}_{B}±0.10	4.20^{a}_{A}±0.09
TLC	Gr-A	7.31^{a}_{A}±0.15	7.29^{a}_{A}±0.13	6.97^{a}_{B}±0.12	6.73^{a}_{C}±0.09
	Gr-B	7.6^{b}_{A}±0.16	7.56^{b}_{A}±0.15	7.28^{a}_{C}±0.12	7.02^{b}_{D}±0.12
	Gr-C	7.38^{ab}_{A}±0.15	7.43^{ab}_{A}±0.15	7.06^{b}_{B}±0.11	7.05^{b}_{BC}±0.11
Neutrophil(%)	Gr-A	63.59^{a}_{AD}±0.39	62.95^{a}_{A}±0.24	62.00^{a}_{B}±0.39	61.00^{a}_{C}±0.28
	Gr-B	63.86^{a}_{A}±0.38	63.00^{b}_{B}±0.31	62.18^{a}_{C}±0.37	$62.00C^{b}_{D}$±0.35
	Gr-C	63.36^{ab}_{B}±0.41	62.95^{a}_{A}±0.34	$61.50A^{b}_{B}$±0.34	61.41^{c}_{BC}±0.31
Lymphocyte (%)	Gr-A	32.5^{a}_{A}±0.44	32.86^{a}_{A}±0.33	33.50^{a}_{B}±0.40	34.45^{a}_{C}±0.26
	Gr-B	31.63^{b}_{A}±0.41	32.82^{a}_{B}±0.39	33.32^{a}_{C}±0.34	33.54^{b}_{CD}±0.27
	Gr-C	32.41^{a}_{A}±0.44	32.5^{a}_{A}±0.37	33.95^{b}_{B}±0.37	33.82^{b}_{B}±0.33
Monocyte(%)	Gr-A	1.18^{a}±0.12	1.04^{a}±0.14	1.04^{a}±0.10	1.18^{a}±0.10
	Gr-B	1.32^{a}±0.16	1.23^{b}±0.16	1.36^{b}±0.13	1.27^{a}±0.16
	Gr-C	1.32^{a}±0.16	1.45^{c}±0.13	1.22^{ab}±0.11	1.36^{ab}±0.12
Eosinophil(%)	Gr-A	2.36± 0.14	2.27±0.16	2.54±0.16	2.64±0.18
	Gr-B	2.50±0.19	2.68±0.16	2.41±0.18	2.73±0.20
	Gr-C	2.59±0.17	2.50±0.17	2.50±0.17	2.77±0.14
Basophil(%)	Gr-A	0.73±0.15	1.00±0.13	0.77±0.15	0.73±0.09
	Gr-B	0.86±0.16	0.73±0.15	0.73±0.15	0.45±0.13
	Gr-C	0.59±0.14	0.59±0.14	0.68±0.14	0.64±0.12

Group A- treated with Diminazine aceturate;Group B- treated with Clindamycin phosphate;Group C- treated with Imidocarb dipropionate. Figures bearing same superscript in a column do not differ significantly. The effect of treatment between groups, days and interaction between day and treatment were statistically significant (P <0.05).

persisted after 7th post treatment day. The level of eosinophil, monocyte and basophil count did not show significant variation. Kalra and Singh (1984) recorded neutrophilia and lymphocytosis in canine babesiosis. The cause of neutrophilia might be due to inflammatory response in the reticuloendothelial system, particularly in liver and kidney. The cause of lymphocytosis may be due to chronicity of canine babesiosis. There might be active lymphopoiesis. The lymphoid organ and bone marrow are stimulated and resulted in increase number of lymphocytes in blood (Schalm *et al.,* 1986).

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Therapeutic management of Amphistome infection in bovine

Ranveer Kumar Sinha, Bibha Kumari and Bipin Kumar*
Department of Veterinary Medicine
Bihar Veterinary College, Bihar Agricultural University
Sabour, Bhagalpur-813210, Patna -800014, Bihar

Abstract

Seventy two bovines of either sex suffering from amphistomiasis were randomly selected for oxyclozanide combination study. Animals were divided into three treatment group of 20 animals each (10 cattle and 10 buffaloes) and a control group of 12 animals (6 cattle and 6 buffaloes). Oxyclozanide, oxyclozanide + tetramisole (1:1) and oxyclozanide + albendazole (1:0.5) were administred once orally @ 10, 20 and 15mg/kg.b.wt. to the animals of group I, II, and III respectively. The EPG was carried out on pretreatment (o day),6 and 12th day of post treatment of all treatment group including control group. The percent efficacy was estimated to be 81.02, 84.91 and 92.01 in cattle and 82.52, 86.06 and 92.74 in buffalo of group I, II and III respectively on the basis of reduction in EPG on 6th day of post medication. The percent efficacy was estimated to be 90.07, 93.00 and 99.61 in cattle and 92.03, 94.00 and 99.42 in buffalo of group I, II and III respectively on the basis of reduction in EPG on 12th day of post medication. So, oxyclozanide + albendazole combination @ 15mg/kg.b.wt. is recommended as the drug of choice in amphistomiasis in bovine.

Key word: Albendazole, EPG, Oxyclozanide, Tetramisole

Anthelmintic actions of oxyclozanide have been tried and reported against amphistomosis in bovine with variable results (Galdhar and Roy, 2004). Tetramisole is presently not a wide choice of drug against fluke infection in cattle whereas albendazole alone has low flukicidal effect (Das *et al* . 1990). The present day attitude to chemical control of parasite to adopt technologies which maximize drug efficacy and avoid development of resistance on repeated exposure. In this context combination of anthelmintic compounds may contribute for prolong effectiveness against flukes. Keeping this fact in view, the present study was chalked out to compare the efficacy of combination compounds oxyclozanide, oxyclozanide + tetramisole and oxyclozanide + albendazole against amphistome infection in bovine.

Materials and Methods

Seventy two (72) bovine of either sex suffering from amphistomiasis were randomly divided into three treatment groups (I, II & III) of 20 animals each (10 cattle and 10 buffaloes). While fourth group consisted of six cattle and six buffaloes were kept as infected untreated control for comparision of EPG with different treatment groups. Oxyclozanide, oxyclozanide + tetramisole (1:1) and oxyclozanide + albendazole (1:0.5) were administered once orally @ 10, 20 and 15 mg/kg. b.wt to the animals of group I, II and III respectively. The EPG was carried out by stoll's dilution method (Soulsby, 1982) on pretreatment (0 day), 6 and 12th days of post treatment. EPG of infected untreated control group was also calculated on similar days of pre and post treatment.

The efficacy of the different drug combinations were evaluated on the basis of percent reduction in faecal egg count (EPG) and was calculated by using the formula

$$\text{Efficacy (\%)} = \frac{\text{Pre-treatment mean EPG} - \text{Post treatment mean EPG}}{\text{Pre treatment mean EPG}} \times 100$$

Results and Discussion

The mean EPG's of different treatments and control group at various days of interval along with percent mortality rates are depicted in Table.

In group I, the percentage efficacy on 12th day post treatment was found to be 90.07 and 92.03 in cattle and buffalo respectively. The high percent reduction in EPG count might be due to high flukicidal activity of oxyclozanide, as it interferes with the phosphorylation process (i.e. generation of ATP) of parasite by interruption in electron transport associated events. Following absorption, oxyclozanide reaches the highest concentration in liver and kidney and mainly excreted

*S.M.S.(Veterinary Science), K.V.K., Arwal, Bihar

Table 1: EPG counts in faeces of cattle and buffalo with amphistomiasis in different Therapeutic groups

Group	Drug (mg/kg. b.wt orally)	Species	EPG and % efficacy at various days interval				
			0 Day (Pretreatment)	6th day	% Efficacy	12th day	% Efficacy
Control (Infected untreated)		Cattle	627.33 ± 10.25	687.66^{a} ± 8.41	—	708.33^{a} ± 14.24	—
		Buffalo	550.83 ± 11.65	600.50^{a} ± 22.80	—	632.66^{a} ± 8.91	—
I	Oxyclozanide (10)	Cattle	643.60 ± 10.29	122.1^{b} ± 3.95	81.02	63.90^{b}± 2.29	90.07
		Buffalo	537.30 ± 8.97	93.9^{b} ± 2.50	82.52	42.8^{b}± 1.16	92.03
II	Oxyclozanide (10)	Cattle	636.20 ± 9.57	96.0^{c} ± 2.14	84.91	44.5^{c} ± 1.25	93.00
	+Tetramisole (10)	Buffalo	528.20 ± 5.93	73.6^{b} ± 1.80	86.06	31.7^{b} ± 1.11	94.00
III	Oxyclozanide (10)	Cattle	652.40 ± 11.39	52.10^{d} ± 1.36	92.01	2.50^{d} ± 0.30	99.61
	+ Albendazole (5)	Buffalo	525.10 ± 6.57	38.10^{c}± 2.10	92.74	3.0^{c} ± 0.51	99.42

Note : Mean with different superscripts (column wise) differ significantly (P<0.01)

through bile, is the possible cause of high flukicidal activity (Booth and Mc Donald, 1982). The present results are also in agreement with the reports of Keyyu *et al.* (2008). The efficacy on 12th day post treatment in group II was found to be 93 and 94 % in cattle and buffalo respectively. The higher efficacy might be due to the added action of tetramisole included in the preparation. Bharti (2000) also reported very high efficacy of oxyclozanide + tetramisole against amphistomiasis. The efficacy on 12th day post treatment in group III was found to be maximum i.e. 99.61 and 99.42 % in cattle and buffalo respectively. The highest efficacy of this combination (oxyclozanide + albendazole) might be due to additive flukicidal effect as both works against flukes. Albendazole has a wide range and it acts on nematode, cestode and trematodes (both adult and larval stage). In the body, albendazole rapidly metabolized to sulphone and sulphoxide which kill the flukes (Brander *et al.*, 1991). However, albendazole alone have low flukicidal effect as reported by Keyyu *et al.* (2008) but reports on this combination is seen to be scanty.

Mild signs of inappetance and diarrhoea was seen in two animal in oxyclozanide treated group and one in oxyclozanide + tetramisole treated group, otherwise no unusual clinical symptom was exhibited by animals of all treatment group upto 12th day post treatment. Increase in milk yield as well as appetite were marked in almost all treated animals after 12th day, post treatment.

As per the analysis of results, of present trial, oxyclozanide + albendazole combination was the most efficacious combination against natural amphistomiasis followed by oxyclozanide + tetramisole and then oxyclozanide alone in bovines.

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Clinical propaedeutics of renal failure in dogs

Ramakant, Mukesh Srivastva and H.P. Lal
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry
Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwa Vidyalaya Evam Go Anusandhan Sansthan
Mathura -281001, U.P.

Abstract

The present study was conducted to record the clinical signs, associated with renal failure irrespective of staging of disease in 24 dogs, presented to Teaching Veterinary Clinical Complex, DUVASU, Mathura. The most consisting clinical findings was vomiting, which was observed in 87.5% cases along with anorexia in 75%, melena in 70.83%, oliguria in 66.66% and dehydration in 50% of clinical cases. Other less common clinical findings were hypothermia in 45.83% and dyspnoea and uremic breath in 41.66% of dogs. Least common findings were ulceration on the tongue and gum (uremic stomatitis), hematemesis, arterial hypertension and profound depression with progressive weight loss in 33.33% of cases followed by anemia, paleness of mucous membrane, epistaxis and polydipsia in 25% of dogs with renal failure irrespective of staging of disease.

Key words: Clinical propaedeutics, Dogs, Renal failure

Renal failure is a common clinical problem occurring in 2-5 % of dogs (Lund *et al.,* 1999), third leading cause of death in dogs. Clinical signs only appear, when at least 75% or more of total nephron becomes non functional (Di Bartola, 2005). Robertson (2001) reported that by the age of five years, nearly 60 % of dogs show renal lesions and decrement in renal function, and as dog ages ten years and older, this approached 90 %. Present study reports the manifestation of clinical signs by dogs suffering from renal failure irrespective of staging of disease.

Materials and Methods

Screening of 100 dogs, irrespective of age, breed and sex was done for renal failure on the basis of history of vomition, melena, anorexia, dehydration, pale mucous membrane and profound depression presented to TVCC, DUVASU, Mathura. Serum creatinine level (>2.0mg/dl) and blood urea nitrogen (>30mg/dl) levels were used as inclusive criteria for dogs to be selected for study. Out of 100 suspected cases, 24 cases were found positive for renal failure.

Results and discussion

The most striking clinical finding in dogs suffering from renal failure was vomition, which was observed in 87.5% cases followed by anorexia in 75% and melena in 70.83% cases. Frequency of vomition in present study was less than previously recorded by Robinson *et al.,* (1989), who reported vomiting in 67 % cases English (1974) and Mary (1992) reported the same finding in clinical cases of renal failure. Suggested reasons for vomiting in renal failure may be direct effects of uremic toxins on D_2- dopaminergic receptors in the chemoreceptor trigger zone (Washabau *et al.,* 1995) and uremic gastritis, while, possible reason for melena may be gastrointestinal ulcerations (Craig, 2003) and throbrocytopathy in response to uremic toxins. Mary (1992) and Forrester and Brandt (1994) reported melana in case of renal failure.

Other more common clinical findings were oliguria in 66.66 % cases, dehydration in 50 % cases. Oliguria was due to decreased renal blood flow which accompanied by decreased GFR and subsequent decreased urine output (Guyton and Hall, 2006). Clement *et al.* (1993) previously reported the same finding in uremic dogs. Parallel to finding of present study Robinson *et al.* (1989) reported dehydration in 40% of renal failure dogs, which was possibly due to vomiting, diarrhea and reduced oral intake of water (Harris *et al.,* 1960). The finding of anorexia was in accordance with Robinson *et al.* (1989), who reported anorexia in 80% cases of renal failure and thought to be due to gastrointestinal upset as reported by Krawiec (1996).

Uremic breath was observed in 41.66 % of clinical cases, while uremic stomatitis causing ulceration on the tongue and gum was noticed in 33.33% of clinical cases. Uremic breath results from bacterial degradation of urea to ammonia Grauer, (2005). Froster and Brandt (1994) reported oral ulceration in some cases of renal failure along, while Robinson *et al.* (1989) found oral ulceration in 20 % cases of renal failure.

Table 1: Clinical propaedeutics of dogs with renal failure

S. No.	Clinical sign	No. of the dogs	Percentage of clinical sign
1.	Vomition	21	87.5%
2.	Melena	17	70.83%
3.	Oliguria	16	66.66 %
4.	Dehydration	12	50.0 %
5.	Anorexia	18	75.0 %
6.	Uremic breath	10	41.66 %
7.	Uremic stomatitis	8	33.33%
8.	Anemia and paleness of mucous membrane	6	25.0%
9.	Hematemesis	8	33.33 %
10.	Polydypsia	6	25.0 %
11.	Arterial hypertension	8	33.33 %
12.	Progressive weight loss	8	33.33 %
13.	Epistaxis	6	25.0%
14.	Profound depression	8	33.33 %
15.	Hypothermia	11	45.83%
16	Hyperthermia	8	33.33%
17.	Dyspnoea	10	41.66%

Anemia and Paleness of mucous membrane was observed in 25% cases, may be due to depressed renal erythropoietin factor production from diseased kidney, blood loss in the form of hematemesis or melena and decreased RBC survival time as an upshot of uremic intoxication. Hematemesis was recorded in 33.33% cases, which was consistent with Krawiec (1996). Suggested reason for which includes gastric ulceration in response to hypergastrinemia as a result of increased secretion or reduced renal clearance of gastrin hypersecretion of gastric acid and direct damage to the gastric mucosa, submucosa, and vasculature by uremic toxins contribute further to the gastritis Graur (2005). Polydipsia and polyuria was recorded in 25% clinical cases, while Robinson *et al.* (1989) reported polydipsia/ polyuria in 67% cases. Possible causes for polydipsia includes vomiting, diarrhea and ploryuia. Haller (2002) suggested polyuria and as first clinical sign of renal disease in dogs.

Arterial hypertension was recorded in 33.33% cases, which was due to the decrease in the ability of the kidneys to excrete sodium and water. Cowgill (1986) reported hypertension in 50 to 93 % of dogs with chronic renal failure. The large quantities of secretion of rennin lead to the formation of angiotensin II which can cause hypertension (Guyton and Hall. 2006). Epistaxis was observed in 25 % cases, which was possibly due to impaired platelet –vessel wall interaction induced by uremia toxins (Boccardo *et al.,* 2004) and thrombocytopathy. Progressive weight loss was observed in 33.33 % dogs, may be due to anorexia in response to oral ulceration, gastritis and entities. Lower temperature observed in 45.83% dogs as previously reported by Joshi *et al.* (1989) which might be due to nephrosis, depression and sedative effect of toxicants. The other suggested reasons for hypothermia in renal failure may be severe uremia (Larry *et al.,* 1995). Hyperthermia was also seen in 33.33% of dogs, the reason of which may be infectious. Dyspnoea was recorded in 41.66% of clinical cases of renal failure in dogs, possibly due to development of metabolic acidosis (Cowgill and Francy, 2005) and terminal dehydration (Joshi *et al.,* 1989). These findings are in accordance with English (1974), Mahajan (2000) and Kraje (2002), who reported metabolic acidosis in dogs with renal failure.

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Prevalence of udder and teat lesions at machine milked dairy farms

A.Q. Mir, B. K. Bansal and D.K. Gupta
Department of Veterinary Medicine,
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana- 141 004, Punjab

Abstract

Udder and Teat lesions affect the dairyman by interfering with the milking process or by increasing the likelihood of IMI. The objective of this study was to find out various lesions of udder and teat in crossbred cows at machine milked dairy farms that affects the milking process and may cause mastitis in the future. The present study involved 872 quarters of 218 lactating dairy cows (HF × Sahiwal crosses) at 10 machine milked dairy farms in various districts of Punjab state. The prevalence of udder and teat lesions was about 23 per cent with warts (6.01%) and dryness (5.54%) as the major lesions. The other lesions observed were Vesicle/ papules (2.83%), leaky teats (2.59%), eversion (2.36%), nodules/hard tip (1.30%), wound/laceration (1.30%) and scab (0.70%).

Keyword: Machine milking, Teat lesions, Mastitis, Warts and Dryness Dogs

Teat-end or orifice is the first line of defence against invading bacteria and changes or damage to this part of the udder may reduce its effectiveness in preventing intramammary infections (IMI) (Gleeson *et al*., 2004). Marcela *et al.* (2012) observed that both teat sphincter and teat canal should be in perfect physical and hygienic conditions to prevent intramammary infection. Maintenance of good condition of teat skin and tissues surrounding the canal is important to obtain high quality milk (Mosaferi *et al.*, 2011). Teat lesions affect the dairyman by interfering with the milking process or by increasing the likelihood of IMI and the risk of mastitis increases as the lesions approach the teat canal. In one study, Agger and Willeberg, (1986) found that at least 7% of cases of subclinical mastitis would have been avoided if no teat lesions had occurred in the herd.

Milking equipment malfunction has traditionally been considered a cause of teat lesions (Farnsworth, 1996). Lesions involving the teat barrel do not generally directly cause mastitis, but do interfere with the milking process and may cause secondary problems (Farnsworth, 1996). Teat lesions are readily colonized by bacteria and thus serve as an important reservoir of infection which microbial colonization of the teat canal may promote the development of mastitis (Haveri *et al*., 2008). Furthermore lesions which affect the teat end and orifice frequently result in increased mastitis problems because of interference with the protective effect of the teat orifice, which is a major barrier preventing bacteria from entering the gland. Economically, losses may be severe as animals usually resist milking and must be culled. The objective of this study was to find out various lesions of udder and teat in crossbred cows at machine milked dairy farms that affects the milking process and may incite the occurrence of mastitis.

Material and Methods

The present study was conducted at 10 machine milked dairy cow farms in Ludhiana, Patiala, Moga, Bathinda and Ferozpur districts of Punjab. A total of 872 quarters from 218 HF × Sahiwal cross-bred dairy cows in milk were studied. The farms were visited during the routine afternoon milking hours (between 3 and 5 pm). The various udder and teat lesions like warts, dryness supernumerary teats, eversions, vesicles, scabs, etc were recorded. The observation at the farms was carried out by one of the authors only to minimize the chances of human error.

Results and Discussion

Teat lesions affect the dairyman by interfering with the milking process or by increasing the likelihood of IMI and the risk of mastitis increases as the lesions approach the teat canal. In one study, Agger and Willeberg, (1986) found that at least 7% of cases of subclinical mastitis would have been avoided if no teat lesions had occurred in the herd.

The overall prevalence of udder and teat lesions was 23 per cent. The lesions observed were warts (6.38%), dryness (5.54%), vesicles/papules (2.83%), leaky teats (2.59%), eversion (2.36%), nodules/hard tip (1.30%), wound/laceration (1.30%) and scab (0.70%). This is similar to but comparatively lower than the prevalence of 34.24 per cent observed by Boro (2002).In the present study warts were the main lesions which is in agreement with the findings of Jhand *et al*. (1994)

who also reported warts to be the most common teat lesions (50%) at machine milked dairy farms followed by teat chaps (18.29%), blind teats (9.76%), teat injuries (6.10%), teat stenosis (3.66%), foot and mouth disease lesions (2.44%), supernumerary teats (2.44%), leaky teats (1.22%), teat with double orifice (1.22%), udder skin injury (1.22%) and udder skin abscess (1.22%). Similarly, warts were also observed to be the main lesions by Singh *et al*. (1994) and Lindholm *et al*. (1994). Under conventional hand milking method, Boro (2002) has also observed the warts (8.43%) as the main lesion followed by blind teats (1.45%), supernumerary teats (1.17%) and wounds (1.17%). The higher incidence of teat warts at machine milked dairy farms might be due to transmission of infection from one animal to the next by the teat cup due to the lack of disinfection. Warts can interfere with the working of the liner and if they become damaged they may be colonized by *Staphylococcus aureus*, *Actinomyces pyogenes* or *Streptococcus dysgalactiae* (Ohnstad *et al*., 2003). The higher percentage of dry teats in present study may be due to lack of teat diping at most of the farms. Skin conditioners and emollients used in teat dips either reduce evaporation from the skin or act as humectants to maintain and improve the teat skin condition (Rasmussen and Larsen, 1998). The leaky teats and eversions may have occurred due to improper management of milking machine. High peak milk flow rate, short teats, teat canal protrusion, inverted teat ends, and early lactation increased the risk of milk leakage in multiparous cows (Klaas *et al.*, 2005). Milk leakage has been associated with increased risk of udder infection and mastitis in dairy cows (Persson *et al*., 2003).

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Micro mineral profile in fodder, soil and serum of dairy cow

S.P. Waghmare, D.B. Sarode, A.Y. Kolte, N.P. Dakshinkar, S.G. Mode, S.H. Vyavahare and Namrata Babhulkar
Department of Veterinary Clinical Medicine,
Post Graduate Institute of Veterinary and Animal Sciences Akola -444104, Maharastra

Abstract

The present study was conducted in saline affected villages of *Purna* river valley of Akola district to determine the micro mineral status in soil, fodder and cows. Total 104 soils, 71 fodders and 360 serum samples of lactating, pregnant, non-pregnant cows and heifers were collected from selected villages and subjected for mineral estimation. The pH of soil of these villages was in alkaline range (7.8 to 8.6). The overall mean concentration (ppm) of soil Cu, Zn, Fe and Mn were 0.50 ± 0.07, 0.79 ± 0.12, 5.88 ± 0.42 and 4.73 ± 0.46, respectively. The overall mean concentration (ppm) of Cu, Zn , Fe and Mn in fodder were 32.43 ± 2.17 , 26.29 ± 2.31, 191.28 ± 9.64 and 49.06 ± 2.84, respectively. The serum Fe level found to be significantly low in pregnant cows than lactating, non-pregnant cows and heifers. No significant variation was observed in serum Cu, Zn and Mn levels between different physiological stages of cows. The overall study concluded that the soil of saline tract area of Akola district is deficient in Zn and marginally low in Fe and Mn. Fodders samples of this area were deficient in Zn, whereas, marginally low in Cu and Mn and adequate in Fe. Lactating and pregnant cows was deficient in Zn, whereas non-pregnant cows were low in Zn, Cu and Mn. The heifers of this area were deficient in Zn and marginally low in Mn. The soil, fodder and animals of saline affected area of Akola district were found to be highly deficient in Zn.

Key words: Dairy cows, Micro minerals, Saline tract area, Soil-fodder-serum.

The mineral deficiency disorder of farm animals are found to be mostly area specific and differs from one region to another region due to different soil composition. In Vidarbha region of Maharashtra State (India), the *Purna* river valley is the unique tract of vertisols having native salinity /sodicity. It has been reported that the salinity of soil affects the crop yield and interferes the uptake of nutrients to the plants. Intensive research related to soil characteristics and yield of crops and its management have been already conducted by several workers (Sagare *et al*., 1991 and Babhulkar, 1999). However, very scanty information is available on the micro mineral profile of soil, fodder/ feed in this area. The status of micro mineral in serum of animals has not been studied so far.

In view of the fact, the present study was undertaken to estimate the status of micro-macro mineral in dairy cow as influenced by soil-plant relationship in saline affected villages of Akola district.

Materials and Methods

The present study was conducted in saline affected villages of Purna river valley of Akola district to estimate the micro- mineral status in soil, fodder and cows. Total seven villages from saline tract area of Akola district. *Gopalkhed, Gandhigram, Hata, Hingna-Tamaswadi, Karanja-Ramzanpur, Andura* and *Nimbhora* were selected by adopting multistage stratified sampling technique

Total 104 soil samples (approximately 100-150 gm) were collected for mineral analysis. A total of 71 fodder samples i.e. sorghum straw, wheat straw, soyabean straw, Arhar straw, mung straw, gram straw, maize stover and pasture available for feeding animals were collected from the owners of animals. A total of 360 serum samples from lactating, pregnant, non-pregnant cows and heifers were collected for mineral estimation.

Soil pH was determined in soil suspension (1:2) by digital pH meter (Jackson, 1979). Available micro-minerals Cu, Zn, Fe and Mn were determined by using DTPA (Diethylene Triamine Penta Acetic Acid) as extractant (Lindsay and Norvell, 1978) using Atomic Absorption Spectrophotometer (AAS) model 1202 (Varian).

Micro minerals in serum viz., Cu, Zn, Fe and Mn in fodder were determined by using Atomic Absorption Spectrophotometer (AAS) model 1202 (Varian) and SHIMADZU-AA 6300 after digesting the samples. Micro-minerals in serum viz., Cu, Zn, Fe and Mn were estimated on Atomic Absorption Spectrophotometer (AAS) model 1202 (Varian) after digesting the samples.

Corresponding Author:
Dr. S. P. Waghmare, M.V.Sc., Ph.D., Hospital Superintendent, Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary & Animal Sciences, Akola (MS), India-444 104

Collected data was analyzed statistically by factorial completely randomized design (FCRD) and completely randomized design (CRD) as per the method described by Snedecor and Cochran, 1994.

Results and Discussion

Micro mineral content and pH of the soil samples collected from saline affected area of Akola district are presented in Table 1. The average pH of soils samples of saline affected area ranged from 7.8 to 8.6 (8.2 ± 0.093), which tended to be higher, indicating that these soils are moderately to very strongly alkaline in reaction. A high pH value could be attributed by virtue of sodium carbonate and carbonate mineral present in alkali soil (Nakeyama, 1970). Sagare *et al*. (2000) reported that the pH of saline affected soil of *Purna* river valley found elevated with corresponding to the depth of the soil. The salinity and sodicity of soils usually affects the physical parameters of Purna valley vertisols thereby leads to impaired normal crop growth as well as nutrient uptake. This observation is in corroborate with those reported by Sarkar *et al.* (1994) who suggested that slight alkalinity of soil might interfere with the availability and uptake of essential micro-minerals to the forage plants.

The mean micro mineral values of soil Cu, Zn, Fe and Mn were 0.50 ± 0.07, 0.79 ± 0.12, 5.88 ± 0.42 and 4.73 ± 0.46 ppm, respectively (Table 1). The average values observed of Cu (0.50 ± 0.07) and Mn (4.73 ± 0.46) content of soil were found higher than the critical levels of < 0.2 ppm and < 2.0 ppm, respectively, whereas, soil Zn (0.79 ± 0.12) and Fe (5.88 ± 0.42) content recorded declined trend. These findings are in close agreement with the findings of Patil (2006), who also reported low soil Zn and Fe level in saline affected area of Akola district. The mean mineral concentration (Cu, Zn, Fe and Mn) in fodder were 32.43 ± 2.17 , 26.29 ± 2.31, 191.28 ± 9.64 and 49.06 ± 2.84 ppm, respectively (Table 1). The overall mean concentration of Zinc (ppm) in fodder of saline affected villages of Akola district was low (critical value < 30 ppm). The low concentration of Zn in fodders might be due to deficiency of Zn in soil of saline tract area of Akola district. Udar (2000) and Hussain Kafil (2006) reported lower Zn concentration in fodder of Western Vidarbha region. The overall mean concentration of iron (ppm) in fodder of saline affected villages of Akola district was found well above the critical level (< 50 ppm). Higher levels of iron in feed stuffs have also been reported in India (Mondal, 2003, Ramana *et al.*, 2001 and Sharma, 2003), which could be due to its high uptake by plants. Earlier reports by McDowell (1992) also indicates that plants grown on acid, neutral and even slightly alkaline soil often contained high level of iron. Excess iron reduces availability of phosphorus by the formation of insoluble ferric phosphate (Suttle, 1987) inhibiting the utilization of Zn and Cu (Underwood, 1977 and Burk, 1978).

The mean serum micro mineral levels (ppm) in cows at different physiological stages of saline affected area of Akola district are presented in Table-2. In the present study serum Cu level did not show any variation between different physiological stages of cows. Parshad Omkar *et al.* (1979) also observed non-significant differences between blood copper levels in heifers,

Table 1: Soil and fodder micro mineral profile of saline affected area.

Samples	No. of samples	pH	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
Critical values (Soil) Patil (2006)	0.2 (ppm)		0.6 (ppm)	4.5 (ppm)	2.0 (ppm)	
Soil	104	8.2±0.09	0.50± 0.07	0.79± 0.12	5.88± 0.42	4.73± 0.46
Critical values (Fodder) Mc Dowell (1992)	8 ppm		30 ppm	50 ppm	40 ppm	
Fodder	71	-	32.43± 2.17	26.30± 2.31	191.28± 9.64	49.06± 2.84

Table 2: Serum micro minerals in animals reared in saline affected areas.

Sr.No.	Elements	Lactating cows	Pregnant cows	Non-pregnant cows	Heifers	Pooled Mean
1	Cu (ppm)	0.78± 0.04	0.75 ± 0.04	0.75 ± 0.04	0.77 ± 0.05	0.76 ±0.01
2	Zn (ppm)	0.59 ± 0.19	0.58± 0.09	0.57 ± 0.07	0.55 ± 0.22	0.57 ±0.02
3	Fe (ppm)	1.67^{c} ± 0.11	1.30^{a} ± 0.09	1.81^{b} ± 0.11	1.85^{b} ± 0.14	1.65 ± 0.02
4	Mn(ppm)	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.20	0.37±0.002

Means bearing same superscript do not differ significantly with each other within respective rows (P<0.01).

lactating, pregnant and non-pregnant animals. However, the highest serum Cu level was found in lactating cows and lowest in pregnant cows (Rajora and Pachauri, 1998 and Hussain Kafil, 2006). It is attributed that such variations due to deposition of copper in developing foetus, which increases constantly throughout the gestation period (Hussain Kafil, 2006). The serum Zn level also showed non-significant differences between different physiological stages of cows. Rajora and Pachauri (1998) recorded significant decrease in serum zinc level in pregnant when compared with non-pregnant cows during early lactation. Parshad Omar *et al.* (1979) recorded significantly higher serum zinc level in heifers and lactating buffaloes than in pregnant and non-pregnant buffaloes. The result of the present study showed that the average serum Zn concentration was found apparently close to critical level (<0.6), attributing marginally low level of Zn concentration in animals. Similar findings were also recorded by Udar *et al.* (2003) and Hussain Kafil (2006) in buffaloes and cows of Akola district of Vidarbha region, respectively. This might be due to low content of Zinc in feed/fodders and soil of saline tract area of Akola district. The alkaline pH of soil (7.8 to 8.6) which might interfere with the availability and uptake of Zn to the forage plants. The optimum pH range of soil for availability of Zn is 5.5 to 7.0 (Pfander, 1971 and Lindsay, 1972).

The pregnant cows had lower serum iron level in comparison to lactating, non-pregnant cows and heifers. This decline in serum iron levels might be due to higher metabolic demand for trace elements greatly exceeding the requirement for the growing foetus. The findings were in accordance with Hussain Kafil (2006), who reported decrease in serum iron concentration with advancement of pregnancy. No significant differences were observed in level of serum Mn between different physiological stages of cows.

In conclusion soil of saline tract area of Akola district is deficient in Zn and marginally low in Fe and Mn. Fodders samples of this area were deficient in Zn, whereas, marginally low in Cu and Mn and adequate in Fe. Lactating and pregnant cows were deficient in Zn, whereas non-pregnant cows were low in Zn, Cu and Mn. The heifers of this area were deficient in Zn and marginally low in Mn. Thus, soil, fodder and animals of saline affected villages of Akola district were found to be highly deficient in Zn and significant positive association between soil-fodder-animal was found for Zn in saline tract area of Akola district.

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The forthcoming 33rd Annual Convention and International Symposium of the Society is scheduled to be held during 22 -24th Jan. 2015 on Theme: **"NEW DIMENSIONS IN VETERINARY MEDICINE: TECHNOLOGICAL ADVANCES, ONE HEALTH CONCEPT AND ANIMAL WELFARE CONCERN"**. This mega event is being organized jointly by the Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Trissur, Kerala and the Organizing Committee College Of Veterianry and Animal Sciences Pookode, Lakkidi P O, Wayanad - 673576. KERALA as well as ISVM. On this momentous occasion, you are invited to attend the scheduled International Symposium at College of Veterianry and Animal Sciences Pookode, Lakkidi, P O, Wayanad- 673576, KERALA. The contact details of the Organizing Committee are as follows:
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Dr. Usha Narayana Pillai (Co-Organizing Secretary): 09947659891, 09847803271 and Dr. P.V. Tresamol,
Co-Organizing Secretaries, Email: isvm2015@gmail.com and Website: www.isvm2015.in.

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Prevalence of ascites in canine

S. K. Upadhyay, P.C. Shukla and Amita Tiwari
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Jabalpur-482001, M.P.

Abstract

The present study was conducted to know the prevalence of ascites in dogs that were presented to the Teaching Veterinary Clinical Complex, College of veterinary science and animal husbandry, Jabalpur (M.P) from December 2006 to July 2007 with the complaint of anorexia and abdominal enlargement. Complete history of each affected dog with regard to the breed, age, sex, hygiene, course of illness and nutritional status was recorded. The results showed the overall prevalence of ascites to be 0.5 % (28/5681). Further the results revealed that the maximum cases of ascites were reported in the age group of more than 5 years (12.35%) , in the females (60.71%) and in the larger breeds i.e. Doberman (33.33%). The dogs that are fed vegetarian homemade diet i.e. protein deficient diet suffered more (12.5%).

Keywords: Age wise, Ascites, Breed wise, Diet, Prevalence, Sex wise

Ascites is a manifestation of either a transudate or a modified transudate accumulation (Gelen and King, 1992). Ascitic fluid production is a consequence of venous stasis and consequently venous hypertension. Ascites may be due to various etiological factors but main causes are hepatic, cardiac and renal in origin. The first noticeable sign of this ailment is the enlargement of abdomen which may be symmetrical or pear shaped. The bulging of umbilicus, on pressure undulating movement of the fluid, poor condition with sunken eyes, anemia and prominent ribs are other signs (Chakrabarti, 2006). The present work was, therefore, planned to study the prevalence of ascites and its correlation with the age, breed, sex and feeding habits of the affected dogs.

Material and Methods

The clinical study was conducted on dogs presented to the TVCC, CVSc&AH, Jabalpur from December 2006 to July 2007. Complete history of each affected dog with regard to the breed, age, sex, hygiene, course of illness and nutritional status was recorded.

Result and Discussion

In the present study, a total of 5681 dogs belonging to different breeds, age and sex were screened to determine the prevalence of ascites and 28 dogs were suffering from ascites. Therefore, the overall prevalence of ascites during December 2006 to July 2007 was 0.5 %. Age wise prevalence of ascites reported that the ailment was 8.1% in the age group of upto 1 year, 10.52 % in the age group of 1 year to 5 year and 12.35% in the age group of 5 year and above than the young ones. Chakrabarti (2006) also reported the lower incidence in the age group below 8-16 months.

The dogs included in the study belonged to the Pomeranian, German shepherd, Doberman, Lhasa apso and non- descript breeds and ascites was found to be 10.71%, 9.86%, 33.33%, 22.22% and 6.25% respectively. Sex wise prevalence was higher in female (60.71%) than male (39.28%).

The results of the study related to the nutritional status of the dogs showed 12.5% prevalence in the group fed vegetarian/homemade diet (protein deficient diet), 8.82 % in the group fed non vegetarian diet with egg and 9.52% in the group fed non vegetarian diet including chicken, mutton and soya (Protein rich diet). More prevalence of ascites in the dogs fed vegetarian diet was probably due to the lack of essential aminoacids in cereal protein, resulting in hypoproteinemia. Wadhwa *et al*. (1995) also reported hypoproteinemia in the cases of ascites related to the liver involvement.

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Prevalence of jaundice in dogs of Jammu

H.A. Tantary, J.S. Soodan, S. Chirag, A. Tikoo and S. Beigh
Veterinary Clinical Medicine Ethics and Jurisprudence
Faculty of Veterinary Science and Animal Husbandry
Sher-E-Kashmir University of Animal Science and Technology of Jammu, R.S. Pura-181102, J & K

Abstract

The study was conducted in clinical cases of dogs presented at Small Animal Medicine OPD of Referral Veterinary Hospital FVSc & A.H R.S.Pura Jammu. The prevalence of jaundice was found to be 32.65 per cent (16/49). Anaemia, weight loss, diarrhoea, with hepatic encephalopathy was the chief clinical signs observed. Haemogram revealed anaemia, thrombocytopenia and increased clotting time. Biochemical studies revealed significanly increased GGT, ALP and total bilirubin levels with increased oxidative stress levels. Jaundice was mainly pre hepatic which was confirmed by Van Den Berg reaction and dip stick test of urine.

Keywords: Dogs, Diarrhoea, Jaundice, Oxidative stress, Therapeutic, Van Den Berg Reaction, Dip Stick.

Jaundice (icterus) is a syndrome characterised by a yellow discolouration of the mucosae and teguments caused by an increase in the serum concentration of bilirubin (hyperbilirubinemia), although often indicative of hepatic disease, extrahepatic conditions can also result in icterus (Eddlestone, 2005). Icterus can be classified into three pathophysiological and etiological types as pre-hepatic or hemolytic icterus (as it is a consquence of hemolysis) occurs when the increase in bilirubin production exceeds the capacities of hepatocytes to conjugate and excrete it, hepatic icterus results from intrehepatic cholestasis, associated with diffuse disease of bile ducts or hepatocytes principally in the periportal zone and post-hepatic icterus results from extrahepatic cholestasis due to impaired or obstructed flow of bile downstream from liver. The normal total bilirubin concentration in blood is less than 0.4mg/dl. The tissues start to discolour when the concentration exceeds 2mg/dl and icterus becomes frank at 4mg/dl and above.

Materials and Methods

The present study was conducted on the dogs presented in the Small Animal Medicine OPD of Referral Veterinary Hospital of the FVASc &AH, SKUAST of Jammu between June 2010 and August 2011. Six apparently healthy dogs in the age group of 3-5 years irrespective of sex and breed were used control for study. Conjunctival or gingival mucous membrane was examined and dehydration status was ascertained by state of dryness of muzzle/nostrils and skin tenting time. Blood samples were collected for haematological and biochemical estimation. Hematological parameters were estimated as per the methods described by Jain *et al*. (1986). Whereas biochemical estimations viz. alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP), gama glutamyl transferase (GGT), plasma glucose, plasma cholesterol, total protein, plasma albumin and globulin, A:G ratio, blood urea nitrogen (BUN), creatinine and bilirubin were carried out spectrophotometrically using commercial kits. Van Den Berg reaction was done to ascertain the type of jaundice using Ehrlich's diazo reagent

Results and Discussion

Out of 49 dogs with hepatic disorders 21 (42.85%) dogs aged between 6 months to 13.5 years were suffering from jaundice as noticed on clinical examination, confirmed by dipstick test of urine and Van Den Berg reaction which was done in nine animals and haematobiochemical estimation. Two dogs had acute hepatitis, 11 dogs had chronic hepatitis and eight had cholestasis/cholangiohepatitis. Highest bilirubin concentration observed was 21 mg/dl in a 9 month old male Labrador. Cholestasis / cholangiohepatitis group

Table 1: Type of jaundice observed

Observation	Pre-hepatic/haemolytic jaundice	Hepatic/toxic jaundice	Post-hepatic/obstructive jaundice
Van den berg reaction	Indirect	Biphasic	Direct
No of positive cases	5	0	4
Colour of urine	Intense yellow	Yellowish	Intense yellow
Icteric index	Moderate (++)	Low (+)	High (+++)

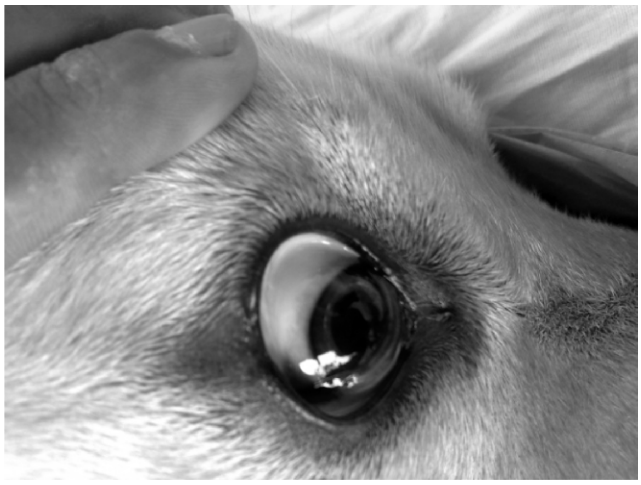

Plate 1. Icteric conjuctival mucous membrane

Plate 2. Icteric skin

followed by chronic hepatitis group showed more increase in total bilirubin level as compared acute hepatitis group. Jaundice was mainly pre-hepatic in origin which was confirmed by direct Van Den Berg reaction along with dipstick test of urine and is shown in Table 1 (Plate 1 and 2).

Haematobiochemical changes (pre treatment and post treatment) in dogs with jaundice are given in Tables 2 and 3. Haematological examination revealed significantly decreased Hb (7.41 ± 0.55 g/dl), PCV (22.89 ± 1.93%), TEC (3.80 ± 0.28 x 10^6/µl) and platelet count (2.30 ± 0.24 x 10^5/µl). TLC (14.21 ± 1.02 x 10^3/µl) increased non-significantly. Clotting time (3.23 ± 0.24 minutes) increased significantly when compared to control group. Differential leucocyte count revealed neutrophils, lymphocytes, monocytes, eosinophils and basophils as 67.20 ± 2.10, 24.70 ± 1.41, 6.10 ± 0.73, 1.84 ± 0.22 and 0.16 ± 0.06 per cent, respectively showing mild neutrophilia as compared to control. Plasma biochemical profile revealed significantly decreased total protein (5.15 ± 0.14 g/dl), albumin (2.23 ± 0.09 g/dl), globulin (2.91 ± 0.11 g/dl), A/G ratio (0.75 ± 0.04), plasma glucose (67.35 ± 3.99 mg/dl) and cholesterol (153.94 ± 10.98 mg/dl). Mean values of ALT (458.61 ± 52.70 IU/L), AST (254.12 ± 12.60 IU/L), ALP (501.2 ± 38.30 IU/L) GGT (25.97 ± 5.40 IU/L), BUN (34.49 ± 2.50 mg/dl) and bilirubin (5.25 ± 1.80 mg/dl) increased significantly. However creatinine (1.87 ± 0.25 mg/dl) was increased non-significantly when compared to control group. Post treatment haematological examination revealed significant increase in Hb (10.08 ± 0.36 g/dl), PCV (29.20 ± 1.75%), TEC, (4.69 ± 0.18 x 10^6/µl)) and platelet (3.50 ± 0.19 x 10^5/µl) count when compared to pre treatment and significant decrease as compared to control group except mean platelet count. TLC (14.50 ± 0.60 x 10^3/µl) increased non-significantly. Clotting time (2.40 ± 0.18 min) decreased significantly as compared to pre treatment and non-significantly as compared to control. Differential leucocyte count revealed neutrophils, lymphocytes, monocytes, eosinophils and basophils as 65.31 ± 1.73, 26.37 ± 1.41, 5.41 ± 0.53, 2.71 ± 0.13 and 0.20 ± 0.03 per cent respectively. Biochemical profile post treatment revealed significant decrease in ALT (50.68 ± 7.98 IU/L), AST (38.74 ± 2.60 IU/L), ALP (59.40 ± 4.80 IU/L), GGT (6.49 ± 0.59 IU/L), BUN (19.35 ± 2.01 mg/dl) and Bilirubin (1.80 ± 0.11 mg/dl) respectively. Plasma glucose (83 ± 2.36 mg/dl), cholesterol (209.20 ± 8.90 mg/dl), total protein (6.27 ± 0.14 g/dl), albumin (2.92 ± 0.05 g/dl) and globulin (3.35 ± 0.12 g/dl) increased significantly as compared to pre treatment and non-significantly decreased as compared to control. Creatinine (1.08 ± 0.68 mg/dl) decreased non-significantly as compared to pre treatment. A/G (0.88 ± 0.03) ratio increased non-significantly compared to pre treatment and was equal to control.

There was significant fall in Hb, PCV, and TEC which was in accordance to findings of Chohan *et al.,* (2009). Decrease in Hb is attributed to increased degradation of erythrocytes due to increased transit time through spleen because of reduced portal blood flow and or increased fragility of erythrocytes due to high levels of bile acids, besides impaired bone marrow responses, decreased erythrocyte survival time, decreased nutrient uptake due to inappetance or anorexia and reduced availability of micronutrients from liver

Table 2: Haematological changes (pre treatment and post treatment) in jaundice

S. No	Parameters	Control (n=6)	0-day (n=21)	15-day (n=18)
1	Hb (g/dl)	$11.95^{a} \pm 0.49$	$7.41^{b} \pm 0.55$	$10.08^{c} \pm 0.36$
2	P.C.V (%)	$34.70^{a} \pm 2.01$	$22.89^{b} \pm 1.93$	$29.2^{c} \pm 1.75$
3	T.E.C (x 10^{6}/µl)	$5.92^{a} \pm 0.28$	$3.80^{b} \pm 0.28$	$4.69^{c} \pm 0.18$
4	T.L.C (x 10^{3}/µl)	$11.53^{a} \pm 0.78$	$14.21^{b} \pm 1.02$	$14.50^{a} \pm 0.6$
5	Platelets (x 10^{5}/µl)	$3.62^{a} \pm 0.19$	$2.30^{b} \pm 0.24$	$3.50^{a} \pm 0.19$
6	Clotting time (min)	$2.06^{a} \pm 0.52$	$3.23^{b} \pm 0.14$	$2.40^{a} \pm 0.18$
7	DLC (%)			
	Neutrophils	$65.00^{a} \pm 1.71$	$67.20^{a} \pm 2.10$	$65.31^{a} \pm 1.73$
	Lymphocytes	$25.66^{a} \pm 1.54$	$24.70^{a} \pm 1.41$	$26.37^{a} \pm 1.41$
	Monocytes	$5.58^{a} \pm 0.31$	$6.10^{a} \pm 0.73$	$5.41^{a} \pm 0.53$
	Eosinophils	$3.25^{a} \pm 0.17$	$1.84^{b} \pm 0.22$	$2.71^{a} \pm 0.13$
	Basophils	$0.16^{a} \pm 0.11$	$0.16^{a} \pm 0.06$	$0.20^{a} \pm 0.03$

Values within a column having superscript with atleast one common letter do not differ significantly at 5% level ($P<0.05$) from each other.

Table 3: Biochemical changes (pre treatment and post treatment) in jaundice

S. No.	Parameters	Control (n=6)	0-day (n=21)	15- day (n=18)
1.	A.L.T. (IU/L)	$47.25^{a} \pm 2.08$	$458.61^{b} \pm 52.70$	$50.68^{a} \pm 7.98$
2.	A.S.T. (IU/L)	$30.22^{a} \pm 1.98$	$254.12^{b} \pm 12.68$	$38.74^{a} \pm 2.60$
3.	A.L.P. (IU/L)	$64.22^{a} \pm 6.60$	$501.20^{b} \pm 38.30$	$59.40^{a} \pm 4.80$
4.	G.G.T. (IU/L)	$8.80^{a} \pm 0.52$	$25.97^{b} \pm 5.40$	$6.49^{ac} \pm 0.59$
5.	Glucose. (mg/dl)	$90.66^{a} \pm 2.10$	$67.35^{b} \pm 3.99$	$83^{c} \pm 2.36$
6.	Cholesterol. (mg/dl)	$214.22^{a} \pm 2.87$	$153.94^{b} \pm 10.98$	$209^{a} \pm 8.90$
7.	Total protein. (g/dl)	$6.30^{a} \pm 0.05$	$5.14^{b} \pm 0.14$	$6.27^{a} \pm 0.14$
8.	Albumin. (g/dl)	$2.97^{a} \pm 0.04$	$2.23^{b} \pm 0.09$	$2.92^{a} \pm 0.05$
9.	Globulin. (g/dl)	$3.35^{a} \pm 0.03$	$2.91^{b} \pm 0.11$	$3.35^{a} \pm 0.12$
10.	A/G ratio.	$0.88^{a} \pm 0.01$	$0.76^{b} \pm 0.04$	$0.88^{a} \pm 0.03$
11.	Bilirubin. (mg/dl)	$1.04^{a} \pm 0.12$	$5.25^{b} \pm 1.17$	$1.80^{a} \pm 0.11$
12.	B.U.N (mg/dl)	$20.26^{a} \pm 1.84$	$34.49^{b} \pm 2.50$	$19.35^{a} \pm 2.01$
13.	Creatinine. (mg/dl)	$1.35^{a} \pm 0.14$	$1.87^{a} \pm 0.25$	$1.08^{a} \pm 0.68$

Values within a column having superscript with atleast one common letter do not differ significantly at 5% level ($P<0.05$) from each other.

(Bush, 2002). Mean platelet count was also significantly decreased. Several mechanisms have been suggested for thrombocytopenia in patients with hepatic disorder which include increased platelet sequestration in the spleen as a result of congestive splenomegaly, reduced production of thrombopoietin by the liver, increased platelet breakdown due to anti-bodies (Prins *et al.,* 2010) and increased consumption resulting from low grade disseminated intravascular coagulopathy. Clotting time was significantly increased, which could be due to improper synthesis of proteins by the liver required for clotting mechanisms. Webster (2005) opined that liver is the production site for all coagulation factors. Reduced hepatic synthesis results in a clinically significant hypocoagulable state. The activities of ALT, AST, ALP and GGT were significantly elevated . Elevations of plasma transaminases such as ALT and AST were indicative of altered hepatocellular membrane permeability, hepatocellular necrosis and inflammation with degree proportional to number of injured hepatocytes (Kramer and Hoffman, 1997). ALP is a membrane bound enzyme found on hepatocyte cannalicular membrane and luminal surface of biliary epithelial cells and its isoenzymes are present in kidneys, intestine, placenta and bone, but elevation in its level in more than one year old dog is usually indicative of hepatic origin unless bone disease coexists, since isoenzymes from other organs are having extremely short half lives. Marked increase in activities of ALP and GGT has been reported in conditions causing cholestasis, cholangiohepatitis, biliary cirrhosis, biliary obstruction and cholecystitis of cholelithiasis (Bandyopadhyay, 2003). A significant increase in total bilirubin was observed in the present study. Hyperbilirubinmemia is due to disturbance of the balance between rate of production of bilirubin and metabolism and excretion

of bilirubin. In the present study the increase might be as a result of diminished excretion due to extensive hepatocyte damage or biliary obstruction or a combination thereof (Vijayakumar *et al*., 2008). Also a significant decrease in total protein, albumin, globulin level and A/G ratio was observed. Liver being the main site of synthesis and degradation of most of the proteins, any hepatic disorder (chronic hepatitis and cirrhosis) are responsible for decrease in albumin concentration. Total plasma protein might also have decreased due to marked decline in the diet intake, malabsorption and ongoing protein losing enteropathies like gastroenteritis, gastrointestinal ulcerations and chronic gastritis. Inflammation also results in increased bowel permeability leading to fluid, electrolyte, protein and cell loss. The findings of the present study were comparable to that of Sevelius (1995. Significant decrease in cholesterol level was observed which may be attributed to decrease in synthesis or absorption from the gut or excessive conversion of cholesterol into bile acids (Hall, 1985). There was a significant decrease in the plasma glucose levels which corroborated with the findings of Varshaney and Hoque (2002) in dogs with hepatic dysfunctions. Hypoglycaemia in the affected dogs might be due to inappetance/anorexia complemented by malabsorption from intestine. Hypoglycemia in patients with hepatic disorders results from decreased glycogenolysis and gluconeogenesis combined with hyper-insulinemia due to decreased hepatic metabolism. Blood urea nitrogen increased significantly which corroborates with the findings of Chohan *et al.* 2009. Ammonia loading may occur in dogs as a result of haemolysis, blood transfusions and gastrointestinal haemorrhage. This could lead to non-renal related elevations in serum urea concentrations via hyperureagenesis. Haemolysis may produce substrates in the form of proteins that would require deamination and consequently lead to hyperammonaemia Creatinine levels were non-significantly increased. Similar observations were made by Chohan *et al.* 2009. This might be due to the renal abnormalities and the urinary retention due to obstruction.

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Serum Sodium and Potassium status in dogs with renal disorder

Ramakant, Mukesh Shrivastva and H.P. Lal
Department of Veterinary Clinical Medicine,
College of Veterinary Science and Animal Husbandry,
Deen Dayal University of Veterinary and Animal Science, Mathura-281001, U.P.

Abstract

The present study was undertaken for comparative evaluation of serum electrolytes particularly sodium and potassium, in 12 renal failure dogs and in 18 apparently healthy. Selection of renal failure dogs were done on the basis of serum level of creatinine (>2.0mg/dl) and blood urea nitrogen level (>30mg/dl). Sodium (Na), the most abundant cation in extracellular fluid, and potassium is the major intracellular cation. Dogs with renal failure showed significantly lower serum sodium than healthy dogs whereas serum potassium values of renal failure dogs were significantly higher than healthy dogs.

Keywords*:* Dog, Electrolyte, Sodium, Potassium and Renal failure.

Renal failure can cause alteration in body fluid and electrolyte balance in dogs (Cowgill, 1986). Electrolyte imbalances are the frequent most life threatening complication of acute uremia. Hyperkalemia in particular causes early morbidity and mortality and must be quickly identified and corrected. Hyperkalemia is caused by inadequate potassium excretion related to decrease filtration, decease delivery of sodium to the cortical collecting duct, injury to potassium secretary sites along the nephron and inability to augment renal potassium secretion (Barsanti, 1997). Hyperkalemia is a consistent feature associated with acute renal failure. Serum K^+ concentration over 6.5 mEq/L are likely to cause a deleterious effect on the heart. Altered ECG pattern or serum K^+ values over 7 to 8 mEq/L necessitate prompt medical attention. Present study reports electrolyte alteration in dogs suffering from renal failure.

Materials and Methods

The present study was carried out at the Department of Veterinary Medicine, Teaching Veterinary Clinical Complex, DUVASU, Mathura. In addition, referred cases from the practicing veterinarians in and around Mathura region were also included. The present study was performed in two groups. Group I was comprise of eighteen apparently healthy dogs of different sex, breed, aged between 3-4 years. Group II Group was comprised of 12 canine patients with renal disorder of any sex and breed**.** The inclusive criteria for selection of the animals in group II was serum creatinine level (>2.0mg/dl) and blood urea nitrogen level (>30mg/dl). About 5 to 6 ml blood was collected from the cephalic vein of all the animals with the help of disposable syringe and blood was collected in a clean and dry centrifuge tubes for blood was subjected for harvesting of serum for estimation of serum electrolyte. Estimation of serum Na^+ and K^+ were done with the help of instrument digital clinical flame photometer (Model-391, ESICO Pvt. Ltd.) and using the kits supplied by M/S span diagnostic limited, plot No. 336-340, road No. 3, GIDC, sachin – 394230 (Surat), India. All the data were statistically analyzed by using "t" test (Snedecor and Cochron, 1994).

Results and Discussions

Serum electrolyte analysis in healthy and renal failure dogs were carried out and results were shown in (Table-1 & Fig.-1). Dogs which suffering from renal failure (Group–II) showed significantly lower Mean S.D. of sodium (Na^+) (138.2±1.30) than (Group – I) healthy dogs (143.79±0.65). It may results from iatrogenic administration of fluids low in sodium for volume restoration or maintenance requirement, but these alteration in renal failure may be due decreased excretory function (Mary, 1992), in spite of their continued ingestion. Hyponatremia is a proven cause for central nervous system dysfunction (Conger and Anderson, 1983) and is one of the factors responsible for letharginess in dogs suffering from renal failure.

Serum K^+ values of renal failure dogs (Group II) were significantly higher (10.49 ± 1.66 mEq/L) in comparison of (Group – I) healthy dogs (4.16 ± 0.1 mEq/L). These findings were pinpointing for hyperkalemia, which was possibly caused by inadequate potassium excretion related to decrease filtration, decreased delivery of sodium to the cortical collecting duct, injury to potassium secretory sites along the

Table 1: Serum electrolyte analysis of healthy dogs.

Parameters	Healthy (n=18)(Mean± S.D.)	Diseased (n=12)(Mean± S.D.)	t value	t tab
Sodium (Na^+) MEq/L	143.79^b±0.65	138.20^a±1.30	4.219**	2.763
Potassium (K^+) MEq/L	4.16^a±0.14	10.49^b±1.66	4.676**	2.763

** - Highly significant ($p<0.01$) a, b, shows the superscript highly significant ($p<0.01$) between the conditions. (MEq/L)

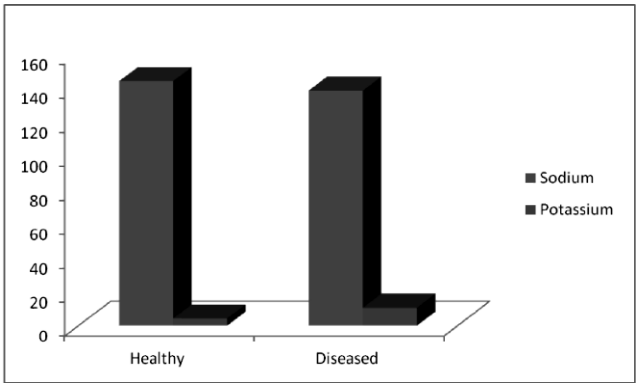

Fig. 1. Serum electrolyte values of healthy and renal failure dogs

nephron, and inability to augment renal potassium secretion (Harrison,*et al.,* 1960). Rastegar and DeFronzo, (1997) reported that hyperkalemia may be further aggravated by cell lysis and release of potassium from intracellular stores (Crush injuries, tumorlysis, myositis). Cellular shift of potassium associated with systemic acidosis, increased potassium load related to the diet or potassium containing enteral or parentral solutions may be other possible causes for hyperkalemia. Hyperkalemia is typically indicative of metabolic acidosis and most commonly seen in oliguric patients (Stanley, 1995). These findings were similar to the findings of (Jayathangaraj, *et al.,* 1995), who observed hyperkalemia and metabolic acidosis in patients with renal failure.

The present study was conducted for comparative evaluation of serum electrolytes (**Na^+** and K^+) in 18 healthy and 12 renal failure dogs. Dogs with renal failure showed significantly lower serum sodium than healthy dogs whereas serum potassium values of renal failure dogs were significantly higher than healthy dogs.

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Dry cow therapy in mastitis: Comparison of efficacy of an antibiotic and a non antibiotic teat sealant treatment in buffalo

Swati Tiwari[1], Anshu Sharma[2], V. K. Jain[1] , Mahavir Singh[3] and Gaurav Charaya[1]
Department of Veterinary Medicine
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004. Haryana

Abstract

A total of 168 apparently healthy quarters of buffaloes were treated at drying off with either 5 lac IU Colistin Sulphate and 200 mg Cloxacillin Sodium or 4 g of an internal teat sealant containing Bismuth Subnitrate. The quarters were sampled before drying off and after parturition and subjected to somatic cell count and bacteriological examination. The mastitis cases were monitored from 0 to 60 days from calving. The odds of getting newer infections were lesser in the quarters infused with teat seleant (1.19%) than in those infused with antibiotic combination (2.34%). The overall clearance rate is 50% for teat sealant and 52.9% for the intramammary antibiotic. Treatment with bismuth subnitrate resulted in lesser infections within 60 days of calving so it can be considered as equally efficacious for dry cow teat sealant therapy.

Keywords: Bismuth subnitrate, Cloxacillin Sodium, Colistin Sulphate, Dry cow therapy and Mastitis

Mastitis is the inflammation of the mammary gland associated with physical and chemical changes in milk, counts amongst the most important diseases in dairy herds (Radostits *et al*., 2007, Beheshti *et al*., 2010). Approaches to its therapy on farm have evolved considerably from pre antibiotic era where symptomatic treatments like massage, embracations and stripping out were used to the modern indiscriminate use of antibiotics Beheshti *et al*. (2010). To cure mastitis infection, various preventive therapies have been used; amongst them is selective dry cow therapy. Dry cow therapy has two aims. First is to cure the existing infections and second is to prevent the establishment of new infections during dry period (Huxley *et al*., 2002). It is reported that the rate of new infections is high around the time of involution, mostly during first 21-60 days of calving (Neave *et al*., 1950). It is thought that the infections gain access to the gland via the teat canal, which is likely to be open or incompletely closed particularly during involution and around the time of colostrogenesis (Oliver and Sordillo, 1988).

Intramammary application of long-acting antibiotics is an essential element in the drying off routine of many dairy operations, and has been recommended worldwide for many years (Dingwell *et al*., 2003). Although internal teat sealants have been shown to be effective in preventing new infections in dry period (Huxley *et al*., 2002; Woolford *et al*., 1998) their use has been less studied. A formulation for this purpose comprising containing 65% wt/wt bismuth subnitrate in a paraffin base was developed and examined to reduce the number of new infections acquired by the mammary gland during the non-lactating period by several authors (Berry and Hillerton, 2002; Godden, *et al.,* 2003). In animals that are unlikely to be sub-clinically infected at dry off, the product alone has been shown to be at least as effective as antibiotic dry cow therapy alone on a quarter comparison basis (Woolford *et al*.,1998) and on a cow comparison basis (Huxley *et al*., 2002). The purpose of this study was to investigate whether in animals deemed to be infected at drying off; an internal teat sealant would reduce intramammary infections after calving or incidence of mastitis within 60 days of parturition.

Materials and Methods

Total 34 buffaloes were selected from organised LUVAS farm and a private farm which were apparently in good health as determined by clinical examination and other methods, had four quarters free of significant teat lesions and having somatic cell count (SCC) less than 5 lac/ml before drying off. They could not have received intramammary or systemic antibiotics or anti inflammatory drugs within 30 days of last milking. Samples were collected aseptically after cleaning the teats with ethanol and proper drying as described by Huxley *et al.* (2002) from all quarters. The samples were then subjected to bacteriological examination and somatic cell count. For bacteriological examination a standard loopful (0.01 ml) from each milk sample was inoculated on the surface of 5% sheep blood agar plates

[1]Department of Veterinary Medicine, [2]College Central Laboratory, [3]Department of Veterinary Microbiology

and MacConkey's lactose agar plates separately. All plates were incubated aerobically at 37 ºC and examined for growth at 24 *h*. The initial cultivation of milk on blood agar (BA) plates offers an overview of all bacteria present in the sample. A further advantage of BA is that hemolysis around colonies, an important diagnostic criterion in classical bacteriology. Somatic cell count (SCC) on milk samples was performed as described by Schalm *et al*. (1971) and the milk smears were stained with Newman-Lampert stain.

After sampling on the day of drying off, the teats were scrubbed with cotton wool soaked in 70% ethanol and allowed to dry. In 84 quarters 5 lac IU Colistin Sulphate and 200 mg Cloxacillin Sodium was infused in teat cistern of each quarter and massaged into the udder. In 84 quarters 4 g of the teat sealant containing 65% bismuth subnitrate in light liquid paraffin was instilled. Bismuth subnitrate is found in a powdered form and so was sterilised using UV light. The animals were managed according to normal healthy husbandry practices. The milk samples were again subjected to bacteriological examination and somatic cell count within 60 days of calving using the same procedure as described above. A successful outcome was defined as a quarter that had no pathogens cultured from it in either of samples examined after calving. Therefore a quarter which did not show any infection before and after the dry period within 60 days of calving was considered as a successful one. The quarters that were infected before drying off but were cured and did not acquire a new infection were also successful.

Results and Discussion

Total 168 apparently healthy quarters were treated at drying off with either 5 lac IU Colistin Sulphate and 200 mg Cloxacillin Sodium or 4g of an internal teat sealant containing Bismuth Subnitrate. The quarters were sampled before drying off and after parturition and subjected to SCC and bacteriological examination. The mastitis cases were monitored from 0 to 60 days from calving. The odds of getting newer infections were lesser in the quarters infused with teat seleant (1.19%) than in those infused with antibiotic combination (2.34%). The overall clearance rate is 50% for teat sealant and 52.9% for the intramammary antibiotic as encapsulated in table 1. Therefore, on treatment with bismuth subnitrate, there were lesser infections within 60 days of calving.

These findings were found to be in complete agreement with Woolford *et al*. (1998) who obtained 73% reduction in intramammary infections. This finding of effectiveness of teat sealer was also similar to the findings of Bhutto *et al*. (2011) who found 59% of the infection cleared on treatment with teat sealer containing bismuth subnitrate. Maeney *et al*. (1977) also observed about 61% of non clinical recoveries in teats treated with bismuth subnitrate teat sealer alone. On the contrary to our study, Godden *et al*. (2003) found only 27.7% reduction in the teats sealed with Bismuth subnitrate.

Bismuth-based teat seals are biologically inert, with high relative density, insoluble in milk and does not set or solidify in the teat, being removed at calving either by the calf or by manual stripping. But in the current study, quarters which were infused with the teat sealant although were bacteriologically healthy but some of these showed swelling and hardening of teats immediately after calving for a few days. In accordance with our study, Meaney (1997) showed that the seal material infused at drying off gave a 90% reduction in the incidence of new IMIs during the dry period. On the contrary in terms of seal persistence, X-ray imaging (bismuth being an X-ray contrast medium) indicated that the seal remained lodged in the lower teat for at least 3-4 weeks after drying off, and that little of the material was lost via the teat orifice during the dry period, although some intramammary dispersion did eventually occur within the dry cow secretions.

The teats sealed with antibiotic infusion were found to be 52% cleared, this was in slight difference with Godden *et al*. (2003); Newton *et al*. (2008); Petzera *et al*. (2009); and Bhutto *et al*. (2011) who reported very high cure rate of 85.5%, 91.0%, 78.9%, and 70.0% respectively in quarters treated with antibiotics teat infusion.

The study indicated that the likelihood to acquiring an infection during dry period is controlled more by animal factors than by farm factors. Several factors have shown to affect the susceptibility of newer infections in the dry period, for e.g. rate of formation of keratin plug (Williamson 1995, Dingwell *et al.*, 2004), parity (Zadoks *et al.*, 2001, Huxley *et al.*, 2002, Green *et al*., 2005), age (Dingwell *et al*., 2002). Differences in environmental and managemental conditions also affect the emergence of newer infection (Williamson *et al*., 1995).

Dingwell *et al.* (2002) and Green *et al.* (2005) observed that animals with a high mean before drying off had higher SCCs in the subsequent lactation as compared to cows with a lower SCC. Thus, use of dry cow therapy should be implied on animals more likely to respond to treatment to maximise cure rate. So, factors like infection status and parity should be taken into account as done by Berry *et al*. (2003).

There is a slightly increased expected risk of new infections with the use of intramammary antibiotic alone during the dry period which finds its reason either as antibiotic being inadequate or efficiency decreases with concentration also, an important aspect of the prophylactic treatment of dry animals with antibiotics is the risk of residues in the milk supply after calving. Therefore a strategy which does not involve antibiotics can also be of considerable importance (Bradley *et al*., 2003). The numbers of quarters infected at drying off with various pathogens and dry period cure rate in the quarters treated with antibiotic and teat sealant is shown in table 1 and table 2.

The reduction in the incidence of mastitis (1.19%) is the most important observation because it is suggestive of improvement by the use of this therapy (table 3). The results were in agreement with results of Godden *et al*. (2003) who reported that odds of a teat sealant and antibiotic treated quarter affected with mastitis in first 60 days of infection was 67 per cent compared with cloxacillin alone. The study indicated that the likelihood to acquiring an infection during dry period is controlled more by animal factors than by farm factors. Several factors have shown to affect the susceptibility of newer infections in the dry period, for e.g. rate of formation of keratin plug (Williamson 1995, Dingwell *et al.*, 2003), parity(Zadoks *et al.*, 2001, Huxley *et al.*, 2002, Green *et al*., 2007), age (Dingwell *et al*., 2003). Dingwell *et al.* (2002) and Green *et al.* (2005) observed that animals with a high mean before drying off had higher SCCs in the subsequent lactation as compared to cows with a lower SCC. Thus, use of dry cow therapy should be implied on animals more likely to respond to treatment to maximise cure rate. So, factors like infection status and parity should be taken into account as done by Berry *et al*. (2003).

There is an increased expected risk of new infections with the use of intramammary antibiotic alone during the dry period which finds its reason either as

Table 1. Effect of antibiotics (Group A) and a teat sealant (Group B) and dry period cure rate (%)

Treatment	Total no. of quarters examined	No. of infected quarters	No. infected quarters after calving	Clearance rate (%)
Group A	84 (21 animals)	17	8	52.94
Group	84 (21 animals)	8	4	50.00

Table 2. Numbers of quarters infected at drying off and after calving with different pathogens in the quarters treated with antibiotic and teat sealant and dry period clearance rate (%)

Treatment	Quarters examined	Quarters infected	Organism (No. of infected quarters at drying off)	Organism No. infected quarters after calving	Clearance rate(%)
Intramammay Antibiotic Colistin Sulphate and Cloxacillin Sodium	84 (21 animals)	17	Staphylococcus (8) Streptococcus (9)	Staphylococcus (6) Streptococcus (2)	25.00 77.78
Intramammay Teat sealant Bismuth subnitrate	84 (21 animals)	8	Staphylococcus (6) Streptococcus(2)	Staphylococcus (3) Streptococcus(1)	50.00 50.00

Table 3. Newer infection rate with different pathogens after calving showing the effect of antibiotics (Group A) and a teat sealant (Group B) and dry period prevention rate (%)

Treatment	Total newer quarters infected	Staphylococcus	Streptococcus rate	Newer infection prevention rate (%)	Dry period
Group AIntramammay Antibiotic Colistin Sulphate and Cloxacillin Sodium	2	1	1	2.3% (2/84)	97.70
Group BIntramammay Teat sealant Bismuth subnitrate	1	1	0	1.19% (1/84)	98.81

antibiotic being inadequate or efficiency decreases with concentration also, an important aspect of the prophylactic treatment of dry animals with antibiotics is the risk of residues in the milk supply aftercalving. Therefore a strategy which does not involve antibiotics is of considerable importance (Green *et al*., 2007).

In conclusion, it can be stated that closure of the teat canal from day one of the dry period as achieved by the teat sealer was as effective in reducing new dry period infections as the infusion of a dry cow antibiotic formulation. The lower incidence of new infections in the ensuing lactation among the infused quarters implies that fewer subclinical infections persisted from the dry period. Use of teat sealers at drying off appears to offer almost the same prophylactic efficacy as the dry cow antibiotic approach.

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Effects of herbal and synthetic anthelmintics in goats

Pratibha Sharma, M.L.V. Rao, P.C.Shukla, Pooja Dixit, Shashi Pradhan
Department of Veterinary Medicine
College of Veterinary Science and Animal Husbandary, Jabalpur- 482001, M.P.

Abstract

In the present study, Hb, PCV, TEC increased significantly in Fenbendazole fed group, whereas a positive increase was observed in Banana and Bathua fed groups. The level of TLC decreased significantly in Fenbendazole treated group than that of the other groups. In DLC, a significant decrease in eosinophil count was observed in Fenbendazole treated group. As regarding the biochemical parameters, mean values of total serum protein increased significantly in Fenbendazole and Bathua fed groups.

Key words: Anthelmintics, Banana, Bathua, Fenbendazole, Goats, Herbal

Helminthiasis is considered as a major constraint in livestock productivity around the globe (Githiori *et al.,* 2004). An infection caused by gastrointestinal helminths parasites is economically important in grazing animals. Nematodes are the most important due to prevalence and adverse effects. Haematological changes in goats infected with *Haemonchus longistipes* and *Trichostrongylus colubriformis* was evaluated (Singh, 1993). Clinical signs observed were weakness, loss of body weight and diarrhoea. There was a rapid fall in haemoglobin and packed cell volume indicating anaemia in goats. Haemato-biochemical study of gastrointestinal nematodiasis in Bengal goats showed that the total leucocyte count value was significantly increased in younger as compared to the older goats (Jas *et al.*, 2008) and serum albumin level was declined significantly. Akanda *et al.* (2012) observed the effect of anthelmintics against gastrointestinal nematodiasis on live weight and haematological indices. Total erythrocyte count, haemoglobin and packed cell volume were decreased significantly in untreated control goats. On the other hand, total leukocyte count was decreased in all treated goat. Hence, the present study was undertaken to compare the haemato-biochemical changes in goats treated with synthetic and herbal anthelmintics.

Material and Methods

The study was conducted at Amanala farm, Department of Veterinary Medicine, College of Veterinary Science and A.H., Jabalpur, NDVSU, M.P.

A total of 30 goats were selected in the experimental trial and were randomly divided into 5 groups comprising of 6 animals in each group. The therapeutic trial was conducted by using Fenbendazole as synthetic anthelmintic @ 7.5 mg/kg b.wt. PO single dose and Banana (*Musa paradisiaca*) leaves @ 8 g/kg b.wt. PO single dose and Bathua (*Chenopodium album*) whole plant @ 3 g/kg b.wt. PO single dose as herbal anthelmintics. These plants were procured from the local farmers of the area. Plant material of both plants were dried in an oven at 40° C, grinded to a fine powder and stored in polythene bags until use. 50-100 g of feed was mixed with crude powder of herbal anthelmintic and was fed to the animals under supervision. In case of refusal by the animal, the herbal powder was given in drinking water.

For haematology, five ml blood was collected aseptically on day 0 (pre treatment) and day 28 (post treatment). The anticoagulant heparin was used. The haematological parameters done as per the method described by Benjamin (2001) were haemoglobin concentration (g/dl), total erythrocyte count (millions/µl), packed cell volume (%), total leukocyte count (thousands/µl) and differential leukocyte count (%). For differential leukocyte count (DLC), the smears from fresh whole blood were prepared and stained by Leishman's stain and examined under oil immersion objective.

The biochemical parameters were analysed by commercial kit (Erba-Chem-5 plus V2) with semi auto analyzer using the standard protocol. The different biochemical parameters estimated were blood glucose concentration (mg/dl) done by glucometer, serum total protein (g/dl), serum albumin (g/dl), serum globulin (g/dl) and albumin/globulin ratio. The various treatment groups were compared using multiple comparison tests i.e. Duncan's Multiple Range test (DMRT).

Table 1: Haemato-biochemical changes in different groups

Parameters	Pre treatment (day 0) and Post treatment (day 28)	Groups			
		Control (untreated)	Fenbendazole @7.5 mg/kg b.wt. PO single dose	Banana (*Musa paradisiaca*) @ 8 g/kg b.wt. PO single dose	Bathua (*Chenopodium album*) @ 3 g/kg b.wt. PO single dose
Haemoglobin (g/dl)	Pre treatment	$6.41^{c} \pm 0.09$	$7.30^{b} \pm 0.03$	$6.44^{c} \pm 0.07$	$6.56^{c} \pm 0.05$
	Post treatment	$6.15^{d} \pm 0.11$	$8.23^{a} \pm 0.06$	$6.48^{c} \pm 0.07$	$6.59^{c} \pm 0.04$
Packed cell volume (%)	Pre treatment	$23.00^{bcd} \pm 0.45$	$22.16^{d} \pm 0.31$	$22.66^{cd} \pm 0.42$	$23.58^{bcd} \pm 0.45$
	Post treatment	$22.16^{d} \pm 0.48$	$28.00^{a} \pm 0.52$	$24.00^{bc} \pm 0.45$	$24.50^{b} \pm 1.02$
Total erythrocyte count (millions/µl)	Pre treatment	$8.37^{b} \pm 0.12$	$8.38^{bc} \pm 0.03$	$8.35^{bc} \pm 0.02$	$8.46^{b} \pm 0.11$
	Post treatment	$8.15^{c} \pm 0.14$	$9.39^{a} \pm 0.15$	$8.44^{bc} \pm 0.02$	$8.56^{b} \pm 0.07$
Total leucocyte count (thousands/µl)	Pre treatment	$12.80^{a} \pm 0.21$	$12.87^{a} \pm 0.22$	$12.88^{a} \pm 0.12$	$12.85^{a} \pm 0.24$
	Post treatment	$12.90^{a} \pm 0.14$	$12.20^{b} \pm 0.13$	$12.56^{ab} \pm 0.08$	$12.50^{ab} \pm 0.21$
Neutrophil (%)	Pre treatment	34.83 ± 0.95	34.00 ± 1.06	35.33 ± 1.67	34.33 ± 0.76
	Post treatment	34.43 ± 1.60	32.50 ± 0.88	34.16 ± 1.62	33.00 ± 0.77
Eosinophil (%)	Pre treatment	$6.00^{a} \pm 0.45$	$4.67^{ab} \pm 0.61$	$5.83^{ab} \pm 0.87$	$5.00^{ab} \pm 0.82$
	Post treatment	$6.33^{a} \pm 0.84$	$2.66^{c} \pm 0.83$	$3.14^{bc} \pm 0.51$	$5.33^{a} \pm 1.02$
Lymphocyte (%)	Pre treatment	54.50 ± 0.99	57.67 ± 1.66	55.33 ± 1.85	57.00 ± 2.42
	Post treatment	53.33 ± 1.73	62.50 ± 0.76	57.50 ± 1.45	62.00 ± 2.38
Monocyte (%)	Pre treatment	2.66 ± 0.54	2.50 ± 0.43	2.33 ± 0.03	2.50 ± 0.56
	Post treatment	1.66 ± 0.33	3.00 ± 0.26	2.83 ± 0.40	2.83 ± 0.40
Basophil (%)	Pre treatment	0.33 ± 0.21	0.33 ± 0.21	0.33 ± 0.21	0.33 ± 0.21
	Post treatment	0.33 ± 0.21	0.33 ± 0.21	0.50 ± 0.22	0.16 ± 0.16
Blood glucose (mg/dl)	Pre treatment	63.66 ± 1.71	63.50 ± 0.62	64.33 ± 1.14	63.50 ± 0.85
	Post treatment	60.83 ± 1.35	66.00 ± 0.51	65.50 ± 1.02	64.83 ± 1.14
Total serum protein (g/dl)	Pre treatment	$6.03^{bc} \pm 0.19$	$6.34^{bc} \pm 0.13$	$6.07^{bc} \pm 0.09$	$5.91^{c} \pm 0.18$
	Post treatment	$5.86^{c} \pm 0.17$	$6.80^{a} \pm 0.19$	$6.14^{bc} \pm 0.05$	$6.40^{ab} \pm 0.16$
Serum albumin (g/dl)	Pre treatment	3.46 ± 0.16	3.62 ± 0.10	3.32 ± 0.09	3.39 ± 0.15
	Post treatment	3.38 ± 0.16	4.07 ± 0.17	3.46 ± 0.06	3.66 ± 0.16
Serum globulin (g/dl)	Pre treatment	2.56 ± 0.06	2.72 ± 0.08	2.65 ± 0.07	2.51 ± 0.05
	Post treatment	2.48 ± 0.04	2.75 ± 0.05	2.70 ± 0.08	2.75 ± 0.05
Albumin globulin ratio (%)	Pre treatment	1.33 ± 0.06	1.31 ± 0.06	1.28 ± 0.06	1.31 ± 0.04
	Post treatment	1.32 ± 0.06	1.36 ± 0.05	1.28 ± 0.06	1.31 ± 0.07

The mean values in the rows and columns (a,b,c) with the same superscripts did not differ significantly (p d" 0.05).

Results and Discussion

Haemato-biochemical changes are presented in Table 1. Banana and Bathua fed group showed increase in haemoglobin concentration while in Fenbendazole treated group, there was a significant increase from pre treatment i.e. 7.30±0.03 to 8.23±0.06 post treatment. It may be due to prevention of further damage to the gastrointestinal tract and leakage of blood from the site of attachment by the adult nematode parasites. The findings are in agreement with Islam *et al.* (1999) and Sharma *et al.* (2000).

In Fenbendazole treated group, the PCV value increased significantly from pre treatment i.e. 22.16±0.31 to 28.00±0.52 post treatment. The findings are in correlation with Pandit *et al.* (2009) and Tariq *et al.* (2010). The mean value of TEC was also significantly increased in Fenbendazole treated group from pre treatment i.e. 8.38±0.03 to 9.39±0.15 post treatment. This increase might be due to increased erythropoietic activity to compensate the blood loss by the parasites. The findings are in coherence with Islam *et al.* (2003). TLC value decreased significantly in Fenbendazole treated group from pre treatment i.e. 12.87±0.22 to 12.20±0.13 post treatment. The observations are in conformity with the findings of Akanda *et al.* (2012) and Aktaruzzaman *et al.* (2012). Eosinophil count value decreased significantly from pre treatment i.e. 4.67±0.61 to 2.66±0.83 post treatment in Fenbendazole treated group. The findings recorded are in coherence with Ghulam *et al.* (1995) and Pal *et al.* (2001). The mean values of total serum protein in Bathua and

Fenbendazole treated group were increased significantly from pre treatment to post treatment. It may be due to hepatoprotective activity of Bathua (Nedialkova *et al.*, 2009).

In the present study, Fenbendazole was found to be highly effective against gastrointestinal nematodiasis followed by Bathua and Banana. Therefore, Bathua and Banana can be used for a long period of time as prevention therapy for gastrointestinal nematodiasis.

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Therapeutic management of hemogalactia in cows

S. V. Singh, J. P. Singh, Ramakant and H.N.Singh
Department of Veterinary Medicine, College of Veterinary Sciences & A.H.
Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad 224229, U.P.

Abstract

Haemogalactia is seldom seen in milch animals. The affected milk is often discarded and thus causes economic loss to the farmers. Conventional therapy involves use of antiobiotics, hemostyptics and antihistaminics that costs more. Homeopathy offers a safe and cheap system of alternate medicine in the treatment of haemogalactia. Earlier reports suggest the use of ipecacuanha and some combination medicines. Literature available mentions the use of some single homeopathic medicines like ipecacuanha, buforana and nitrum acidum in treatment of haemogalactia. The present study compares the efficacy of these drugs with the conventional therapy. The results suggest homeopathic medicines ipecacuanha and buforana as an effective alternate system in the treatment of hemogalactia when administered orally.

Keywords: Haemogalactia, Cows, Homeopathy, Ipecacuanha, Buforana, Acidum nitrum

Haemogalactia refers to passing of blood or blood clots in the milk (Sharma, *et al.*, 2010). The presence of blood, however, imparts an insipid quality to milk. The milk that contains blood is discarded and delay in treatment often causes heavy economic loss to farmers. Homeopathy has gained the reputation of an effective alternative therapy in veterinary practice (Day, 1998). Combination medicines have been introduced in homeopathy for safe and quick recovery (Reilly, 1986). Recently, promising results with a homeopathic combination remedy in the management of udder affections (fibrosed/nonfibrosed mastitis, and udder oedema) have been reported in dairy cows (Ram Naresh *et. al*., 2002). Various researchers have suggested the use of homeopathic remedies in the treatment of cows showing blood tinged milk. The present study was conducted to compare the therapeutic efficacy of different homeopathic medicines administered per os in the treatment of hemogalactia.

Materials and method

Twenty four animals showing blood in milk were included in this study. The quarters were normal in consistency and milk from unaffected quarters were normal in color and negative for California Mastitis Test and Whiteside Test. Animals were divided in four groups (Table 1) according to the treatment given.

The response was adjudged by regular testing of milk. The duration of the treatment was not defined at the beginning but was decided on the clinical response of the drug within first 2–3 days of the treatment. If there was no response homeopathic treatment was discontinued and the animal counted as a failure. No supporting therapy except stripping twice daily was given during the course of the treatment.

Results and Discussion

The animals of group A and B showed remarkable improvement after 2-3 days. In group A, 4 animals recovered completely after 2 days and one animal showed recovery after 3 days. One animal did not respond to the treatment.

In Group B, 1 animal recovered completely after first day of treatment and improvement was observed in 4 animals. Out of these 4 animals, another 1 animal recovered completely after 2 days and 3 animals became normal after 3 days of treatment. However in one animal improvement was noticed but complete recovery could not be seen.

In Nitric acid treated group, recovery was observed in 2 animals only and hence the treatment was discontinued after 3 days in remaining 4 animals when no improvement was noticed.

In group D, 2 cows recovered completely after 2 days and 4 animals showed recovery after 3 days of treatment. The details of percent recovery is shown in table 2.

The use of homeopathic single medicines in the management of mastitic dairy cows has previously been reported by a number of workers with variable results from various parts of the world (Upadhya and Sharma, 1999). The results of the present study are in agreement

Table 1: Treatment protocol in different groups

Group	Number of animals	Treatment given
A	6	Ipecac 200 C potency QID for 5 days
B	6	Buforana 200 C potency QID for 5 days
C	6	Nitric acid 200 C potency QID for 3 days
D	6	Inj Styptochrome 10ml IM OD

Table 2: Recovery rate in different treatment groups

Groups	% Recovery			
	Total	Day1	Day2	Day3
A	83.33	Improvement	66.66	83.33
B	83.33	16.67	33.33	83.33
C	33.33	Improvement in 1	Improvement in 1	33.33
D	100	Improvement	33.33	100

with Ruddock, 1999 who earlier suggested the oral use of homeopathic medicines. Singh, *et. al.,* 2004 reported 100% recovery after 2 subcutaneous injections of ipecacuanha. Varshney and Naresh (2004) reported 100% cure rate in the treatment of blood in milk with an average recovery period of 4 days using a homeopathic complex of which ipecacuanha 30 C was one component. Chandel *et. al.* (2009) observed 94.52% efficacy in treating blood in milk after oral administration of homeopathic complex medicine.

Homeopathic medicines ipecacuanha and buforana proved highly effective in the treatment of hemogalactia when administered per os.

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Effect of high level of roughage in total mixed ration on health and performance of crossebred cattle and buffaloe

S.K. Sinha, V.B. Chaturvedi, P. Singh, Swati Shivani*[1]
Energy Metabolism and Respiration Calorimetry Laboratory, Division of Animal Nutrition
Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, U.P.

ABSTRACT

Twelve adult crossbred cattle and buffalo were divided into 3 equal groups (T_1, T_2 and T_3) each consisting two crossbred cattle, and two buffalo were fed experimental diets in 3×3 switchover design for 28 days respectively. The concentrate and roughage (wheat straw: green maize) ratio in the diet of groups T_1, T_2 and T_3 was 60:20:20, 40:30:30 and 20:40:40, respectively. Daily dry matter intake was significantly ($P<0.01$) higher in group T_1 than in group T_3, which also resulted in significantly higher ($P<0.01$) total body weight change in the former group. Protein, albumin, globulin, Hb and PCV in blood were comparable in the treatment groups and both species but ALT and AST level was higher in the buffalo than crossbred cattle. The level of glucose and blood urea nitrogen was comparable between both species and their treatment groups. There was no difference in serum calcium and inorganic phosphorus between the treatments and species, which indicated that body health status of animals is not significantly influenced by restricted concentrate feeding. Blood was collected at the end of all three metabolic trials. All haemato-biochemical changes were within the normal range and these values were not being affected by dietary treatment.

Keywords: Biochemical, buffalo, cattle, concentrate, haematological, roughage

In our country both cattle and buffalo are maintained mainly as dairy animals. They play an important role in farmer's economic life, being an integral part of the farming system. Being ruminants they both have certain similarities in nature and type of feed intake digestion and metabolism. They have the unique ability to utilise both roughages and concentrate to meet their body demand for maintenance and production. There was not much difference in the digestive tract of the cattle and buffalo as both have fully developed rumen and the gastrointestinal tract which is also the similar. Generally livestock owners in India belong to the low-income group and they are not able to feed the recommended amount of concentrate to their animals. Straw and other fibrous feeds like green maize, berseem were commonly available as major feed resources for ruminants. The accurate determination of metabolism is important in the formulation of animal diets to promote efficient production. Previous studies have shown that the concentrate content of diets positively affects voluntary feed intake, health status and body weight change of ruminants (Johnson *et al.* 1992; Murphy *et al.* 1994). Malnutrition of the animals may result in lower productivity and lower disease resistance. A protein and energy deficient diet may adversely affect the health status of growing animals (Gribell *et al.* 1987). Therefore, the present study was conducted to evaluate the effect of high roughage and low roughage based total mixed diets on health status of crossbred cattle and buffalo. The objective of this study was to evaluate the health status and blood metabolites of two different genetic groups fed with the different ratio of diets based on concentrate: wheat straw: green maize.

Materials and Methods

Six adult male crossbred cattle (*Bos taurus* × *Bos indicus*) and six adult male buffalo (*Bubalus bubalis*) age 12-18 months with average body weight of 244.77±10.56 and 297.11±14.39 kg, respectively were distributed in three dietary groups(2each) and were fed on experimental diets in 3×3 switch-over design. Wheat straw and green maize was used as the basal roughage. Experimental feeding consisted of three ratio of concentrate (C), wheat straw (W) and green maize (G) i.e. 60C:20W:20G (T_1), 40C:30W:30G (T_2) and 20C:40W:40G (T_3) fed as TMR diets alternately to each group. Animals were fed *ad libitum* three TMR diets consisting of concentrate mixture (crushed maize grain, 37%; solvent extracted soybean meal, 20%; wheat bran, 40%; mineral mixture, 2% and salt, 1%.), wheat straw and green fodder (Maize) in define ratios. All the animals were fed *ad lib*. Clean drinking water was offered to the animals twice daily in the morning and evening. Blood samples were collected from all animals of three trials after 28 days of experimental feeding respectively.

***Corresponding Author**- Ph.D Scholar, Division of Animal Nutrition, IVRI Izatnagar.
[1] Ph.D Scholar, Division of Animal Nutrition, NDRI, Karnal.

Blood was collected from each animal through jugular vein puncture in clean and dry vials containing heparin as anticoagulant for haematological analysis whereas in plain glass tube for sepration of serum which was stored at -20-°C till analysis.

Haematocrit or packed cell volume (PCV) and total haemoglobin were estimated by standard method as per Jain (1986).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were determined as per the method given by Reitman and Frankel, (1957), total protein by Doumas, (1975), albumin by Gustafsson, (1978), Glucose by (Trinder, 1969), Urea by Rahmatullah and Boyd, (1980) using commercially available kits.

The data were analysed using the statistical software SPSS (version 20.0). All data were subjected to ANOVA and differences among treatments were analysed by Duncan's Multiple Range using Generalized Linear Model (Snedecor and Cochran, 1967).

Results and discussion

The concentrate mixture, wheat straw and green maize contained 92.51, 92.65 & 91.21% organic matter, 20.13, 2.95 & 9.22% crude protein, 37.36, 74.52 & 62.97% neutral detergent fibre, 10.35, 52.84 & 39.61% acid detergent fibre and 4.24, 3.71, 4.04 kcal gross energy per gram dry matter (DM), respectively. Daily DM intake was significantly higher ($P<0.01$) in the group T_1 (2.62±0.05 kg/100 kg BW or 105.49±1.76 g/$kg^{0.75}$) compared to group T_3 (2.13±0.08 kg/100 kg BW or 86.33±2.35 g/$kg^{0.75}$) owing to the higher concentrate content in the former group (Slabbert *et al.* 1992; Johnson *et al.* 1992). This difference in feed intake was reflected in body weight change in crossbred cattle and buffalo. There is a positive relationship between body weight change and feed intake that increases with an increase in dietary concentrate intake by ruminants (Mallikarjunappa *et al.* 1983; Nachtomi *et al.* 1991).

The results of various blood haematological and biochemical constituents presented in Table- 3 and 4 and discussed below. The haematological parameters like Hb and PCV are indicators of erythrocytes normality and general well being of animals. There was non-significant difference among the two groups for blood haemoglobin levels (g/dL) and haematocrit or packed cell volume (%) i.e. 13.02±0.57 and 32.61±1.03 in crossbred cattle and 13.93±0.79 and 34.78±0.85 in buffalo, respectively (Table- 3). Among the treatments haemoglobin levels (g/dL) were non-significant with each other. There was non-significant gradual decrease in mean values of PCV with decreasing amount of concentrate in diet. The packed cell volume (%) was found to be non-significant between the respective treatments. The Hb values in all the three groups were in normal range (Singh *et al.* 1988). The obtained values of Hb and PCV were within normal range of 8-15 g/dl and 28-37% (Kaneko *et al.* 1997). The blood biochemical parameters of this study indicated that cattle and buffalo can well accept the total mixed ration diets as different ratios without any adverse effect on their physiology.

The ALT and AST is both cytoplasmic and mitochondrial enzyme, which is released even by mild degenerative changes (Evans and Whitehorn, 1995). The activity of AST and ALT is an indicator of damage to liver and muscles (Casteel, 1994). The Alanine aminotransferase ALT (GPT) activity (IU/L) was significant among the different treatment within the groups or it was varied significantly ($P<0.05$) between

Table 1: Chemical composition (%) of feeds and fodder offered to crossbred cattle and buffalo

Attributes	Concentrate Mixture(C)	Wheat Straw(W)	Green Maize(G)
Proximate components			
OM	92.51	92.65	91.21
CP	20.13	2.95	9.22
Cell wall components			
NDF	37.36	74.52	62.97
ADF	10.35	52.84	39.61
Energy Value			
GE (Kcal/g)	4.24	3.71	4.04

OM = Organic matter; CP = Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; GE = Gross energy; C=Concentrate; W =Wheat straw; G=Green maize.

Table 2: Intake of Dry Matter in Crossbred Cattle and Buffalo Fed Various TMR Diets

60C:20W:20G	40C:30W:30G	20C:40W:40G	Mean ± SE		SEM	P-Values		
T1	T2	T3	Cattle	Buffalo		T	S	T×S
Total Dry Matter Intake (kg/d)								
6.93^{b}±0.34	6.71^{ab}±0.34	5.83^{a}±0.32	5.91^{q}±0.27	7.06^{p}±0.25	0.20	*	**	NS
Dry Matter Intake (g/kg$W^{0.75}$)								
105.49^{b}±1.76	101.52^{b}±2.77	86.33^{a}±2.35	95.79±2.88	99.77±2.51	1.91	**	NS	NS
Dry Matter Intake (kg/100 kg BW)								
2.62^{b}±0.05	2.52^{b}±0.06	2.13^{a}±0.08	2.43±0.07	2.42±0.07	0.05	**	NS	NS

Mean bearing different superscripts in a column and row differ significantly, *P<0.05; **P<0.01; SEM, standard error of the mean (n=36); T = Dietary treatment; S = Species (Crossbred cattle and buffalo); T×S = Interaction between species and dietary treatments; C=Concentrate; W =Wheat straw; G=Green maize; Kg/d = kilograms per day; BW = Body weight; g/kg$W^{0.75}$ = Grams nutrient per kg metabolic body weight.

Table 3: Effect in Haematological Profile in Crossbred Cattle and Buffalo Fed Various TMR Diets

60C:20W:20G	40C:30W:30G	20C:40W:40G	Mean ± SE		SEM	P-Values		
T1	T2	T3	Cattle	Buffalo		T	S	T×S
Haemoglobin (g/dL)								
13.62±0.67	13.86±0.71	12.95±1.13	13.02±0.57	13.93±0.79	0.49	NS	NS	NS
Packed cell Volume (PCV %)								
35.25±1.18	33.58±1.26	32.25±1.14	32.61±1.03	34.78±0.85	0.68	NS	NS	NS

Mean bearing different superscripts in a column and row differ significantly, *P<0.05; **P<0.01; SEM, standard error of the mean (n=36); T = Dietary treatment; S = Species (Crossbred cattle and buffalo); T×S = Interaction between species and dietary treatments; C=Concentrate; W =Wheat straw; G=Green maize; g/dL = Grams per decilitre; PCV = Packed cell volume.

the two species i.e. crossbred cattle (25.64±1.38) and buffalo (30.29±1.86) being higher for buffalo than crossbred cattle. The ALT activity was higher due to higher intake of total dry matter intake. Among the various treatments it was non-significant for T_1 and T_2 and both groups are significant with T_3 group. Further, none of these parameters were outside the normal ranges SGPT: 11-40 and SGOT: 78-132 IU/L) suggested for bovines (Kaneko *et al.* 1997). The AST (GOT) activity (IU/L) was 70.64±2.98 and 95.23±3.43 in crossbred cattle and buffalo groups, respectively and was higher in buffalo than cross cattle. The AST activity was found to be non-significant between the respective treatments being as depicted in the Table- 4.

There was non-significant difference between the serum Total protein (g/dL) level of group buffalo (7.38±0.13) and group crossbred cattle (6.99±0.18) and serum Total protein level was also non-significant within the treatments T_1 (7.16±0.20), T_2 (7.23±0.25) and T_3 (7.17±0.16), respectively, as given in Table- 4. There was non-significant result between group and species interaction for serum total protein (g/dL). These values were within the normal range as suggested by Kaneko *et al*. (1999). The serum protein level indicates the balance between anabolism and catabolism of protein in body. The plasma protein concentration at any given time in turn is a function of hormonal balances, nutritional status, water balance and other factors affecting the state of health. There was a general increase in total protein, a small decrease in albumin and an increase in globulin and advancing age in all the species of animals (Tumbleson *et al*. 1972; Jain, 1986).

For albumin (g/dL) there was non-significant difference among the two species i.e. 3.380±0.068 in crossbred cattle and 3.29±0.06 in buffalo. There was significant difference in the serum albumin (g/dL) level of cattle and buffalo when given three different treatments of concentrate and roughage being higher in T_1 (3.45±0.06) and lowest in T_3 (3.19±0.09) and T_2 (3.35±0.08) lied between them and non-significant both of them. Albumin is a major labile storage reservoir of proteins and also transporter of its constituent amino acids. Its synthesis is diminished during fasting, malnutrition, hormonal imbalance and poor condition of liver (Jain, 1986). The mean values of serum albumin were comparable to normal range of 3 to 3.8 g/dL. These observations were in parallel with the findings of Sahoo *et al.* (1999), Mondal *et al.* (1996) and Pattaniak, (1997) in young crossbred calves. There was non-significant result in the serum globulin (g/dL) level of cattle and

Table 4: Effect in Blood Biochemical Profile in Crossbred Cattle and Buffalo Fed Various TMR Diets

	60C:20W:20G	40C:30W:30G	20C:40W:40G	Mean ± SE		SEM	P-Values		
	T1	T2	T3	Cattle	Buffalo		T	S	T×S
ALT	31.00^{b}±1.67	29.21^{b}±2.42	23.68^{a}±1.61	25.64^{q}±1.38	30.29^{p}±1.86	1.20	*	*	NS
AST	84.26±5.85	86.38±3.46	78.16±6.29	70.64^{q}±2.98	95.23^{p}±3.43	3.05	NS	**	NS
Total Protein	7.16±0.20	7.23±0.25	7.17±0.16	6.99±0.18	7.38±0.13	0.12	NS	NS	NS
Albumin	3.45^{b}±0.06	3.35^{ab}±0.08	3.19^{a}±0.09	3.38±0.06	3.29±0.06	0.04	*	NS	NS
Globulin	3.77±0.25	3.81±0.25	3.97±0.21	3.61±0.21	4.09±0.15	0.13	NS	NS	NS
A:G Ratio	0.98±0.09	0.95±0.10	0.84±0.06	1.02^{p}±0.08	0.83^{q}±0.04	0.05	NS	*	NS
Glucose	76.03±4.82	71.27±7.19	66.63±6.25	74.36±5.56	68.26±4.34	3.51	NS	NS	NS
BUN	25.64±0.86	26.47±0.66	25.39±0.96	25.97±0.58	25.70±0.77	0.47	NS	NS	NS
Ca	9.24±0.20	9.12±0.21	8.88±0.14	9.05±0.17	9.10±0.12	0.10	NS	NS	NS
P	4.91±0.11	4.33±0.24	4.40±0.39	4.77±0.19	4.33±0.25	0.16	NS	NS	NS

Mean bearing different superscripts in a column and row differ significantly, *P<0.05; **P<0.01; SEM, standard error of the mean (n=36); T = Dietary treatment; S = Species (Crossbred cattle and buffalo); T×S = Interaction between species and dietary treatments; C=Concentrate; W =Wheat straw; G=Green maize; IU/L = International unit per litre; mg/dL = Milligrams per decilitre; A: G = Albumin: Globulin.

Table- 5: Change in Body Weight (kg) of Crossbred Cattle and Buffalo fed during experimental diets

Diet	Crossbred Cattle			Buffalo		
	Initial (kg)	Final (kg)	Change in body wt. (kg)	Initial (kg)	Final (kg)	Change in body wt. (kg)
T1	241.33±19.79	251.16±19.43	9.83±0.98	282.33±18.59	294.16±17.48	11.83±1.27
T2	236.50±20.28	244.50±19.03	8.00±1.26	287.33±22.51	296.66±21.67	9.33±0.88
T3	243.66±20.04	251.33±18.18	7.66±2.49	307.16±35.90	314.00±34.76	7.33±1.33

Kg = Kilograms.

buffalo (3.61±0.21 and 4.09±0.15) when given three different treatments of concentrate and roughage. For globulin (g/dL) also there was non-significant difference among the three treatment groups. The albumin globulin ratio was obtained in accordance with all the others above as there was significant (P<0.05) difference in A: G ratio among the crossbred cattle i.e. (1.02±0.08) and buffalo (0.83±0.04), respectively and within treatment groups they all were non-significant difference with each other. The values found in this study were similar to those described by Rebhun and Guard, (2000), which are 6.00 to 8.50 g/dL for total protein and 5.03 to 5.55 g/dL for albumin.

Serum glucose level is an indicator of the physiological condition of the animals. An increased or decreased level of serum glucose is an indicator of stress to the animals. The serum glucose level (mg/dL) was found to be non-significant between cattle and buffalo (74.36±5.56 and 68.26±4.34) and it was also found non-significant within treatment T_1, T_2 and T_3 in both crossbred cattle and buffalo i.e. (76.03±4.82), (71.27±7.19) and (66.63±6.25), respectively. Data revealed to glucose levels obtained in this study showed similar values than those mentioned by Tiwari *et al.* (2001) who observed glucose levels of 51 to 64 mg/dL for growing buffalo and from 74 to 76 mg/dL for Holstein cattle. Kaneko *et al.* (1997) reported range of glucose concentrations for bovines 45-75 mg/dL. All the effect occurs in according to the plane of nutrition.

Blood urea N level is an indicator of protein degradation in rumen. There was non-significant difference in the serum urea level (mg/dL) of crossbred cattle and buffalo (25.97±0.58 and 25.70±0.77) when fed three different TMR diets of concentrate and roughage the results were comparable in T_1 (25.64±0.86), T_2 (26.47±0.66) and T_3 (25.39±0.96). As protein is the major source of ammonia for urea synthesis, the rate of urea formation depends on the rate of protein (i.e. amino acids) catabolism and the rate of utilization of NH_3 for bacterial protein synthesis. An increase in blood urea nitrogen may reflect the accelerated protein catabolism in the liver, and increased urinary excretion of urea of urea nitrogen (Kaneko *et al.* 1999). High and low plasma urea levels in cattle and buffalo are indicative of high and low nitrogen intake and thus reflected their protein status. Norton *et al.*

(1979) reported that plasma urea concentration was higher in water buffalo than cattle, and suggested higher renal resumption of urea in water buffalo. The level of serum calcium (mg/dL) and inorganic phosphorus was in crossbred cattle and buffalo were comparable with each other and their respective treatment groups.

The restricted concentrate feeding in this experiment had no adverse effect on body health status as described by Lebengarts, (1986). From this experiment it appears that even a reasonably low level of concentrate feeding to crossbred cattle and buffalo may provide a satisfactory level of protection against malnutrition.

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The forthcoming 33rd Annual Convention and International Symposium of the Society is scheduled to be held during 22 -24th Jan. 2015 on Theme: **"NEW DIMENSIONS IN VETERINARY MEDICINE: TECHNOLOGICAL ADVANCES, ONE HEALTH CONCEPT AND ANIMAL WELFARE CONCERN"**. This mega event is being organized jointly by the Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Trissur, Kerala and the Organizing Committee College Of Veterianry and Animal Sciences Pookode, Lakkidi P O, Wayanad - 673576. KERALA as well as ISVM. On this momentous occasion, you are invited to attend the scheduled International Symposium at College of Veterianry and Animal Sciences Pookode, Lakkidi, P O, Wayanad- 673576, KERALA. The contact details of the Organizing Committee are as follows:
Dr. Dr. P. C. Alex (Convener): Mob 09446381785, Dr. S. Ajithkumar, (Organizing Secretary) : 09400322511,
Dr. Usha Narayana Pillai (Co-Organizing Secretary): 09947659891, 09847803271 and Dr. P.V. Tresamol,
Co-Organizing Secretaries, Email: isvm2015@gmail.com and Website: www.isvm2015.in.

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Therapeutic efficacy of herbal drugs in treating caprine mastitis

Arpana Raikwar and P.C. Shukla
Department of Veterinary Medicine,
College of Veterinary Science and Animal Husbandary, Jabalpur -482001, M.P.

Abstract

In the present study, a total of 24 mastitic does randomly selected and divided into 4 groups, had been treated with combinations of herbal drugs *Tinospora cardifolia* and *Eclipta alba* along with wisprec spray. Enrofloxacin is taken as standard drug. The combination of *Tinospora cardifolia (Guduchi / Giloe*) + *Eclipta alba (Bhringraj*) were found to be comparable in effectiveness with Enrofloxacin in treating mastitis.

Keywords: *Eclipta alba* , goat, Mastitis, *Tinospora cordifolia*

Mastitis is an inflammation of the mammary gland leading to a chemical and physical reaction in mammary tissues and milk produced by goats (Radostits et al., 2000). The infectious agents enter through the milk canal, interact with the mammary tissue cells and multiply (Green and Bradley, 2004). Mastitis in clinical and subclinical form is a major cause of loss in milk production to goat husbandry, eventually incurring economic losses to the poor farmers. According to Zamin *et al*. (2010), mastitis has caused colossal damage to livestock production by increasing the culling of morbid animals due to poor management and lack of therapeutics and control measures.

Material and Methods

The present investigation was conducted at livestock farm, Adhartal and private goat keepers in the nearby areas of Jabalpur. In this study, 24 mastitic does had been divided into 4 groups each containing 6 animals, the groups were named as G1, G2, G3 and G4. They were subjected to clinical examination of udder, physical and microscopic examination of milk i.e. Somatic Cell count (SCC), Total Viable count (TVC) and Coliform count (CC) before and after the treatment were noticed. Group G1 does were treated with Enrofloxacin @10mg/kg b.wt orally along with topical application of Wisprec spray. G2 animals were treated with herbal drug *T. cardifolia* @ 400mg/kg b.wt by orally, along with topical application of Wisprec spray. G3 animals were treated with *E. alba* @ 400mg/kg b.wt by orally, along with topial application of Wisprec spray. G4 animals were treated with the combination of herbal drugs *T. cardifolia* and *E. alba* @ 200mg/ kg b.wt by orally for 5 days in all the cases.

Results and Discussion

In group G1, a total of 6 animals and 8 halves were treated for 5 days. The curative effect obtained on animal basis was 83.33% and on half basis it was 87.5%. These findings are suggestive of the fact that the combination used during the study was proved to be effective in curing the mastitis. It has been also observed during the study that administration of drugs will help in lowering down SCC, TVC and Coliform count (Table no. I, II &III) as reported by Rao *et al.* (2002)

In group G2 animals were treated with a herbal drug *T. cardifolia* orally along with wisprec spray topically as per the schedule. A total of 6 animals and 7 halves were treated for 5 days. The drug were found to be 66.6% effective on animal basis and 71.4% on halves basis. These findings also of the opinion that this combination is

Table 1: Effect of treatment on Sometic cell count in milk

Groups	Pre-treatment SCC(10^5 cells/ml)		Post-treatment SCC(10^5 cells/ml)		t - value
	Range	Mean ± SE	Range	Mean ± SE	
G1	37.45 – 70.24	54.7± 5.06	12.23 – 20.25	17.3 ± 2.21 *	6.22
G2	40.98 – 80.02	61.6±4.42	22.81 – 44.20	34.51±3.85	3.85
G3	35.59 – 67.90	51.02±3.91	18.52 – 39.42	31.6±1.97	6.70
G4	37.22 – 71.01	50.2±3.32	12.22 – 23.77	19.1±1.32 *	5.66

Significant ($P<0.05$), SE – Standard Error, SCC – Sometic Cell Count

Table 2: Effect of treatment on Total Viable count (10^3 CFU /ml) in milk.

Groups	Pre-treatment TVC (10^3 CFU /ml)		Post-treatment TVC (10^3 CFU /ml)		t - value
	Range	Mean ± SE	Range	Mean ± SE	
G1	9.0 – 18.4	13.3± 6.82	3.1 – 7.9	5.5 ± 3.89 *	10.14
G2	25.3 – 30.5	27.7±4.89	14.2 – 23.6	18.1±4.24	10.62
G3	23.1 – 30.4	27.4±5.42	17.0 – 23.9	21.5±4.51	11.40
G4	16.2 – 24.1	18.1±6.47	10.0 – 17.5	9.9±4.54 *	14.12

Significant (P<0.05), SE – Standard Error, TVC – Total Viable Count

Table 3: Effect of treatment on Coliform Count (10^3 CFU /ml) in milk

Groups	Pre-treatment CC (10^3 CFU /ml)		Post-treatment CC (10^3 CFU /ml)		t - value
	Range	Mean ± SE	Range	Mean ± SE	
G1	2.1 – 9.7	4.0± 1.58	0.3 – 2.5	1.3 ± 0.61 *	6.40
G2	1.6 – 8.7	4.5±1.42	1.0 – 4.2	2.0±0.22	7.24
G3	2.5 – 8.8	4.9±1.63	1.8 – 4.4	2.5±0.28	8.04
G4	2.5 – 8.4	4.1±1.17	0.8 – 3.8	1.7±0.38 *	12.59

*Significant (P<0.05), SE-Standard Error, CC-Coliform Count, CFU- Colony Forming Unit

modrately effective in treating the mastitis. These findings correlates with the Ranjan, *et al.* (2006) who reported ameliorative potential of *T.Cardifolia.* In group G3 animals were treated with herbal drug *E. alba* orally along with wisprec spray topically is givin as per the schedule. A total of 6 animals and 7 halves were treated for 5 days. The overall effectiveness was found to be 50% on animal basis and 57.1% on half basis, in treating mastitis in goat as also observed by Vishnu *et al.* (2010). In group G4 the animals were treated with a combination of herbal drugs *T. cardifolia* and *E. alba* orally along with wisprec spray topically as per the schedule. A total of 6 animals and 9 halves were treated. This combination was found to be 83.33% effective on animal basis and 77.7% on half basis. The same effect was observed by Jawahar, *et al.* (1997).

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Clinical Article

Babesia canis infection in a Labrador pup- A clinical case report

S. Saravanan[1], K. M. Palanivel[2] K. Senthilvel[3] and T. Sathyabama[4]
Department of Veterinary Epidemiology and Preventive Medicine,
Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu

Abstract

A five months old male Labrador pup manifested signs suggestive of babesiosis and examination of the peripheral capillary blood smear revealed B. *canis* and ticks from the pup were identified as *Rhipicephalus sanguineus.* Haematocrit and serum biochemical analysis revealed a reduction in haemoglobin level, RBC count and glucose, and increased urea, creatinine and phosphorous. Diminazene aceturate was found to be effective along with supportive therapy.

Key words: *Babesia canis*, *Rhipicephalus sanguineus*, Diminazene aceturate

Canine babesiosis caused by *Babesia. canis* and *B. gibsoni* is a haemolytic disease of worldwide significance as a cause of biphasic fever, haemolytic anaemia, icterus, haemoglobinuria and death. The large *Babesia*, *B. canis* (4–5 µm) is the widespread species in Europe and Asia and is of intermediate pathogenicity and the signs can range from chronic to per acute and fatal, depending on the virulence of the species and the susceptibility of the host (Schoeman, 2009). The disease affects mostly puppies although dogs of all ages can be affected. However, *B. canis* infection is rare and the information is scanty in India, as the incidence was only 0.65% when compared to *B. gibsoni* (8.26%) among haemoprtozoan infections (Harkirat Singh *et al*., 2012). Hence, the present study was undertaken to study a rare case of babesiosis in a young Labrador Retriever in Namakkal area.

Case history and observations

A five months old male Labrador pup was brought to the Infectious Diseases Ward at Teaching Veterinary Clinical Complex (TVCC), Veterinary College and Research Institute, Namakkal with a history of anorexia, pyrexia (39.5ºC), vomition, greenish diarrhea, small ecchymotic patches on the lower abdomen (Figure 1), blanched conjunctival mucous membrane, popliteal lymphadenitis, hair loss and tick infestation on foot and inside the ears.

A thin blood smear using a drop of blood from the peripheral ear vein was prepared on a clean glass slide, fixed in methanol and stained with Giemsa (Coles, 1986) for microscopic examination under oil immersion objective. Blood samples were collected aseptically with and without EDTA for complete blood cell count and determining the changes in the serum biochemical values.

Treatment and discussion

Diagnosis of acute babesiosis (*B. canis*) was made based on the classic clinical presentation and the demonstration of the piroplasms within red blood cells from thin capillary blood smears and the intraerythrocytic merozoites of the large Babesia (Figure 2) with similar reproducible characteristics as paired bodies with pear shape could be observed. The tick collected from the clinical case was identified to be *Rhipicephalus sanguineus as* Lobetti (2006) reported that *R. sanguineus*, *Dermacentor spp*. and *Haemaphysalis spp.* can transmit the large babesia in dogs.

In the present study, it could be inferred that very young aged pup was found to be at risk, however, Harkirat Singh *et al*. (2012) observed *B. canis* infection in dogs above 6 months of age. The purpural lesion observed in this study might be due to intravascular and extravascular haemolysis. Similar finding was also reported by Furlanello *et al.* (2005). The clinical signs observed in the present investigation concurred with the reports of Mohr *et al.* (2000) who described icterus, vomition and diarrhea in gut form of babesiosis.

The values of haemoglobin (7.3 gm%), PCV (28%) and RBC count (3.6 lakh/cmm), and the serum biochemical values of glucose (62.5 mg/dl), total protein (8.1g/dl), albumin (2.3 g/dl), BUN (15 mg/dl), creatinine (3 mg/dl), calcium (8 mg/dl) and phosphorous (11 mg/dl) obtained in this study were similar to the earlier

[1]Assistant Professor (sarvet_25@yahoo.com) & [2]Professor & Head. [3]Associate Professor, Dept. of Veterinary Parasitology & [4]Assistant Professor, Dept. of Veterinary Biochemistry, Veterinary College and Research Institute, Namakkal-2.

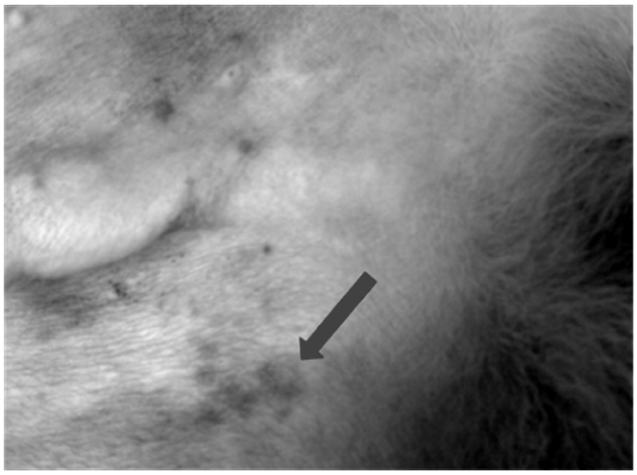

Fig. 1 Ecchymotic patches in the lower abdomen of 5 months old pup infected with *B. canis*

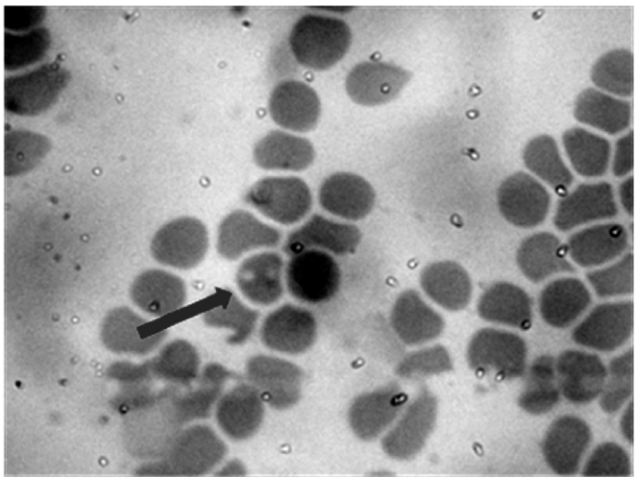

Fig. 2 Intraerythrocytic *B. canis* (large Babesia) in a *Labrador Retriever* pup (x1000)

reports (Keller *et al*. 2004). However, hypoglycaemia, hyperkalemia, and elevated urea and creatinine levels were similar to the findings of De Scally *et al*. (2006) and Keller *et al.* (2004), and this could possibly be due to the altered renal function and glomerular filtration. The pup responded to a single injection of Diminazene aceturate (Berenil) @.5 mg/ kg bodyweight, deep I/M with supportive treatment including multivitamins and fluids (Miller *et al*., 2005). Control of the ticks by acaricide spray or using tick collars is the only effective way in preventing the exposure of young dogs to the 'window of vulnerability' due to this pathogenic *Babesia* species.

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Caecal impaction in Kathiawari horse and its management

P. Pothiappan, S. Parthiban[1], B. Gowri, M. Thangapandiyan[2]and S. R. Srinivasan[2]
Resident Veterinary Services Section, Madras Veterinary College
Chennai- 600 007, Tamil Nadu

Abstract

A 10 year old Kathiawari horse was brought to the Madras Veterinary College Teaching Hospital with the history of frequent lying down, rolling on the floor and absence of defecation. On examination of the animal was found to be dull and depressed, congested mucous membrance. The animal had an elevated heart rate (78/minute) and respiratory rate of 28/minute. On rectal examination the caecum was distended and hard in nature. Auscultation of the right flank revealed no borborygmous sound. The hematological and biochemical parameters were within the normal range. The case was tentatively diagnosed as colic due to caecal impaction. The horse was treated with fluid therapy and Flunixin meglumine@1.1mg / kg b.wt IV and liquid paraffin @10ml / kg body weight through nasogastric intubation. Animal was under fluid therapy for the next 5 days and the horse had an uneventful recovery.

Key words: Colic, Caecal impaction, Horse

The caecum in the horse is a large blind end portion of the gastrointestinal system between the ileum and the large colon. A primary impaction is when the bowel becomes full of ingesta and is very firm upon palpation. This type of colic has an insidious onset usually over several days and the horse will have a significant decrease in fecal output and exhibit mild but progressive signs of discomfort (Blikslager, 2009). Caecal impactions, as opposed to large colon impactions, have the propensity to rupture which is always fatal. A secondary caecal impaction is when the bowel becomes distended with fluid ingestia. This may be more appropriately called caecal dysfunction resulting from abnormal motility. The reasons for impaired motility are unclear but may be related to postoperative pain or associated with nonsteroidal anti-inflammatory drugs (Little *et al.,* 2001 and Sellon *et al*., 2004). Factors associated with impactions include poor dentition, lack of access to water, coarse feeds, acute cessation of routine exercise with confinement, and treatment for musculoskeletal diseases (White and Lopes, 2003). The present paper discusses the caecal impaction and its emergency medical management in kathiawari horse.

Case history and observations

A 10 year old kathiawari gelding horse was brought to the Madras Veterinary College Teaching Hospital with the history of frequent lying down, rolling on the floor (Fig:1) and absence of defecation. On clinical examination, the animal was found to be dull and depressed, congested mucous membrane. The animal had an elevated heart rate (78/minute) and respiratory rate of 28/minute. Auscultation of the right flank revealed no borborygmous sound. Rectal examination revealed a firm, distended, harden caecum, faeces was hard like a bolus and indentable mass in the caecal base. The hematological and biochemical parameters were within the normal range. Based on the history, clinical observation and rectal examination the case was diagnosed caecal impaction.

Treatment and Discussion

The affected horse was treated with Inj. Flunixin meglumine@1.1mg / kg b.wt i.v, Inj. RL 10 ml/kg b.wt i.v, and rectal enema 3L of paraffin mixed water was given. On nasogastric intubation there is no gastric reflux and liquid paraffin @10ml / kg body weight was administered through nasogastric intubation (Fig: 2). Animal was under fluid therapy for the next 5 days and the horse had an uneventful recovery. Medical therapy can be successful for treating solid-filled impactions. Early detection is important because horses with caecal impaction need either a greater degree of medical attention on the farm or early referral for further treatment, possibly including surgery. The basic premise for treating colon impaction is relieving pain, softening the consistency of the impacted ingesta, and stimulating motility to increase fecal transit. Flunixin meglumine (flunixin) is the most effective of the non steroidal anti-inflamatory drug (NSAID) used to treat acute abdominal disease in the horse. It blocks the production of

[1]Assistant professor, Dept of Microbiology, VC& RI, Tirunalveli.
[2]Assistant professor, Dept. of pathology, Madras Veterinary College, Chennai-7.

Fig. 1 Rolling on ground

Fig. 2 Nasogastric intubation

prostaglandins, specifically thromboxane and prostacyclin, for 8 to 12 hours after a single dose (Blikslager, 2009).

Horses with solid-filled caecal impactions should be well hydrated for detectable dehydration (>6% of body weight), intravenous fluids should be administered. The most important aspects of fluid therapy are increasing circulating volume and rehydrating tissues, which can be accomplished by using a balanced electrolyte solution. Recent research has shown that while intravenous fluid therapy is critical for horses with marked hypovolemia (White and Dabareiner, 1997) Additional treatments include laxatives, such as mineral oil has been shown to be more effective for hydrating intestinal contents, whereas mineral oil lubricates impactions but tends to move around them. Mineral oil should be seen around the anus 18 to 24 hours after administration if gastrointestinal motility is normal (Blikslager, 2009).

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Lead toxicity in a two year old heifer- a case report

Ipsita Mohanty, Amit Raj Gupta[1] and Kautuk Kumar Sardar
Department of Veterinary Pharmacology and Toxicology
College of Veterinary Science and Animal Husbandry
Orissa University of Agriculture and Technology, Bhubaneswar-751003, Odisha.

Abstract

A two year old, crossbred Jersey heifer weighing around 200 kg was presented to the Teaching Veterinary Clinical Complex, OUAT, Bhubaneswar with history of ingestion of lead paint, anorexia, restlessness, hyper-reflexia, staggering gait and diarrhoea. Physical examination revealed dullness and depression with forced breathing, heavy jugular pulse, lacrimation and papillary dilation. Haematological examination revealed a fall in blood haemoglobin, TEC, TLC and rise in PCV and ESR level. The biochemical analysis showed significant decrease in blood glucose, L.H. and F.S.H level and increase in AST, ALT level without any change in the serum total protein, urea and creatinine level. Sodium calcium edentate was used as the preferred antidote against lead poisoning. Atropine Sulphate counteracted cholinomimetic symptoms and Vitamin B supplement was used as nervine tonic. The poisoning was controlled within 15 days post-treatment with complete recovery.

Key words: Lead toxicity, Therapeutic management, Cattle.

Lead poisoning is a matter of great concern for veterinarians and clinicians all around the globe, not only because of severe economic loss and food safety concerns, but also due to its adverse effects towards public health (Galey *et al*., 1990; Gordon *et al*., 2001). Acute lead toxicity in the young heifers is a commonly occurring phenomenon which is due to inquisite nature of the young heifers who are attracted towards the salty taste of lead contaminated feed or old batteries, paints etc. Morever, cow's reticulum is a honey comb like compartment which traps ingested foreign objects and slowly dissolve and release the lead into the cow's body.

It is the single toxicological problem which has ever received widespread attention from the point of view of basic research, therapeutics and control (Hammond and Aronson, 1964). Poisoning in animal populations may serve as a sentinel to assess the extent of environmental contamination and human health problems related to lead (Sharaf *et al*., 2008). From a public health point of view, the danger from lead in the environment continues to be a matter of concern, for their subtle effects on intelligence quotient and blood pressure (Gordon *et al*., 2001). This article reports a case of lead poisoning in a 2 year old heifer and efforts to provide a better understanding of this disease at a rather intensive level.

A 2 year old, crossbred Jersey heifer weighing around 200 kg with history of ingestion of lead paint about 12-14 hours back was presented to the Teaching Veterinary Clinical Complex, OUAT, Bhubaneswar, with clinical signs of dullness, depression, anorexia, restlessness, hyper-reflexia, forced breathing, lacrimation, papillary dilation, and diarrhoea. The cow was walking through fences with uncoordinated movement and staggering gait and falling down with head pressing behavior. Clinical examination revealed hyperthermia (104°F), increased respiration rate (38/ min) and heavy jugular pulse (80/ min).

Blood was collected from jugular venepuncture in EDTA for haematological examination (Iain, 1986). Part of the blood was collected in EDTA free vial and serum was harvested after clotting of blood for biochemical estimation. Glucose, total protein, urea, creatinine, AST and ALT were estimated using commercially available kits.

In the present study, haematological examination as presented in Table 1 revealed a fall in blood haemoglobin, TEC, TLC and rise in PCV and ESR level which may be due to the fact that lead inhibits haemoglobin synthesis and decrease the life span of RBC in the body (Potula, 1996). Lead has a high affinity for sulfhydryl groups and thus inhibits sulphydryl dependent enzymes such as 5-aminolaevulinic acid dehydratase (ALAD, EC 4.2.1.24), ferrochelatase (EC 4.99.1.2) and also of pyrimidine 5k-nucleotidase, EC 3.2.2.10) which may be attributed to the presence of Basophilic stippling in RBC (Rempel, 1989).

The biochemical analysis showed significant decrease in blood glucose and increase in AST, ALT

[1] Teaching Veterinary Clinical Complex,

Table 1: Haematological parameters before and after treatment of lead toxicity.

Parameters	Before treatment	After treatment
Hb (g/dL)	6.91	10.5
TEC (per 10^6 /cmm)	4.22	6.51
TLC (per 10^3 /cmm)	11.35	8.1
PCV	37	32
ESR	1.95	1.21

Table 2: Biochemical analysis before and after treatment of lead toxicity.

Parameters	Before treatment	After treatment
Glucose (mg/dL)	43.21	114.43
Total Protein (g/dL)	6.56	6.76
Urea (mg/dL)	3.92	3.01
Creatinine (mg/dL)	1.02	0.96
AST (U/L)	102.41	45.8
ALT (U/L)	38.91	11.82
LH (mμ/mL)	4.02	8.79
FSH (mμ/mL)	0.91	2.07

level as indicated in Table 2 which may be due abnormal liver function (Swarup *et al*., 2007; Sharap *et al*., 2008). However, there was no change in the serum total protein, urea and creatinine level (Table 2) in the patient, thus suggesting a normal kidney function which may be due to early stage of lead poisoning.

The level of L.H. and F.S.H, presented in Table 3, showed significant decrease from normal values again validated the present case to be an early stage of lead poisoning (Swarup *et al*., 2007; Sharap *et al*., 2008).

Both clinical signs and ocular changes as observed in the present case might be attributed to the toxic effect of lead on the CNS, characterized by cerebral disturbances leading to partial blindness, which may be due to cerebrocortical oedema, or may be due to associated optic neuritis (Radostits *et al*., 2007).

The heifer was treated initially with sodium calcium edentate (6.6 % disodium calcium ethylenediamine-tetra-acetic acid) as the preferred antidote against lead poisoning which acts as a chelating agent @70 mg/kg/day im divided in 3 doses. It forms complexes with lead, prevent its binding to cell constituents and being hydrophilic are eliminated in the urine (Kety, 1942). On the first day of treatment, the animal was administered DNS (Dextrose Normal Saline) iv to counteract the dehydration and also replenish the lost nutrients due to anorexia. In addition, Atropine Sulphate @ 0.2 mg/kg b. wt iv was administred with DNS to counterbalance the nervous depression due to cholinomimetic symptoms. The same line of treatment was repeated for 5 more days with Atropine Sulfate @ 0.05 mg/kg b. wt iv. From, 6th day onwards till 12th day the treatment was continued with an addition of administration of Vitamin B1 supplement @50mg/kg b wt on alternate days as a nervine tonic.

The animal responded to treatment and the poisoning was controlled within 15 days. Other clinical signs had almost completely resolved within 10 days of treatment. Haematological examination after treatment revealed improvement in blood parameters and the animal was recovered completely.

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Therapeutic management of Anaplasmosis in a Jaffrabadi buffalo

B. R. Maharana, K. B. Vala, P N Panigrahi[1], S.K.Behra[1]
College of Veterinary Science & A.H, JAU, Junagadh-462001, Gujarat

Abstract

A ten year old Jaffrabadi buffalo was brought to Veterinary Polyclinic with symptoms of anorexia, reduced milk yield, dullness, congested mucus membrane, and high fever. Blood sample was collected and microscopic examination confirmed the case of Anaplasmosis. The animal was managed with Oxytetracycline @20mg/kg (Pfizer) for four days followed by acaricide spray. The present study reports a case of bovine anaplsmosis, its clinical variants, diagnosis, hematology and therapeutic management.

Key words: Anaplasma, Buffalo, Haemoprotozoa, Oxytetracycline.

Anaplasma marginale is the most pathogenic of its species which poses a serious constraint to animal health. This organism has been recorded in domestic and wild ruminants (Kuttler, 1984; Kocan *et al*., 2010). The incidence of anaplasmosis has been reported 2.82% in dairy cattle in Kaira and Anand districts of Gujarat, however no cases in buffaloes (Vahora *et al.,* 2012). Anaplasma is distributed throughout the tropical and subtropical countries like India corresponding to the distribution of tick vectors. This can also mechanically transmitted either by biting flies or by contaminated surgical instruments. Being a rickettsiale obligate intracellular organism, it requires a host in order to survive. The pathogen multiplies within the tick and can be carried over to later stages of the tick's life cycle, but the infection is not transmitted to the eggs. As carriers, ticks are unaffected by this but when they feed on an animal, this parasite enters through the site of the bite, infecting the animal.

In the blood, the organism enters the red blood cell by penetrating the cell membrane so that a vacuole is formed. Then it divides to form an inclusion body containing up to eight initial bodies packed together which under compound microscope looks as a single dot inside RBC. Cattle that recover from anaplasmosis remain carriers of the organism but are immune to further infection. *Anaplasma* like *Babesia* shows inverse age resistance. As calves from immune mothers receive maternal antibodies against *A. marginale* through colostrum they show resistance to infection. So susceptibility increases gradually as the animal became older. Animal aged 2-3 years develop typical and often fatal anaplasmosis. The severity of the disease increases with age showing the per-acute and possibly fatal disease (Taylor *et al*., 2007). Simultaneously the severity of infection is higher in imported cattle than the indigenous one (Bundza and Samagh, 1982).

[1]Indian Veterinary Research Institute, Izatnagar, Bareilly-243122

Case history and observations

A ten year old Jaffrabadi buffalo was presented to veterinary polyclinic, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh with symptoms of dullness and depression, anorexia and reduced milk yield. The visible mucous membrane was congested. There was high temperature (105ºF), increased heart and respiration rate.

Blood samples were taken from jugular vein along with EDTA as anticoagulant for specific diagnosis and to estimate hematological parameters. A peripheral blood smear examination stained with Giemsa stain revealed *Anaplasma marginale* as intraerythrocytic irregular shaped dots at the periphery of the erythrocytes (Fig. 1). The hematological profiles revealed marked anaemia, leucocytopenia and coincidental decrease in packed cell volume (Table 1).

Treatment and Discussion

After confirmatory diagnosis, the animal was treated with Oxytetracycline @ 20mg/kg (Pfizer) for four days along with multivitamin injection @ 10 ml I/M and B-complex @ 5 ml I/M for three days followed by acaricide spray (0.1% aqueous solution of deltamethrin, Butox) as it is a tick borne disease. Clinical improvement was noticed within 24hrs and complete recovery was noticed by 96 hrs from the initiation of treatment.

In the present case report, anaplasmosis was documented in a buffalo depending upon the clinical sign and microscopic findings in veterinary polyclinic, Junagadh. Fever is usually the first recorded sign of

Table 1: Hematological profiles of Jaffrabadi buffalo

Hematological profiles	Infected (Anaplasmosis)
Hemoglobin (gm%)	5.8
HCT (%)	19.2
MCV (fl)	45.4
MCH (pg)	13.7
MCHC (g/dl)	30.2
Total erythrocytes count (10^6/µl)	4.1
Total leucocytes count (10^3/µl)	5.2
Packed cell volume (%)	21

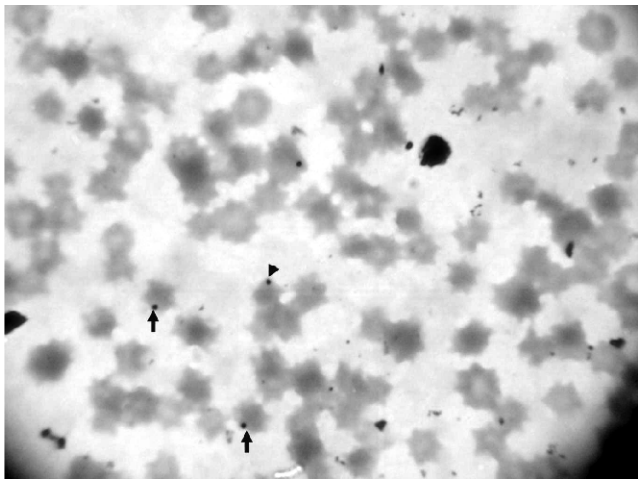

Fig. 1 *Anaplasma marginale* (indicated by arrow) in erythrocytes.

anaplasmosis which may be due to infection of large number of erythrocytes with the organism. The declined packed cell volume with increased parasitemia may be associated with the clinical symptoms of dullness or depression, anorexia and reduced milk yield. Here, marked anemia may be associated with phagocytosis of large number of parasitized erythrocytes and coincidental decreases in packed cell volume.

Tetracycline has been the drug of choice for anaplasmosis control since the 1950s. Administration of low levels of tetracycline prior to infection of cattle prevents or reduces clinical disease. Tetracycline inhibits rickettsiale replication by binding to the 30s subunit of the ribosomes interfering with attachment of aminoacyl-tRNA and thus resulting in inhibition of protein synthesis (Reynard, 1992).This is the way by which Oxytetracycline appears to kill the organism by interfering with the ability of the organism to complete its replicative cycle within the parasitophorous vacule in the host RBC. Hence, Oxytetracycline is considered as more effective and safe drug of choice for anaplasmosis (Atif *et al*., 2012) and administration through intramuscular route @ 20 mg/kg for 3-7 days was effective in eliminating acute anaplasma infections successfully. In the present case report, the buffalo was observed to be recovered by 4th day and there was no complain of relapse of infection. Buffalo just like cattle are susceptible to anaplasma infection but the incidence and severity is less as the earlier acts as reservoir host (Kocan *et al*., 2010). Previous study also reported less susceptibility to anaplasma in buffalo compared to cattle (Vahora *et al*., 2012). The lower susceptibility may be due to natural wallowing behaviour of buffaloes which protect the animal from tick infestation. The present case could be due to tick biting which may transmit the infectious agent.

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Guttural pouch empyema in thoroughbred horse and its management

P. Pothiappan[1], K.Sridhar[2], S. Kavitha[3] P. Selvaraj[4], A. P. Nambi[5] and S. R. Srinivasan[6]
Department. of Veterinary Clinical Medicine, Ethics and Jurisprudence
Madras Veterinary College, Vepery, Chennai-600 007, Tamil Nadu

Abstract

An eight year old Thoroughbred gelding was presented to the Madras Veterinary College Teaching Hospital with the history of inappetance, bilateral purulent nasal discharge, respiratory noise, painful swelling below the ear since one month. Clinical examination revealed elevated temperature (39.50C), firm retropharyngeal swelling, purulent bilateral nasal discharge, stertorous breathing, respiratory noise, dysphagia and lymphadenopathy of local lymph nodes. Blood parameters were within the normal limits. Upper respiratory tract endoscopy was performed at rest which showed purulent material and tympanitis in the guttural pouch. Based on endoscopy the case was diagnosed as Guttural Pouch Empyema. Guttural pouch were washed with normal saline weekly twice for two weeks. Based on ABST result Inj. Ceftriaxone @ 10 mg / kg b.wt, i.v was administered twice daily for 15 days. The animal showed clinical improvement after therapy.

Key words: Guttural pouch empyema, Horse, Management

Guttural pouches are large diverticula of the eustachian tubes that connect the pharynx to the middle ear. They are located in the caudal area of the head (Baptiste *et al*, 2000). Guttural pouch empyema this is the most common disease of guttural pouches. It is most frequently a result of an upper airway infection, which can extend into the guttural pouch.

Alternatively, retropharyngeal abscesses can drain into the ipsilateral guttural pouch, resulting in guttural pouch empyema. Bacteria most commonly isolated from bacterial infections include S equi var equi. (Hardy and veille, 2003). The present case deals guttural pouch empyema and its emergency medical management in Thoroughbred horse.

Case history and observation

An eight year old Thoroughbred gelding was presented to the Madras Veterinary College Teaching Hospital with the history of inappetance, bilateral purulent nasal discharge, respiratory noise, painful swelling below the ear since one month. Clinical examination revealed elevated temperature (39.50C), firm retropharyngeal swelling, purulent bilateral nasal discharge, stertorous breathing, respiratory noise, dysphagia and lymphadenopathy of local lymph nodes.

Animal was subjected to guttural pouch radiograph and its showed retropharyngeal swelling, thickening of ventral wall and fluid lines across the guttural pouch. Blood parameters were within the normal limits. Upper respiratory tract endoscopy was performed in standing position under sedation (xylazine 0.5mg/kg i.v) which showed purulent material and tympanitis in the guttural pouch. Nasal swab was sent for ABST which positive for gram positive and gram negative organism. Based on history, clinical signs radiograph and endoscopy the case was diagnosed as Guttural Pouch Empyema.

Treatment and Discussion

Based on ABST result the animal was treated with Inj. Ceftriaxone @ 10 mg / kg b.wt i.v twice daily for 15 days, Inj.Flunixin meglumine 1.1mg/kg i.v for 5 days. Double lumen soft end catheters were inserted through biopsy channel of endoscopy to the guttural pouch and flushed with normal saline (300ml) twice a week for three weeks. After therapy the animal showed clinical improvement and had an uneventful recovery.

Treatment of guttural pouch empyema includes systemic and local therapy as well as addressing secondary complications. Systemic antibiotics are usually recommended for two reasons: drainage of the pouch can infect the lower airways, and treatment of lymph nodes abscesses can be helped with antibiotics. Catheterization helps in daily flushing of the affected

[1]**Corresponding author:** Assistant professor, University research farm, Madhavaram milk colony, Chennai-51. Email: vetpothi@yahoo.co.in, [2]Veterinary Assistant Surgeon, VeterinaryDispensary, Senthamangalam, Vellore, 631051.
[3]Associate professor, Dept. of VCME &J, Madras Veterinary College, Chennai-7., [4]Associate professor, Dept. of VCME &J, Madras Veterinary College, Chennai-7., [5]Professor and Head, Dept. of VCME &J, Madras Veterinary College, Chennai-7., [6]Professor, Director of Clinics, Madras Veterinary College, Chennai-7.

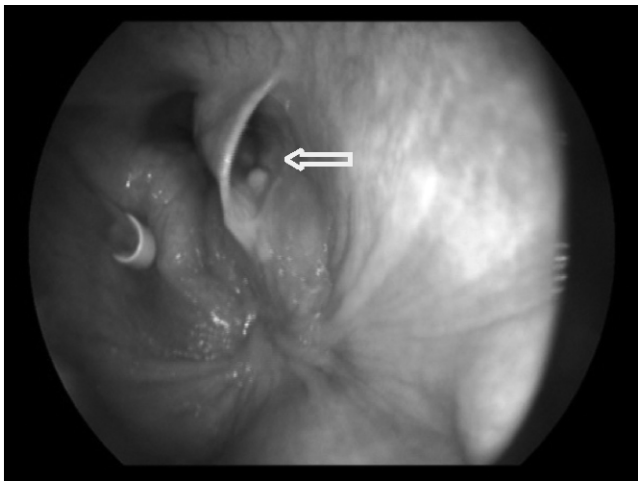

Fig: 1 Discharge around the guttural pouch opening

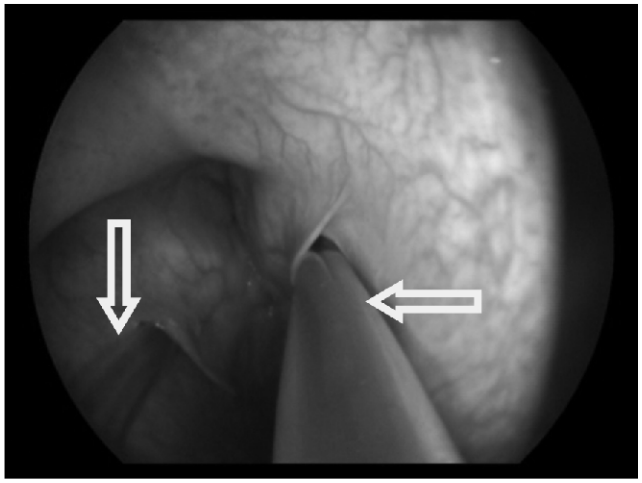

Fig: 2 Double lumen soft end catheters were inserted to the guttural pouch for flushing

pouch. Balanced electrolyte solutions are recommended to avoid further irritation of the pouch. Instillation of antiseptics into the pouch is not recommended, because antiseptics are usually irritating (Hardy and Veille, 2003). Treatment options include guttural pouch culture and lavage (Adkins *et al.* 1997 and Seahorn and Schumacher 1991). There is no information in the literature indicating whether the duration of treatment affects the rate of resolution, although repeated lavage is commonly reported (Freeman, 1980).

Guttural pouch endoscopy yields the most information regarding guttural pouch disease. The flexible endoscope can be introduced in the pouch using the biopsy channel and instrument, or the pharyngeal opening can be pried open using a Chambers catheter. The biopsy forceps are introduced into the biopsy channel of the endoscope. On most endoscopes, the biopsy channel is located in an eccentric location on the end of the scope such that the endoscope has to be manipulated to open the pouch maximally (Judy *et al.* 1999). Although many theories have been postulated as to the etiology of this condition, *Streptococcus* species are by far the most common pathogens isolated from affected pouches (Chiesa *et al.* 1999). Empyema, there were no significant differences between the results of endoscopy and radiography for detection of empyema, with or without, chondroids (Judy *et al.* 1999). However, endoscopy as part of a complete airway examination will allow direct visualization of the contents as well as the lining of the guttural pouch.

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Idiopathic vestibular syndrome in a Labrador dog and its therapeutic management

S. Mahajan, K. Mahendran, A. Kumar and S. Dey.
Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243 112, Bareilly (U.P.), India

Abstract

A 3 year old, female, Labrador was presented with a history of sudden onset of nervousness, tilting of neck towards right side, walking only short distance, then falling and difficulty in rising. Clinical samples were collected and processed for routine hematological and biochemical parameters. Results of hematological and biochemical estimation were unremarkable and were in normal range with the negative reports of screening tests for canine distemper and haemoprotozoa. Keeping in mind the laboratory and clinical findings, the case was presumably diagnosed as idiopathic vestibular syndrome and was treated with high dose of methyl prednisolone 80 mg total dose IM along with ascorbic acid 500 mg slow IV in 500 ml of dextrose saline, cefotaxime-sulbactam 750 mg slow IV, multivitamin (inj neurobion forte 2 ml) and complete clinical recovery in was seen after 2 weeks of treatment.

Key Words: Dog, idiopathic vestibular syndrome and methyl prednisolone.

The vestibular system is the major sensory system that along with the general proprioceptive and visual system maintains balance (Brandt and Strupp, 2005). Vestibular syndrome can have multiple etiologies such as infections, either from the brain or middle ear, cancer, poisonings, parasitism, immune disorders, occasionally as a sign of hypothyroidism and commonly as an idiopathic event. Idiopathic vestibular syndrome is something that comes on unexpectedly and for unknown reason (Rossmeisl, 2010). The hallmark clinical signs of vestibular syndrome irrespective of location (peripheral or central) are head tilt, vestibular ataxia, tight circling, falling, rolling, spontaneous nystagmus and nausea etc. (Lorenz *et al.,* 2011). The present investigation reports a case of idiopathic vestibular syndrome in a dog and its successful therapeutic management.

Case history and Observations

A 3 year old, female, working Labrador belonging to 36 bn, ITBP, Lohaghat was presented to Referral Veterinary Polyclinic, IVRI with a history of sudden onset of nervousness, tilting of neck towards right side, walking only short distance, then falling and difficulty in rising. The clinical examination of the animal revealed normal rectal temperature, bradycardia, shallow breathing, tilting of head towards right along with circling and unilateral horizontal nystagmus. There was normal menace and withdrawal reflex. Although the animal able to bear its weight on limbs initially but the condition aggravated further to lateral recumbency with head tilt towards right. Clinical samples were collected and processed for routine hematological (TLC, DLC, TEC, Hb and total Platelet count) and biochemical (blood glucose, BUN, serum creatinine, ALT, AST and total protein) parameters. Blood samples were screened for canine distemper and haemoprotozoa by commercially available rapid Immunoassay detection Kits.

Treatment and Discussion

Results of hematological and biochemical estimation were unremarkable and were in normal range with the negative reports of screening tests for canine distemper and haemoprotozoa (Table 1). Keeping in mind the above findings, the case was presumably diagnosed as idiopathic vestibular syndrome. The animal was treated with high dose of methyl prednisolone 80 mg total dose IM along with ascorbic acid 500 mg slow IV in 500 ml of dextrose saline, cefotaxime-sulbactam

Table 1: Hematological and Biochemical parameters before and after therapy

Hematological parameters	TLC/mm^3	TEC/mm^3	Hb (g %)	N	L	M	B	E	Platelets (lac/mm^3)
Before therapy	16300	5.68 x10^6	11.8	74 %	21%	3%	0%	2%	2.95
After therapy	17400	5.46 x10^6	10.3	75%	20%	3%	0%	2%	2.94

Biochemical parameters	SGPT (U/L)	SGOT (U/L)	BUN (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio
Before therapy	36	22	20.8	0.9	62	6.2	3	3.20	0.94
After therapy	35	20	17.6	0.8	71	6	3.1	2.90	1.07

750 mg slow IV, multivitamin (inj neurobion forte 2 ml) slow iv twice daily for 3 days. Prednisolone can be used during acute phase of Idiopathic vestibular syndrome along with other supportive therapy (Fitzmaurice, 2010; Rossmeisl, 2010). On day 4, animal showed signs of improvement and was able to bear its weight along with slight control on its head. The dose of methyl prednisolone was reduced to 40mg IM daily along with same supportive treatment up to day 7. Most animal suffering from acute Idiopathic vestibular syndrome makes complete recovery, although severely affected animal may occasionally maintain a residual head tilt (Schunk, 1990). In the present case, animal showed immense improvement and was able to walk in straight line, no tilting of head and listening to command with normal appetite. A complete hematological and biochemical evaluation was again conduced on day 7 and all the parameters were in the normal range. Animal was then prescribed further with tapered doses of prednisolone @10 mg (tab wysalone) orally along with a multivitamins (tab. neurokind) and hepatoprotectant (Syp. Liv 52) from day 8th onwards. With the above line of treatment, there was complete clinical recovery in 2 weeks and animal was discharged to conduct its duty.

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Hyalomma aegyptium infestation on the lesions of break carapace in turtle (*Testudo graeca* ibera)

Khodadad Pirali Kheirabadi[1], Amir Dehghani Samani[2], Ali Shokohi[3], Hamed Sadeghi Dehsahraei[3]
Department of Pathobiology, Faculty of Veterinary Medicine
Shahrekord University, Shahrekord, 115, Iran.

Abstract

The main goal of this study is showing that parasites can be in uncommon locations than their common choice localities on their host's body. With this in mind, if we don't attention to this subject, it is possible that we infested by our pet's parasites and these parasites may carry the zoonotic pathogen. A damaged turtle (*Testudo graeca* ibera) with the break carapace referred to Faculty of veterinary medicine of Shahrekord University, for treatment the fracture of carapace. In first examination of carapace, 6 ticks removed from lesions and then samples were sent to the laboratory. Ticks species were identified and confirmed as *Hyalomma aegyptium* (Acari: Ixodidae). This study revealed that care about this parasite should be considered, because this parasites can infested many different animal even turtles, and could carry the important zoonotic pathogens. So we recommend to considering a suitable control of these parasites as a major problem in animals and important vectors of infectious agents to livestock and humans.

Key words: Break carapace, *Hyalomma aegyptium*, *Testudo graeca*, Zoonotic pathogens.

Testudo graeca Linnaeus, the Spur-Thigh Tortoise, in North Africa, from Morocco to Libya, as well as in Europe, from southern Spain, the Balearic Islands, to Sardinia and Sicily; east of a gap in the Italian and western Balkan Peninsula, the range area continues from eastern Romania, Serbia, Bulgaria, Macedonia and Greece across most of Turkey, into the Transcaucasia countries, and as far as Lebanon, Syria, Jordan, Iraq and Iran in the Near East is distributed (Ananjeva et al., 2006; Bonin et al., 2006). *Testudo graeca* lives from sea level to about 2700 m and occurs on dry open steppes, barren hillsides and wastelands, where vegetation varies from sea dune grasses to scrub thorn or dry woodlands (Vatansever et al., 2008), but also in vineyards and gardens. Hyalomma ticks are often the most abundant tick parasites of livestock, in hot, arid, and semiarid area. There are 30 *Hyalomma* spp. known as important vectors of infectious agents to livestock and humans. Hyalommines are mostly moderately large to large ticks with long mouthparts (Fraser, 1986). *Hyalomma aegyptium* is a hard-tick with a typical three-host life cycle. The main hosts for adults are Pale arctic tortoises of the genus Testudo (Mihalca et al., 2011). Also rare reported from other hosts like hedgehogs and hares in adult stage of tick. Nevertheless, larvae and nymphs of *H. aegyptium* are less host-specific and feed on various vertebrates: tortoises, lizards, birds, small mammals and even humans (Bursali *et al.*, 2010).

Several pathogens were detected in *H. aegyptium* contain *Theileria annulata* (Ray, 1950), *Borrelia turcica* (Güner *et al.*, 2004), Rickettsia spp and *Borrelia burgdorferi* (Kar *et al.*, 2011). Experimental trials are usually long and difficult to perform, hence the need for a preliminary assessment of the carrier status in natural populations. Until now, experimental proof of the vector capacity of *H. aegyptium* was shown for several pathogens: *Hemolivia mauritanica* (Sergent and Sergent, 1904), *Hepatozoon kisrae* (Paperna *et al.,* 2002*), Rickettsia aeschlimannii (*Bitam *et al.*, 2009) and *Coxiella burnetii* (Široký *et al.*, 2010).

The report describe a rare report of *Hyalomma aegyptium* in a turtle with damaged carapace which sometime lives near humans and even be as pet animal in homes, and point that *Hyalomma aegyptium* may carry many important zoonotic pathogens.

Material and Methods

During October 2012, a damaged turtle (*Testudo graeca* ibera) with the braked carapace and about 8 years old (Figure 1) find in the mountains around of Shahrekord town (latitude, 32° 19' 32" N and longitude, 50° 51' 52" E), in south western of Iran, then it was referred to the Faculty of Veterinary Medicine of Shahrekord University to treatment the fracture of

[1] Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, 115, Iran.
[2] Department of Clinical sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, 115, Iran.
[3] Under graduated student of Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, 115, Iran.

carapace. In first examination of carapace, 6 ticks removed from lesions and for the confirmed identify, ticks were sent in ethanol 70%, contain 5% glycerine to the laboratory. There were no any ticks on other parts of turtle's body. Removed ticks were washed with Potassium hydroxide 10% then imaged by optical microscope.

Results and Discussions

Removed ticks species were identified and confirmed as *Hyalomma aegyptium* (Acari: Ixodidae), according to the Walker et al. 2003, 4 ticks were female and 2 ticks were male. Female ticks show in Figure 2 and male ticks (Fig 3).

Fig 4 show the mouths appendix and arrow show external spur and internal spur of ticks. In figure 5 broad ad-anal plats and reduced sub-anal plates showed by arrow.

Ticks species were identified as *Hyalomma aegyptium* (Acari: Ixodidae), according to the Walker *et al*. 2003. Ticks are a suborder that named metastigmata, belong to the order of Acarina. These ticks

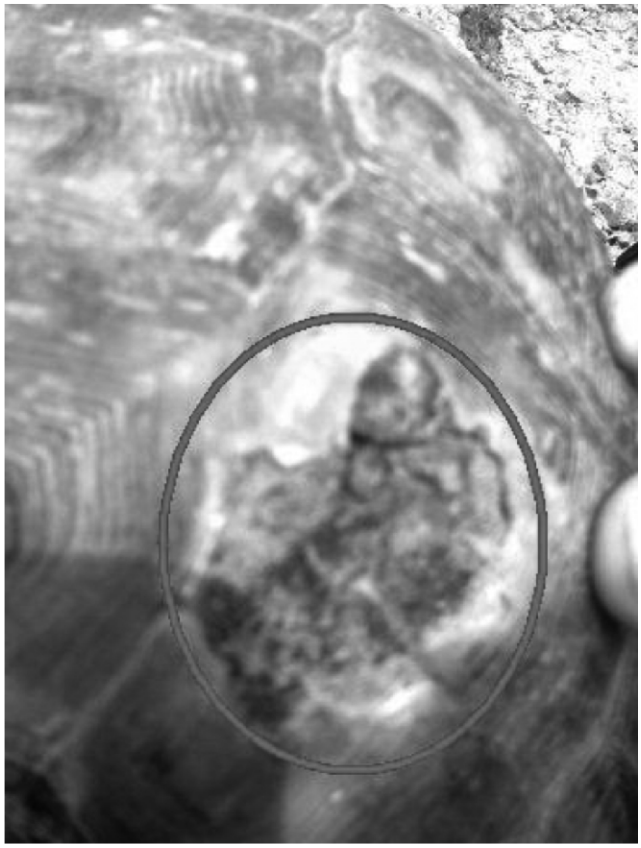

Fig 1. Damaged carapace of turtle (*Testudo graeca* ibera)

can bite humans and pets, and can carry several pathogens, which cause relapsing fever and western equine encephalitis viruses in humans. Results of current studies have shown the case with which exotic ticks have been introduced into other countries on imported reptiles and disseminated from importers to breeders, zoos, wildlife theme parks, pet stores and private hobbyists (Walker *et al.*, 2003).

Hyalomma aegyptium has been commonly recorded on cattle and buffaloes from Balkan countries, Pakistan, Russia, India, and southern Marmara Region of Turkey.

The geographical distribution and habitats of several generalist tick species have expanded in the recent years. Major drivers for this trend include climate changes and globalization (Harrus and Baneth, 2005). On the other hand, for certain tick species which are co-distributed with their endangered hosts, like the case of *H. aegyptium*, the trend is a decreasing geographical range (Mihalca *et al.*, 2011). However, in general, a decrease in the availability of natural host populations could lead to host-switching behavior (Keesing *et al.*, 2010).

In Romania, all stages of *H. aegyptium* were found on only two hosts, the Spur-thigh tortoise, *Testudo graeca* and the Northern White-breasted hedgehog, *Erinaceus roumanicus* and its distribution matches the one of the tortoise host (Mihalca *et al.*, 2012).

In southern Europe the hosts of *H. aegyptium* are primarily tortoises but also lizards, dog, horse, hedgehog, hamster and birds. In Italy *H. aegyptium* has occurred on partridge, in Egypt, on quail, pigeon, chats and warblers (Hillyard, 1996). *H. aegyptium* were reported from cattle and buffaloes from, Pakistan, Turkey and India (Aydn, 2000). It has been known for many years that reptiles imported into other locations were on occasion infested with ticks (Burridge, 2001). At least eight exotic tick species were being imported into Florida on reptiles (Norval, 1985).

In this study ticks species were identified as *Hyalomma aegyptium* (Acari: Ixodidae) and this parasite may infect the skin of pet,

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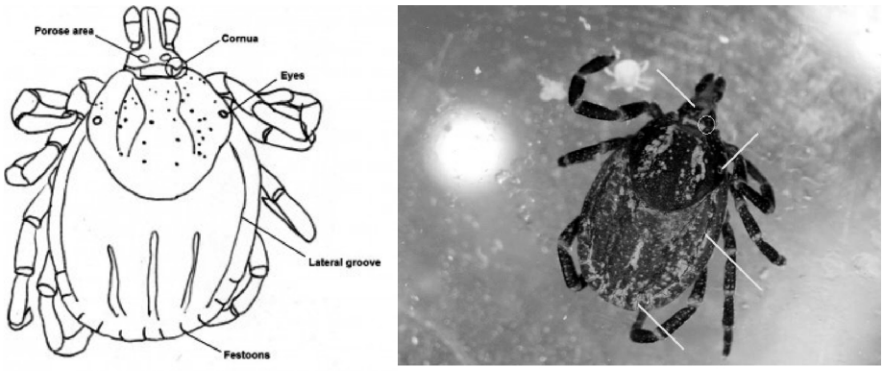

Fig 2. Female tick

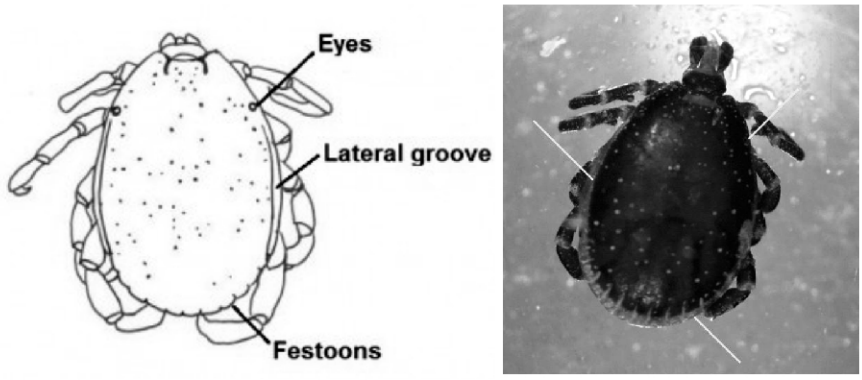

Fig 3. Male tick

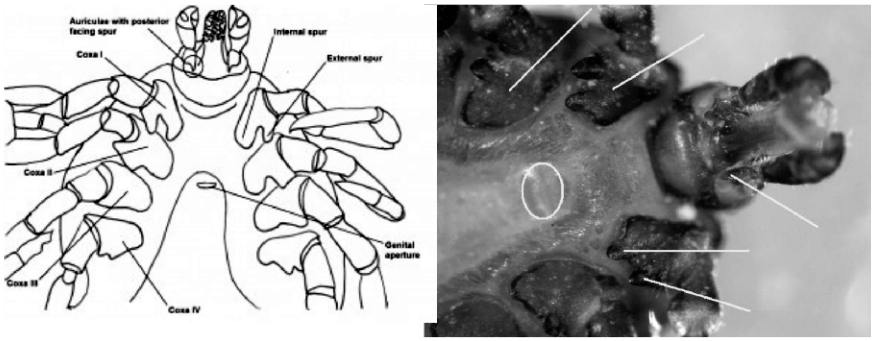

Fig 4. Mouths appendix of tick

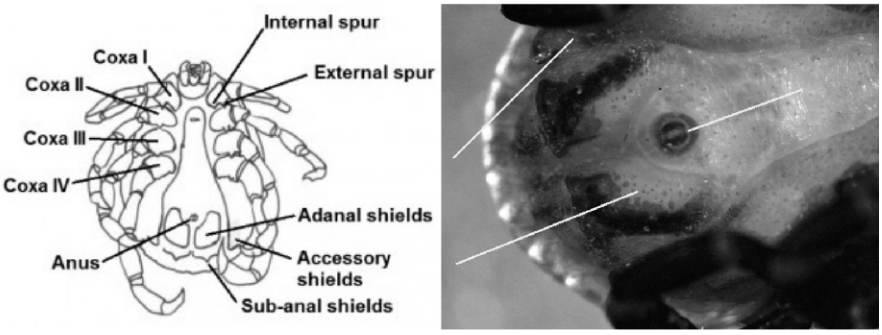

Fig 5. Broad adanal plats and reduced subanal plates

persons whose help us to do this research.

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Pulmonary adenocarcinoma in a German Shepherd dog: ultrasonographic diagnosis

D.Sumathi, M.Chandrasekar, M.Venkatesan, P.Selvaraj and A.P.Nambi
Centre of Advance Faculty Training in Veterinary Clinical Medicine Ethics and Jurisprudence
Madras Veterinary College, Chennai-600007, Tamil Nadu

Abstract

A nine year old male German shepherd dog with a three month history of coughing and dyspnoea for past one month is reported. The investigating protocol, which also included ultrasound guided pulmonary biopsy, allowed a diagnosis of lung adenocarcinoma. The tumor exhibited a nodular-disseminated growth, mimicking the metastatic involvement of the lung, instead of the single-mass appearance. Ultrasonographic study revealed multiple hypoechoic masses diffusely in thorax with irregular borders and pleural effusion. The present report indicates that, although the incidence of canine primary lung neoplasms is markedly low, this condition must be considered in the differential diagnosis of lung diseases that cause coughing and dyspnoea in older dogs.

Keywords: German shepherd dog, Lung tumour, Primary lung adenocarcinoma,

In dog, pulmonary metastatic neoplastic involvement is common than primary tumors (adenocarcinoma and squmous cell carcinoma) which are infrequently reported (only one percent of all cancers diagnosed) but their biological behavior can be juxtaposed to that of humans (Porrello *et al.* 2006). The most common histopathologic diagnosis for primary lung tumors in dogs is adenocarcinoma of bronchial, alveolar, or mixed bronchoalveolar origin.

Case history and observations

Nine year old German shepherd male dog was presented to the Madras Veterinary College Teaching Hospital with the history of mild to moderate dyspnea, vomiting and inappetance for one month. On physical examination, the dog was depressed and showed mild abdominal distension**.** Laboratory examination showed neutrophilic leukocytosis, elevated BUN (55.24mg/dl), Creatinine (2.58mg/dl) and hypocalcaemia (3.11mg/dl). Lateral and ventrodorsal views of chest radiograph showed pleural effusion masking the cardiac silhouette (Fig.1). Abdominal radiographs showed gas filled and mild serosal detail loss. Ultrasonography of thorax revealed multiple 2-3cm sized hypoechoic masses diffusely in thorax with irregular borders (Fig. 2a). Under ultrasonographic guidance, with automatic spring loaded biopsy gun (Bard) core tissue biopsy of thoracic mass was done, which was histopathologically identified as pulmonary adenocarcinoma (Fig.2b).

Discussion

Characteristic clinical signs of small cell anaplastic carcinoma vary depending upon location, size and type of tumors. Pulmonary adenocarcinomas are generally very malignant and intrapulmonary metastases in several lobes, in the bronchial lymphnodes, in the

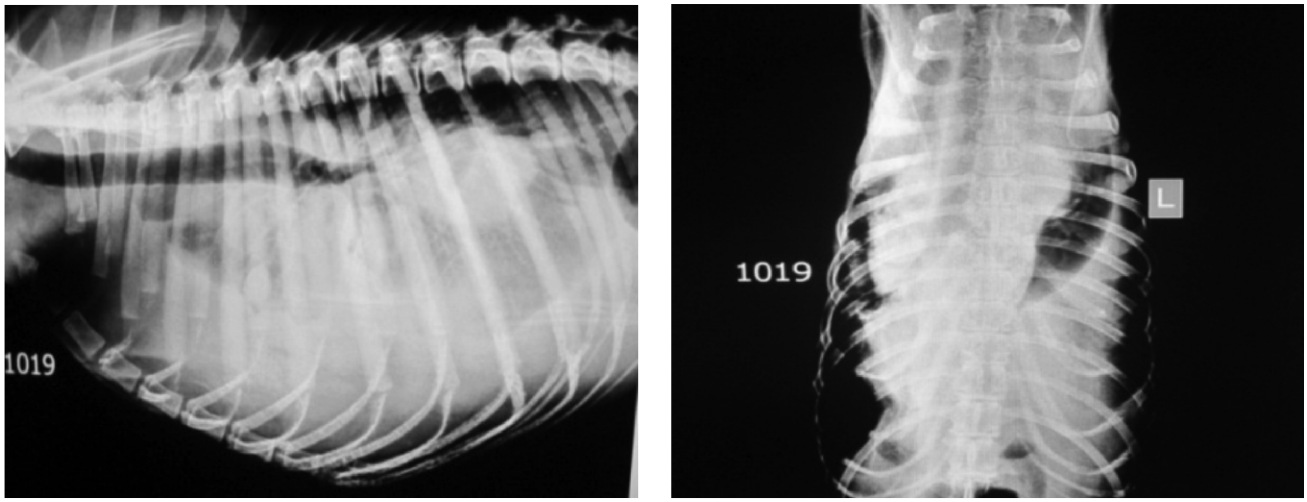

Fig.1. Lateral and ventrodorsal view of chest radiographs showing pleural effusion masking the cardiac silhouette

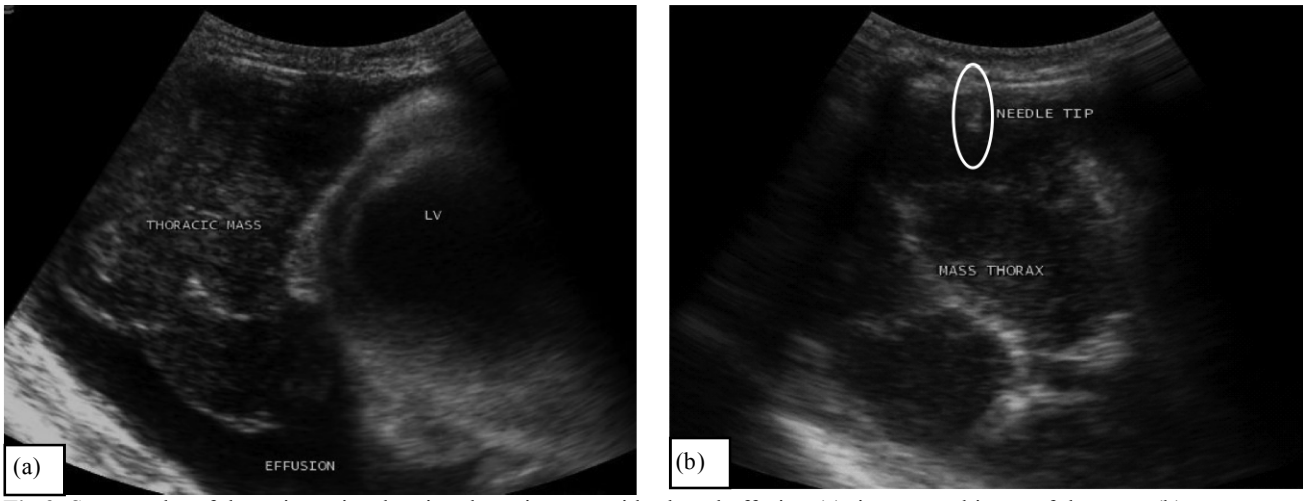

Fig.3. Sonography of thoracic cavity showing thoracic mass with pleural effusion (a) tissue core biopsy of the same (b)

pleura, and even in the brain are not uncommon. Clinically these neoplasms are associated with coughing, dyspnoea, and sometimes fever due to secondary infections (Stunzi *et al.* 1974). Most general sign is nonproductive cough for weeks or months (Mehlhaff and Mooney, 1985). Apart from this, lethargy, anorexia, hemoptysis, and in severe cases dyspnoea, pneumothorax, or pleural effusion is observed (Kim *et al.* 2005). In this study dyspnoea, vomiting, inappetance for one month, emaciation, mild abdominal distension, pleural effusion and depression was observed.

There are no sex or breed predilection (Paoloni *et al*. 2006); the average age of dogs and cats with pulmonary adenocarcinoma is 12 years (Stunzi *et al.* 1974). In this study nine years old male was affected. Pulmonary neoplasia, either primary or metastatic, is most commonly described ultrasonographically as a round, hypoechoic, homogenous nodule or mass (Schwarz *et al.* 1999) which concurred with the present case. With limited treatment options animal succumbed to the condition.

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Dirofilariosis in a dog and its management- A case report

Mritunjay Kumar, Bhabatosh Pal, Feroz Ali Dar and M. Norjit Singh
Department of Teaching Veterinary Clinical Complex
College of Veterinary Science and Animal Husbandry
R. K. Nagar, Agartala-799 008, Tripura

Abstract

A 4½ year old male Labrador Retriever dog was presented with complaints of exercise intolerance, lethargy, weight loss, vomiting and persistent coughing specially during night for last 4 days. Direct and knott's method examination of blood were found positive for microfilaria of *Dirofilaria immitis*. Based on clinical signs the case was diagnosed as class III heart worm disease. Treatment was instituted with a combination product of amoxycillin and potassium clavulanate, chlorpheniramine meleate, diethylcarbamazine citrate and ivermectin. On day 9th of treatment dog showed improvement in clinical signs and blood sample examination revealed absence of microfilaria.

Keywords: Diethylcarbamazine, Dirofilaria, Dog, Ivermectin.

Dirofilariosis is a disease caused by filaria worms of the genus Dirofilaria (Vezzani, 2006). The present paper reports a case of dirifilariosis and its therapeutic management in a 4½ year old male Labrador Retriever dog, presented to the TVCC with complaints of exercise intolerance, lethargy, weight loss, vomiting and persistent coughing specially during night for last 4 days.

Clinical examination showed almost normal rectal temperature (102.2 ^{o}F) and pulse (109/min) and heart rate (112/min). Auscultation of heart revealed right sided heart murmur and split heart sound while lung auscultation showed slight crackles. Haematological studies were conducted as per standard procedures (Jain, 1986). The serum biochemical parameters and enzymatic activities were determined by autoanalyser (RA-50, Bayer Pvt. Ltd., India**)** using standard kits.

Microfilariae was confirmed by modified Knott test (Genchi *et al.,* 2005). Treatment was instituted with a combination product of amoxycillin and clavulanate potassium @ 20 mg/kg b wt im bid and chlorpheniramine meleate @ 1.0 mg/kg b wt im od for 3 days, diethylcarbamazine citrate @ 6.6 mg/kg b wt tid for 21 days followed by ivermectin @ 0.2 mg/kg b wt sc every month for 6 months.

Canine dirofilariosis was diagnosed by identification of microfilarie morphologically. Haematology revealed total leukocytes count 16.5 x10^3/µl, neutrophils 68%, lymphocytes 27%, eosinophils 5%, haemoglobin 8.4 gm%, total erythrocytes count 4.1 x10^6/µl. Abnormalities identified with heart worm infection may include mild nonregenerative anemia, neutrophilia and eosinophilia (Calvert and Rawlings, 1988). The results of biochemical and enzymatic activities revealed serum creatinine 1.7 mg/dl, blood urea nitrogen 36 mg/dl, alanine aminotransferase 25 U/L and aspertate aminotransferase 92 U/L. Liver enzyme elevations, azotemia, and hyperbilirubinemia may be noted in patients with severe heartworm disease (Calvert and Rawlings, 1988). Thoracic radiography showed multifocal alveolar pulmonary pattern along with enlargement of the right cranial lobe artery and right caudal lobe artery compared with their respective vein. Echocardiogram was normal except for inverted T-wave. In the present report, it is concluded that the combination therapy of diethylcarbamazine and ivermectin were found effective and safe in the management of canine heartworm disease.

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Vezzani, D. E., Diego, F. and Cristina, W. 2006. Dirofilariasis in Argentina: Historical review and first report of *Dirofilaria immitis* in a natural mosquito population. *Vet. Parasitol.* **136**: 259–273.

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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.

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The official language of journal is English. The articles should be sent to **"The Associate Editor, Indian Journal of Veterinary Medicine, Division of Medicine, Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, U.P. (India)"** along with a self addressed envelope of A4 size having postage tickets of Rs 30 for further correspondence**.** 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 (2.5 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.

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Authors are requested also to return the original version along with original comments of the reviewer to the editorial office for reference and records. The modified articles should be submitted to editorial office within 30 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.

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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.

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   C. Key words: 4 to 5 Key word.

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   F. Results: The experimental data should be presented clearly and concisely. Information presented in tables and figures should not be repeated

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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 abnormal 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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Abbreviations and Symbols: Metric system should be followed in the text. The quantities should be expressed in SI units. Contributor(s) are requested to use the following abbreviations.

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	od
Decilitre	dl	Parts per million	ppm
Gram	g	Percent	%
Hour(s)	hr	Picogram	pg
Inch	in	Revolution per min	rpm
Intramuscular	im	Second(s)	sec
Intraperitoneal	ip	Square centimeter	cm^2
Intravenous	iv	Subcutaneous	sc
Kilo calories	kcal	Thrice a day	tid
Kilogram	kg	Year(s)	yr
Twice a day	bid	Volts	v

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

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The British spellings must be followed throughout in the text and Oxford English Dictionary may be consulted in doubt.

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Indian Journal of Veterinary Medicine

Vol. 34 (June & December, 2014)

Author Index

Indian Journal of Veterinary Medicine

Vol. 34 (June & December, 2014)

Subject Index

ACKNOWLEDGEMENT TO THE REVIEWERS – 2014

Editorial board of Indian Journal of Veterinary Medicine would like to express their greatest appreciation and gratitude towards the reviewers of the Journal for the year 2013. We acknowledge that their valuable comments and insights have helped us to publish the best among the submitted article.

On behalf of Indian Society for Veterinary Medicine we sincerely thanks them for their time devoted and extremely thoughtful suggestions which immensely contributed to the improvement of the journal and overall for the greater cause that is for the development of Veterinary Sciences.

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Total articles published in 2014	**:**	**56**
Research articles	:	10
Short communications	:	31
Clinical Articles	:	15
Total articles published in June issue (Vol. 34, No. 1, 2014)	**:**	**27**
Research articles	:	5
Short communications	:	16
Clinical Articles	:	6
Total articles published in December Issue (Vol. 34, No. 2, 2014)	**:**	**29**
Research articles	:	5
Short communications	:	15
Clinical Articles	:	9
Total article submitted for publication (Jan 2014 to Dec 2014)	**:**	**96**
Articles accepted for publication	:	49
Articles rejected	:	47
Acceptance rate	:	51.04 %
Rejection rate	:	48.95 %

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1. Place of Publication	:	Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India.
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Dated: 31st December, 2014 **(S. Dey)**

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