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Research Article

Diabetes mellitus in canine of Odisha

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Abstract

Diabetes mellitus is a common endocrine disease of dog wich is manageable with early diagnosis, proper treatment and client education. The aim of this study is to record the incidence of diabetes in view of change in urbanisation and lifestyle of pet dogs. The incidence was found highest among the breeds such as Labrador retriever and pug. Dogs received on commercial pet food, lack of exercise, obesity and concurrent diseases were found positively correlated with incidence of diabetes in dogs. The incidence was higher in geriatric age group, male and obese dogs. Studies in different veterinary hospitals of Odisha suggest there is increase in cases of canine diabetes but decrease in case fatality rate.

Keywords: Diabetes mellitus, Dog, Odisha, Prevalence

Diabetes in dogs shares many similarities to diabetes in man. It is a multifaceted disease and a challenge for the clinician and the researcher. In the recent years, Diabetes mellitus (DM) is one of the common metabolic disorders affecting middle-aged to geriatric dogs characterized by hyperglycemia, glycosuria and weight loss, resulting from absolute or relative deficiency of insulin. DM in human population shows an increasing trend and Asian population are at increased risk. Similar studies on diabetes in canine population of Asian countries are still under preliminary stages or not taken seriously (Kumar *et al*., 2014).

Available literature shows that the prevalence of diabetes melitus in canine in the state of Odisha has not yet been assessed. Therefore the present work was undertaken to record the prevalence of diabetes among canine population in Odisha from 2013 to 2015.

Materials and Methods

A total of 948 dogs were screened at veterinary clinical complex (TVCC), College of veterinary science and animal husbandry, Bhubaneswar, Govt. hospitals and private pet clinics of Odisha during the period of 2013-2015. Information about breed, age, sex, exposure to exercise, body condition (presence of obesity or not), type of diet offered to the dog, concurrent disease status of the animal were collected from the pet owner.

The blood was collected from the ear pina of the dog by use of lancet of the portable blood glucometer (PGBM) to get 3-5 microliter of blood. This blood was touched with the test strip of (PGBM) containing glucose oxidase by patient site blood glucose monitoring method (Stein *et al.,* 2002). The animals showing blood glucose value more than 140 was subjected for detailed examination by collecting blood in sodium fluoride, clot activator, EDTA vials for blood glucose, biochemical parameters and PCV estimation. Date were analyised statistically using SPSS software.

Results and Discussion

Out of 948 dogs screened 34 dogs were found positive for diabetes (Table 1). The overall prevalence rate was recorded as 3.5% All the affected dogs were suffering from insulin dependent type of diabetes mellitus. Diabetes is common in dogs, with an estimated prevalence of 0.32% in UK (Catchpole *et al*., 2005). Diabetes is an emerging endocrinopathy in dogs and its incidence ranges from 1 in 50 to 1 in 400 (Dey, 2011). Recent trends indicate steep rise (32%) in cases of canine DM from 2006 to 2010 according to Banfield Hospital of USA. The canine DM strikes Indian dog population at quite high frequency Kumar *et al*., (2014). The prevalence study reveals that the incidence rate of occurrence of this endocrinopathy has taken an increasing trend (Anon, 2012).

The studied dog population was containing 18 different breeds carrying both purebred as well as mix bred animals (Table 1). Among the studied breeds the Labrador and Pug breeds of dogs showed higher incidence rate of DM compared to others. The breeds with higher prevalence rate than the overall were German shepherd, Pug, Daschound, Spitz, Chihuahua and

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Golden retriever indicating increased risk to suffer from DM. Labrador Retriever and West Highland White Terrier breeds of dogs are more prone to diabetes as they carry MHC genes which are associated with diabetes susceptibility in dogs (Kennedy *et al.,* 2003). Breeds with no incidence of diabetes mellitus in the present study were Greatdane, Desi, Basset hound, Rot weiller, Boxer, Cockspaniel, Dalmatian and Irish setter. Catchpole *et al*., 2005 also described that however dogs are with low risk for DM are Boxer, Papillon, Tibetan Spaniel, German Shepherd, Cocker Spaniels, Collies have low risk of DM. The preference for breeds by the owners is one of cause of increased incidence of DM in canine population Klinkenberg *et al*., 2006.

Aging has been considered as one of the important factor in canine DM. It is mainly a disease of middle-aged and older dogs. The current study reveals higher prevalence of DM in dogs which are more than five years of age (Table 2) which is also supported by Guptill *et al*., 2003; Davison *et al*., 2005 and Catchpole *et al*., 2005. Canine DM mostly reported in middle age to old age dogs between 5 and 12 years of age Hess, 2010. Diabetes mellitus is a disease of middle-aged dogs with a peak incidence around 8 years of age and typically occurs in dogs between 5 and 12 years of age, and is uncommon under 3 years of age (Catchpole *et al*., 2005). It is one of the most frequently diagnosed endocrinopathy found in middle aged and older dogs, the prevalence rate is increasing (Rand *et al.,* 2004).

Juvenile onset (less than 12 months of age) of diabetes is uncommon in dogs but in this study 2 diabetic dogs of less than one year of age were recoreded out of 34 affected animals. Catchpole *et al.,* 2005 has also reported Juvenile onset diabetes is uncommon in dogs. These affected dogs showed clinical signs of diabetes at around 12 weeks of age, also suggesting a congenital beta cell abnormality. Histopathological examination of a pancreases biopsy from one of these dogs demonstrated marked islet hypoplasia and the condition was probably due to an autosomal recessive mutation (Kramer *et al.,* 1988).

The male dogs showed higher incidence of diabetes than female dogs in the studied dog population

Table 1: Prevalence of diabetes mellitus in different breeds of dogs

Breeds	Percentage (%)	Affected/total no of dogs
German shepherd	5.26 (4/76)	4/948
Labrador retriever	13.18 (12/79)*	12/948
Pug	9.09 (3/33)*	3/948
Daschound	5.79 (4/69)	4/948
Spitz	4.38 (3/69)	3/948
Irish setter	0 (0/33)	0/948
Dalmatian	0 (0/49)	0/948
Cocker spaniel	0 (0/10)	0/948
Boxer	0 (0/52)	0/948
Chihuahua	5.55 (2/36)	2/948
Pomeranian	1.72 (1/58)	1/948
Saint Bernard	2.56 (1/39)	1/948
Golden retriever	6.18 (3/44)	3/948
Basset hound	0 (0/29)	0/948
Cross Bred	1.76 (1/85)	1/948
Rot weiller	0 (0/42)	0/948
Local	0 (0/108)	0/948
Greatdane	0 (0/25)	0/948

Chi-squire value- 45.611, , degrees of freedom=17, *-significant at P = 0.01

Table 2: Incidence of Diabetes mellitus based on different risk factors

Risk factors	Variables	percentage	P-valueExact Sig. (1-sided)	Degree of freedom	Chi-squire value
Age*	< 5 year	1.33 (6/449)	<0.01	1	12.491
	> 5 year	5.6 (28/499)			
Sex	Male	4.36 (22/482)	0.115	1	1.886
	Female	2.7 (12/432)			
Exercise	exposed	1.23 * (5/435)	<0.01	1	13.890
	Unexposed	5.67 (29/511)			
obesity	Obese	4.45 (20/449)	0.114	1	1.881
	Non-obese	2.89 (14/498)			
Diet	Commercial	8.15* (27/331)	<0.01	1	30.725
	Home-made	1.13 (7/617)			
Concurrent Disease	Present	5.68 (31/545)	<0.01	1	16.375
	Absent	0.74* (3/403)			

(* - indicates significance at P= 0.01 level)

(Table 2). Foster (1975) and Marmor *et al.,* (1982) reported female dogs (70% of diabetic cases) were more likely to develop diabetes. However, this bias is less apparent in Catchpole *et al*., (2005) in which 53% are female among diabetic dogs. However, the male-female risk ratio has a predominance of females at older ages (Neiger *et al.*, 1996 and Kumar *et al*., 2014). In females are more frequently affected than males. In bitches, this usually occurs during the metoestrus phase of the oestrus cycle which is mainly due to the induction of growth hormone secretion by progesterone and other progestogens. Diabetes in sexually intact female dogs is often associated with the progesterone-dominated phase of dioestrus which resembles gestational diabetes in humans (Catchpole *et al*., 2005).

The prevalence of diabetes is significantly lower (P>0.05) in dogs which are exposed to exercise as they are free in the house 0.23 % than dogs are getting less exposure to exercise as they are tied in cannel by the owner 5.67% in the studied dog population. Exercise can have a dramatic effect on blood sugar levels. Inactive dogs are insulin resistant. Exercise utilizes energy and helps to avoid elevated blood sugar level. The other possible factors contributing to increase incidence of DM are urbanisation of human population and engagement in occupations ensuing change in lifestyle of pet dogs leads to lack of exercise for dogs as one of the risk factor Klinkenberg *et al*., 2006.

The obese dogs showed higher prevalence of diabetes (4.45%) than dogs which are not obese 2.89 % in the studied dog population indicating obese population are at higher risk for development of DM in canine. Risk factors for both dogs and cats include insulin resistance caused by obesity. One of the most common complications of obesity in dogs is the development of diabetes mellitus. Obesity-associated pancreatitis has a role to play in disease progression of DM in dogs Lem *et al*., (2008).

The prevalence of diabetes is significantly higher (P>0.05) in dogs are getting commercial readily available food 8.15% than dogs are getting prepared dog food by the owner at home 1.13 % in the studied dog population. More dependence on commercial pet food and high-fat diets are one of the risk factors for increasing prevalence rate of DM in dogs Klinkenberg *et al*., 2006, Lem *et al*., 2008.

Among the diabetic dogs, 5.68 % were found affected with concurrent diseases like chronic dermatitis, hepatitis, blindness, renal problems, dental problems and geriatric problems are significantly (P>0.05) more prevalent to diabetes than dogs 0.74% which were not apparently sick. The potential factors in the etiopathogenesis of diabetes mellitus in dogs are genetics, immune mediated insulinitis, pancreatitis, obesity, concurrent hormonal disease hyper adrenocorticism, diestrus-induced excess growth hormone, hypothyroidism, drugs (glucocorticoids), concurrent illness, cardiac disease, hyperlipidemia, islet amyloidosis, urinary tract infections, otitis, dermatitis, acute pancreatitis, dental problems and neoplasia (Hess *et al*., 2000), Das *et al*., (2013) has found that dogs presented for chronic dermatitis found positive for diabetes. It is a common condition in dogs with many concurrent complication metabolic acidosis nephropathy, hepatic lipidosis and hepatic failure Hiblu *et al*., (2015) found dogs suffering diabetes along with hepatic necrosis, acute pancreatitis to be associated with DM (Cook *et al*., 1993). Hyperadrenocorticism is a contributing factor for development of DM in dogs.

Conclusion

In conclusion, an increasing trend of diabetes was recorded. The overall prevalence rate was recorded as 3.5% All the affected dogs were suffering from insulin dependent type of diabetes mellitus. The prevalence was higher geriatric age group, male and obese dogs. Studies in different veterinary hospitals of Odisha suggest there is increase in cases of canine diabetes and decrease in case fatality rate.

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Effect of *Aegle marmelos* and *Murraya koenigii* combination on estrus response and fertility in delayed pubertal Sahiwal heifers

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Abstract

A total of 14 sahiwal heifers of age between 27 to 38 month and had history of no breeding despite daily, morning and evening bull parading. All the heifers were randomly divided in to 2 groups viz. G-1 (n=8) and G-2 (n=6). Heifers in G-1 group were treated with shade dried leaf powder of *Aegle marmelos* (AME) and *Murraya koenigii* (MUR) in the concentrate mixture for 9 days. The significantly higher ($P<0.05$) proportion 75 % (6/8) as compare to control 16.67% (1/6) within 15.1 ± 4.63 days after onset of treatment. Animal exhibited standing estrus were inseminated and ultrasonographic scanning after 40 days of insemination revealed pregnancy percent 75 % (7/8) in treatment as compare to control 16.67% (1/6) and all diagnosed pregnant animals calved successfully in both treatment and control group.

Keywords: *Aegle marmelos*, Delayed pubertal, Estrus response, *Murraya koenigii*,

Introduction

In India, majority of rural masses earn their livelihood through dairy animals either by selling of animals, milk and its product and utilisation of dung as fuel and manure to sustain agricultural production. Pubertal anoestrus, a serious problem in dairy heifers that delays the entry to production chain and commonly occurring in both individually reared nondescripts heifers as well as heifers reared under organised herds. Although, many delayed pubertal heifers attained the age, the sexually matured size and optimal body condition even though remained in anoestrus. Trans-rectal examination of such heifers is characterized by poorly developed genital organs with normal size ovaries or with apparently normal size genital organs with pea shaped ovaries and in some absolute cases they may have poorly developed genital organs with pea shaped ovaries (Das, G. K., personal communication). To address such condition, a considerable research attention has been paid over the decades to understand the pathogenesis of the condition and also to treat the condition with various heat inducers including exogenous hormones. However, variable and inconsistent oestrus and fertility response following costly exogenous hormonal administration, non-availability and residual effect forced researchers to develop low cost, easily available and appreciable result oriented alternate therapeutic principles. The use of green medicine of plant origin is nowadays becoming an emerging area of therapeutic research for clinical illness both in human and animals. In that direction, some medicinal plants have recently drawn many research attention for fertility augmentation in animals because of low cost, appreciable results, non-toxic and easily accessible to the farmers (Hegde *et. al.,* 2002; Mehrotra *et.al*., 2004; Jondhale *et.al*., 2007; Dutt *et al.,* 2010; 2011). *Aegle marmelos* (Bel) and *Murraya koenigii* (Curry) are two such important medicinal plants have also drawn some current research attention for the augmentation of oestrus and fertility in anoestrus animals as a combined treatment protocols (Dutt *et. al.,* 2011; Das *et al.,* 2016). Sahiwal is a native breed of cattle of Indian sub-continent, has become popular for their milk production potential, disease resistance and wider adaptability and attracted policy attention under *Gokul Mission* of being native high producing breed. The breed faces some inherent limitation and commonly known as shy breeder. In the present investigation, we attempted to validate the effect of *Aegle marmelos* and *Murraya koenigii* on estrus and fertility response in delayed pubertal Sahiwal heifers reared under farm condition.

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Materials and Methods

Animals

Fourteen apparently healthy delayed pubertal Sahiwal heifers with a mean age of 32.85 ± 0.86 months and body weight: 224.29±9.25kg were selected for the study from the Instructional Dairy Farm of Deen Dayal Upadhyaya Veterinary and Animal Sciences University, Mathura (located at an altitude of 287 meters above sea level latitude: 27° 30' 0" North and longitude: 77° 41' 0" East) based on the clinical history of anoestrus form the data records of the farm during winter month from December to February. Heifers were subjected to daily, morning and evening bull parading and visual observation for oestrus detection.

Housing and management

All the experimental heifers were confined for the entire period of study to a barn with one third cover area and rest uncovered area with free movement inside enclosure. The daily feed consisted of adequate chaffed green fodder, wheat straw, concentrates, mineral mixture (1%) and water available *ad libitum*.

Test herbs

Mature fresh, green leaves of *Aegle marmelos* and *Murraya koenigii* were collected from their natural habitats. The collected plant leaves were shade dried followed by grinding in to powder and stored in a closed bags at room temperature with proper lebels indicating plant name, month and place of collection. The dose was calculated by extrapolation from the 50% ethanolic extract of Aegle and Murraya showing effective (@1000 mg/kg) in augmenting ovarian function in rat (Mehrotra, 2002; Jondhale, 2007) to cattle by the dose equivalent system using Km factor (Van Miert, 1986). Dose of leaf powder per kg body weight was worked out (Dutt *et al*., 2010) for Murraya and Aegle separately and was divided by two to yield half of the dose. Final dose was obtained by mixing both the calculated doses, according to animal's body weight. The calculated powder dose administered orally daily for nine days i.e. from 0 (initiation) to 8 mixed in concentrate feed.

Oestrus detection, ovulation and breeding

The behavioural signs like vulval swelling, cervical discharge and homo- or hetero-sexual mount showed by the heifers and sexual interest of the teaser bull towards them were the criteria to select the animal in oestrus. However, heifers exhibiting distinct oestrus signs along with intense sexual interest by the bull towards them were bred with artificial insemination using frozen thawed semen. Following the herb treatment, ovaries were scanned using a trans-rectal B-mode ultrasonographic scanner (7.5 MHz) in alternate occasion to identify the location of the largest follicle (LF) and presence of corpus luteum in order to confirm the ovulation.

Pregnancy Diagnosis

Pregnancy diagnosis was done by ultra-sonographic scanning of uterus at day 40 post-A.I. followed by per-rectal examination at day 90.

Statistical analysis

Response of the heifers to medicinal herbs treatment was cross tabulated in responder (estrus) versus non-responder and the resultant 2 x 2 contingency table was analyzed by chi-square test corrected for yete's continuity as some cell frequencies were < 5 and significance was set at 5 %.

Result

Results pertaining to the estrus induction and fertility augmentation following treatment with *Aegle marmelos* and *Murraya koenigii* leaf powder in a combination are shown in Table 1, 2 and 3. In this study, the supplementation of *Aegle marmelos* and *Murraya koenigii* leaf powder caused significant oestrus induction and fertility in delayed pubertal Sahiwal heifers.

Estrus response

Proportion of heifers that showed various visual signs of estrus following herb supplementation during treatment and post-treatment cycle is presented in Table 1. All (100%) the treated heifers showed different degree of vulval swelling after herb treatment while only 3 (50%) heifers showed mild degree of swelling in the control group. About 87.5% heifers in herb group showed little to optimum cervical discharge and 50% showed homo-/ hetero-sexual mount while the corresponding values for control heifers were 33.3 and 16.7%, respectively. Characteristically, the teaser bull showed intense sexual interest in only 2 heifers in treatment group and 1 in control group those had optimum cervical discharge and showed either homo-

or hetero-sexual mount, and later on inseminated. Four heifers in herb group that showed homo- or hetero-sexual mount, 2 of them were inseminated but remainder did not because of no to little cervical discharge and poor interest of teaser to female. Four out of 8 heifers in herb group, towards whom the teaser showed no interest, were also not inseminated in this study. In control group, out of 6 heifers, 2 showed little cervical discharge of which one showed homo- or hetero-sexual mount and teaser showed intense sexual interest towards the heifer (Table 1).

Location of LF, ovulation and estrus interval

Ovarian scanning showed about 87.5 (7/8) to 100% (6/6) heifers have the LF in the right ovary in treatment and control group, respectively. However, only 12.5% or 1 out of 8 heifers and in treatment group had LF on the left ovary (Table 2). About 75% (6/8) heifers in the treatment group and 16.7% (1/6) in control group had CL in the ovary during treatment cycle. The mean interval between the start of treatment and the resumption of the estrous cycle was 15.1±4.63 days in treatment group and only one heifer in control group showed resumption of cycle within 6 days since the start of the treatment (Table 2).

Fertility response

Overall, 8 (100%) out of 8 in the treatment group and 2 (33.3%) out of 6 in the control group were showed estrus and subsequently inseminated. The number of services required per conception was 1.38 (11/8) and 1.50 (3/2) in the treatment and control group, respectively. Overall, 100% (8/8) heifers in treatment group and 33.3% (2/6) in the control group finally became pregnant and later on calved in this study (Table 3).

Discussion

Present study demonstrates oestrus induction and fertility response in delayed pubertal Sahiwal heifer after supplementation with *Aegle marmelos* and *Murraya koenigii* leaf powder when used in combination. The present validation trial confirms the earlier finding communicating the promising oestrus and fertility response using the similar medicinal herbs with a similar dose schedule of treatment (Das *et al.,* 2016). The proportion of heifers (50%) that showed homo-or hetero-sexual mount during the treatment cycle in this study was similar to the finding that was reported in the previous study (Das *et al.,* 2016), in which about 54.5%

Table 1: Type of estrus signs, teaser activity and breeding of Sahiwal heifers after treatment with *Aegle marmelos* and *Murraya koenigii*

Group	Treatment cycle						Post-treatment cycles			
	Prominent estrus sign	Weak estrus sign	Overall in estrus	No. (%) inseminated	No. (%) pregnant	No. (%) calved	Prominent estrus sign	No. (%) inseminated	No. (%) pregnant	No. (%) calved
Treatment	2 (25)	3 (37.5)	5(62.5)	2 (25.0)	2 (25.0)	2 (25.0)	6 (75.0)	6 (75.0)	6 (75.0)	6 (75.0)
Untreatment	1 (16.6)	-	1 (16.7)	1(16.7)	1 (16.7)	1 (16.7)	1(20.0)	1 (20.0)	1 (20.0)	1 (20.0)

Table 2: Location of LF, ovulation and mean onset estrus in Sahiwal heifers after treatment with *Aegle marmelos* and *Murraya koenigii*

Group	No. of heifers treated	Treatment cycle			
		Location large follicle (LF) and ovulation			Mean interval to resumption of cycle (days)
		Right ovary	Left ovary	No. (%) heifers ovulated	
Treatment	8	7 (87.5)	1 (12.5)	6 (75.0)	15.1±4.63
Untreatment	6	6 (100.0)	-	1 (16.7)	6

Table 3: Overall estrus and fertility response in Sahiwal heifers after treatment with *Aegle marmelos* and *Murraya koenigii*

Group	No. of heifers treated	Overall (Treatment cycle + Post-treatment cycle)			Overall no. of heifers pregnant	Overall no. of heifers calved
		No. heifers showed estrus	No. of heifers inseminated	No. of services per conception		
Treatment	8	8 (100.0)	8 (100.0)	1.38 (11/8)	7	7
Untreatment	6	2 (33.3)	2 (33.3)	1.5 (3/2)	2	2

heifers showed standing oestrus. And unlike the previous study, we investigated the behavioural signs of oestrus in a greater detail in this study. Although in the previous study, standing oestrus was the only criteria used to denote the heifers in oestrus (Das *et al.,* 2016) and in the previous study, it has been shown that about 90.9% delayed pubertal heifers resumed cycle following the treatment with Aegle and Murraya (Das *et al.,* 2016).

Six heifers those remained un-bred till the end of treatment, all had optimum vulval swelling, cervical discharge and showed homo- or hetero-sexual mount. Interestingly, teaser bull showed intense sexual interest in all the 6 herb treated heifers during post-treatment cycle and bred subsequently through A. I. On the other hand, only one out of 5 un-bred heifers in control group, showed visual signs of oestrus (Table 1). All the animals irrespective of control and treatment bred through frozen semen and confirm pregnancy through ultrasonography at day 40 of insemination. Further these animals were re-examined per-rectally at day 90. All inseminated animal found pregnant and percentage of pregnancy in herbs supplemented group (75 %) significantly high as compare to untreated control group (16.67 %). All diagnosed pregnant animal calved successfully without any peri-parturient complications (Table 1). In the present study, 75% treated heifer exhibited behavioural sign of estrus and success fully detected by teaser bull, compare to earlier report (54.1- 71.4%) finding in delayed pubertal heifers (Das *et al*., 2012a; 2012c, Das *et al*., 2016) and post partum anoestrus (60.0 %) buffaloes (Dutt *et al*., 2011) estrus response observed in the present research was relatively higher after using similar treatment. The reason behind an increased in estrus response was not known.

Earlier it was reported that anoestrus animal show incidence of silent estrus when treated with *Aegle marmelos* and *Murraya koengii* leaf powder and incidence was reported vary between (12.5 -36.3 %) (Das *et al*., 2012a; 2012c, Das *et al*., 2016). In the present study the incidence of silent estrus was within the reported range. But through combine treatment besides the standing estrus it can also induce cyclicity to about 25 % heifers which appeared to be acyclic before the treatment.

The estrus induction response of this study was, however, higher than that of previously reported either with *A. marmelos* (Kumar, 2008) or with *M. Koenigii* (Umashanker *et al.,* 2006) alone in anoestrus buffaloes. Higher estrus response after using both the plant together might be due to synergistic actions of the component parts of the plants and strengthen the previous views of (Dutt *et al.,* 2010; 2011). Previously, it has been reported that ethanolic extracts of *Murraya koenigii* (Mehrotra *et al*., 2003; Nandini *et al.,* 2010) and *Aegle marmelos* (Jondhale *et al.,* 2009a) advances puberty in immature rats. The possible effect is attributed to either the presence of phytoestrogens and high mineral contents in the leaf of the plants or stimulating effect on the endogenous steroidogenic activity under the hypothalamic effect (Mehrotra *et al.,* 2003, 2004; Jondhale *et al*, 2009a; Nandini *et al.,* 2010). The beneficial effect of above said plants on increased follicular turnover, promotion of terminal follicular growth and increased estradiol production (Dutt *et al.* 2010; Kumar *et al* 2012b) probably caused exhibition of estrus in delayed pubertal heifers in this study.

The mean interval between the initiation of treatment and onset of standing estrus in the heifer was 15.1 ± 4.63 days in treatment group. The only heifer that shows standing estrus in control group was within 22 days (Table 1). *Aegle marmelos* in combination with *Murraya koenigii* is reported to induce estrus by 5.33 d and 9.33 d after the end of the treatment in anoestrus goats (Dutt *et al*., 2010) and buffaloes (Dutt *et al.,* 2011), respectively. Similar treatment reported to induce estrus at an interval of 22.8 d and 8.8 d in delayed pubertal heifers under farm (Das *et al*. 2012a) and field (Das *et al.,* 2012b) condition, respectively. And in present report 15.1 ± 4.63 day. The variations in the mean interval to onset of estrus within the delayed pubertal heifers among the allied studies are, however, unclear. Therefore, it can be speculated that individual animal variation may possibly the reason for the difference in the outcome of various studies.

Six out of eight and one out of six found pregnant in treatment and control animals respectively. The number of insemination required 1(6/6) and 1(1/1) in treatment and control group respectively. All the heifers that shown standing estrus where inseminated, they become pregnant and finally calved (Table 1).Thus present study proved the beneficial effect of *Aegle marmelos* and *Murrya koengii* plant leaves for estrus induction and fertility augmentation in delayed pubertal Sahiwal heifers.

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Electrocardiographic and serum biochemical reference values of Black Bengal goats

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Abstract

Electrocardiographic and serum biochemical reference values were established for healthy, adult, female, Black Bengal goats (*Capra hircus*). The standard bipolar and augmented unipolar limb leads electrocardiograms were recorded in 20 goats, and wave forms were analyzed. Harvested sera were subjected to biochemicals analyses. Positive P waves were dominant except for aVR and aVL leads. The rS in I and aVL, and qR in II, III and aVF leads were dominant QRS patterns. Positive T waves in I, II and aVL, whereas, negative T waves in III and aVR leads were dominant. The amplitudes and the durations of P, QRS complex and T waves remained unchanged ($P > 0.05$) among leads. PR, RR and QT intervals were also unchanged ($P > 0.05$) among leads. Recorded heart rates (per minutes) were 93.403±19.815, 96.673±18.687, 93.518±23.654, 89.602±19.601, 89.561±17.171 and 91.456±20.203 in leads I, II, III, aVR, aVL and aVF respectively. Heart rates were unchanged ($P > 0.05$) among leads. Cholesterol, triglyceride, HDL, LDL, calcium, sodium, potassium, LDH, CK and CK-MB concentrations were 91.24±20.91 mg/dl, 31.02±10.71 mg/dl, 19.20±4.45 mg/dl, 65.833±19.34 mg/dl, 2.031±0.25 mmol/L, 129.032±24.33 mmol/L, 3.616±0.74 mmol/L, 202.535±74.14 U/L, 75.221±54.16 U/L and 25.124±20.98 U/L.

Keywords: Black Bengal goats, Electrocardiography, Serum biochemicals

Introduction

Electrocardiography (ECG) is a noninvasive, relatively inexpensive and extremely useful technique for gaining information about the heart in most species. It is initial test of choice to evaluate cardiac problems associated with the initiation and conduction of waves of depolarization (Santamarina *et al.*, 2001). Small body size, easy availability and cheapness are advantages that make the goat more prefe-rable over other ruminants for biological research (Mohan *et al.,* 2005). However, electrocardiographic studies are infrequently reported in goats (Mohan *et al.,* 2005; Ahmed and Sanyal, 2008; Pogliani *et al.,* 2013; Atmaca *et al.,* 2014) when compared to studies performed in dogs and horses. Further, it has been reported that ECG parameters vary among different breeds of goats (Ahmed and Sanyal, 2008; Mohan *et al.,* 2005; Pogliani *et al.,* 2013) due to differences in their body size. Serum biochemical parameters are of great importance to the veterinary practitioner as they provide valuable information that can contribute to the assessment of the health status of the animal and the monitoring of prognosis in pathological disorders (Coles, 1986). Considering the above reports, the present study was conducted to establish the normal reference ECG values for six standard limb leads in Bla ,ck Bengal goats. Besides, serum biochemical reference values were also established.

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Materials and Methods

Animals

Twenty clinically healthy, adult, nulliparous, female, Black Bengal goats (*Capra hircus*) maintained at animal farm of Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India in semi intensive farming system under well ventilated and proper hygienic conditions were used in this study. Goats were aged 26.80 ± 9.07 (12-37) months and weighed 21.90 ± 3.28 (18-27) kg with a range of body condition scores from 2.0 to 3.5.

Electrocardiographic examinations

Standard electrocardiographic (ECG) examinations were performed without chemical restraint of the animals. The ECG recordings were made in right lateral recumbent position using a portable single channel electrocardiograph (Cardiart 6108T; BPL Medical Technologies Private Limited, Bangalore, Karnataka, India), with a 50 mm/s paper speed and a sensitivity of 20 mm/mV. The forelimbs were kept parallel to each other and perpen-dicular to the long axis of the body. Alligator clip elec-trodes were fixed directly to the skin, just below the elbow and stifle joints in forelimb and hind limb respectively as per method

described earlier (Szabuniewicz and Clark, 1967). Electrode gel (Cardijelly; BPL India Limited, Palakkad, Kerala, India) was used to obtain good clip-to-skin contact. All the recordings were made between 10.00 and 11.00 AM. The standard bipolar (Lead I, II and III) and unipolar augmented limb leads (aVR, aVL and aVF) were recorded. The descriptions of various waves form of the electrocardiogram are as per Tilley (1985). The amplitude (mV) and the duration (s) of P, QRS complex and T wave forms were manually measured in all the six limb leads. The PR, RR and QT intervals (s) were also measured in all the six limb leads.

Biochemical analysis

From each animal, 5.0 ml of blood was drawn from the right jugular vein using a 6-ml syringe with a 24-gauge hypodermic needle and immediately transferred to a 15-ml centifuge tube. The blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 10 minutes. Post-centrifuged sera were carefully harvested in eppendorf tubes and stored at -20 °C for biochemical studies. Serum total cholesterol (Chol), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), sodium (Na), potassium (K), calcium (Ca) concentrations, and lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase-MB (CK-MB) activities were measured by standard absorptive spectrophotometric methods using commerical test kits supplied from Span Diagnostics Limited, Surat, Gujarat, India (Chol, LDL, HDL TG, Ca, Na, K test kits) and Coral Clinical Systems, Goa, India (LDH, CK, CK-MB test kits) with a spectrophotometer (Lambda 35 UV/Vis Spectrophotometer; Perkin Elmer, Waltham, Massachusetts, USA).

Statistical analysis

Statistical analysis was performed using Statistics Package for Social Science software version 17.0 for Windows. The results were expressed as mean ± standard deviation of mean (SD). Electrocardiographic values in different leads were analysed by one way analysis of variance (ANOVA) and means were compared by Tukey's test for multiple comparisons. $P < 0.05$ considered statistically significant.

Results and Discussion

Representative recorded electrocardiogram (ECG) of Black Bengal goats is presented in figure 1. An analysis of the morphology of the P, QRS complex, and T waves of electrocardiograms (ECG) in standard bipolar and augmented unipolar limb leads of Black Bengal goats is presented in table 1. Generally positive P waves were found in all the leads, except for aVR and aVL. Our results agree with those from Atmaca *et al.* (2014), which showed a similar P wave morphology in Angora goats. On the contrary to what was reported in Saanen goats (Pogliani *et al.,* 2013), negative P wave was found in lead I (30%), aVR (60%) and aVL (30%) in studied goats. Negative P waves indicating that the impulse was generated from the atrio-ventricular (AV) junc-tion as a junctional premature complex (Ahmed and Sanyal, 2008). In leads I, II, and aVL, the P wave morphology could not be detected in few animals. This may have been due to a lack of coordinated atrial activity, so that P waves were not being formed, or it could be that P waves were present but were just not clear. The rS pattern of QRS complex predominated in I, aVL leads, and qR pattern in leads II, III and aVF. In lead aVR, rS and qRs pattern of QRS complex were equally dominant in studied goats. Predominant rS and pattern of QRS complex in I and aVL leads of studied goats was agree with the results obtained by Smith (1978) who indicate that the rS morphology as being the most frequent in small ruminants. The QRS complex morphology is quite variable within and between the different leads in goats (Mohan *et al.,* 2005). Accor-ding to our findings, variability in the QRS complexes might be due to the differences in the to-pographic anatomy of the heart within the thorax, position of heart in relation to the limbs (Breazile, 1971) and mechanism of ventricles activation (Hamlin *et al.,* 1984). Because of this, Szabuniewicz and Clark (1967) were able to conclude that the characterization of the QRS complex pattern in the normal ECG for goats would be meaningless. Positive T wave was predominant in leads I, II and aVL, whereas, negative T wave was predominant in leads III and aVR. Equal frequency of both positive and negative T was observed in lead aVF of studied goats.

The amplitude (mV) of P, QRS complex and T waves in standard bipolar and augmented unipolar limb leads of Black Bengal goats is presented in table 2. In studied goats, mean P wave amplitude which is signifying atrial depolarization were comparable to Angora goats (Atmaca *et al.,* 2014). On the contrary to

what was reported in Jamunapari goats (Mohan *et al.,* 2005), higher P wave amplitude in bipolar lead I and III, and lower P amplitude in bipolar lead II was recorded in studied goats. The P amplitude was higher in the augmented lead aVL and lower in aVR and aVF in studied goats than those reported in Jamunapari goats (Mohan *et al.,* 2005). This may be genuine species differences. The highest amplitude for P wave was recorded in lead, I and lowest in aVF. The QRS amplitude (mV) of studied goats were lower than those reported in Jamunapari goats (Mohan *et al.,* 2005) but higher than those observed in Kagani goats (Raina *et al.,* 2008). Altogether, QRS complex amplitudes of studied goats in various leads were lower than those

Table 1: Percent (%) morphology of various ECG wave forms in standard bipolar (I, II and III) and unipolar augmented limb leads (aVR, aVL and aVF) in Black Bengal goats (n=20)

	P wave			QRS complex									T wave		
Lead	Positive	Negative	Silent	rS	RS	Rr'	rSr'	W	qR	QR	qRs	qrS	Positive	Negative	Silent
I	50	30	20	30			20		10	20	20		40	30	30
II	100						10		70	10	10		60	40	
III	90		10			10			80		10		30	70	
aVR	40	60		30	10				10		30	20	30	60	10
aVL	30	30	40	50			30	10				10	70	30	
aVF	100								70	20	10		50	50	

Table 2: Amplitude (mV) (mean ± SD) of various ECG wave forms in standard bipolar (I, II and III) and augmented unipolar limb leads (aVR, aVL and aVF) in Black Bengal goats (n=20)

Lead	P	QRS	T
I	0.072 ± 0.026(0.05-0.10)†	0.289 ± 0.136(0.10-0.50)	0.142 ± 0.085(0.03-0.30)
II	0.067 ± 0.025(0.05-0.10)	0.317 ± 0.130(0.20-0.60)	0.117 ± 0.050(0.05-0.20)
III	0.058 ± 0.025(0.03-0.10)	0.372 ± 0.148(0.15-0.60)	0.139 ± 0.055(0.10-0.25)
aVR	0.058 ± 0.018(0.05-0.10)	0.233 ± 0.125(0.10-0.50)	0.122 ± 0.056(0.05-0.20)
aVL	0.058 ± 0.025(0.03-0.10)	0.283 ± 0.144(0.10-0.45)	0.128 ± 0.087(0.05-0.30)
aVF	0.050 ± 0.000(0.05-0.05)	0.322 ± 0.120(0.15-0.50)	0.106 ± 0.063(0.05-0.25)

Values in the column does not differ significantly (P > 0.05).
† Values in the parenthesis indicates range.

Table 3: Duration (s) (mean ± SD) of various ECG wave forms in standard bipolar (I, II and III) and augmented unipolar limb leads (aVR, aVL and aVF) in Black Bengal goats (n=20)

Lead	P	QRS	T
I	0.038 ± 0.007(0.02-0.04)†	0.053 ± 0.017(0.04-0.08)	0.062 ± 0.023(0.02-0.08)
II	0.038 ± 0.007(0.02-0.04)	0.051 ± 0.018(0.04-0.08)	0.073 ± 0.014(0.04-0.08)
III	0.036 ± 0.009(0.02-0.04)	0.058 ± 0.016(0.04-0.08)	0.073 ± 0.014(0.04-0.08)
aVR	0.038 ± 0.007(0.02-0.04)	0.051 ± 0.018(0.04-0.08)	0.067 ± 0.017(0.04-0.08)
aVL	0.036 ± 0.009(0.02-0.04)	0.042 ± 0.007(0.04-0.06)	0.067 ± 0.022(0.02-0.08)
aVF	0.038 ± 0.012(0.02-0.06)	0.062 ± 0.018(0.04-0.08)	0.064 ± 0.022(0.04-0.10)

Values in the column does not differ significantly (P > 0.05).
† Values in the parenthesis indicates range.

Table 4: Duration (s) (mean ± SD) of various intervals in standard bipolar (I, II and III) and augmented unipolar limb leads (aVR, aVL and aVF) in the ECG of Black Bengal goats (n=20)

Lead	PR	RR	QT
I	0.100 ± 0.022(0.06-0.12)†	0.667 ± 0.134(0.44-0.94)	0.338 ± 0.041(0.28-0.40)
II	0.098 ± 0.018(0.08-0.12)	0.640 ± 0.116(0.44-0.86)	0.336 ± 0.044(0.28-0.40)
III	0.102 ± 0.023(0.06-0.12)	0.671 ± 0.136(0.40-0.88)	0.360 ± 0.046(0.32-0.44)
aVR	0.107 ± 0.026(0.06-0.14)	0.689 ± 0.126(0.44-0.90)	0.324 ± 0.033(0.28-0.38)
aVL	0.089 ± 0.032(0.04-0.12)	0.691 ± 0.128(0.48-0.94)	0.333 ± 0.030(0.28-0.38)
aVF	0.098 ± 0.027(0.04-0.12)	0.682 ± 0.139(0.44-0.96)	0.344 ± 0.042(0.28-0.40)

Values in the column does not differ significantly (P > 0.05).
† Values in the parenthesis indicates range.

reported for dogs (Atmaca and Emre, 2010). The low amplitude QRS deflections might be due to high degree of synchronized ventricular depolarization, which results in canceling of various wave of depolarization passing in any given direction. The highest amplitude for QRS complex was recorded in lead III and lowest in aVR. The T amplitude (mV) values were lower than those reported in Jamunapari goats (Mohan *et al.,* 2005). In the studied goats significant variation in amplitudes between animals was not observed. However, considerable variation from time to time in the same animal and between animals of the same and different species is reported especially for QRS complex (Ayalar *et al.,* 1998).

The P, QRS complex and T waves duration (s) in standard bipolar and augmented unipolar limb leads of Black Bengal goats is presented in table 3. The mean P wave durations (s) in all the leads of studied goats was higher than Angora goats (Atmaca *et al.,* 2014) and lower than Jamunapari goats (Mohan *et al.,* 2005). The mean QRS complex durations (s) in all the leads of studied goats was higher than Angora (Atmaca *et al.,* 2014) and Jamunapari goats (Mohan *et al.,* 2005). The mean T wave duration (s) was higher than Atmaca *et al.* (2014) and lower than Mohan *et al.* (2005) and Ahmed and Sanyal (2008).

The PR, RR and QT intervals (s) in standard bipolar and augmented unipolar limb leads of Black Bengal goats is presented in table 4. The PR interval representing the time duration between atrial and ventricular depolarization (or the delay at AV node) observed in all the leads of the studied goats was higher than Mohan *et al.* (2005), Pogliani *et al.* (2013) and lower than Ahmed and Sanyal (2008) and Fakour *et al.* (2013). The RR interval representing the ventricular rate had a wide range in studied goats, which might be due to its close relationship with autonomic nervous activity. The RR interval observed in all the leads of studied goats was lower than Jamunapari goats (Mohan *et al.,* 2005). The AV nodal delays in goats were intermediary to those for swine and dog. The caprine ventricles remained depolarized for about 0.28-0.44 s, as indicated by the QT interval. These values were higher than the value reported by Mohan *et al.* (2005), Fakour *et al.* (2013) and Pogliani *et al.* (2013). The QT interval depends upon the heart rate, which is closely related to RR interval. The QT interval is reported to be of potential clinical value because of its association with ventricular arrhythmias (Hanton *et al.,* 2001).

The mean heart rates (per minutes) calculated from RR intervals were 93.403 ± 19.815, 96.673 ± 18.687, 93.518 ± 23.654, 89.602 ± 19.601, 89.561 ± 17.171 and 91.456 ± 20.203 in leads I, II, III, aVR, aVL and aVF of studied goats. These values were lower than the value reported by Mohan *et al.* (2005), Fakour *et al.* (2013) and Pogliani *et al.* (2013). The heart of the goats is reported to vary in size and form according to the breed and this variation is expected to be reflected in the ECG (Mohan *et al*., 2005), so this different heart rate in Black Bengal goats may be due to its breed differences.

Serum total cholesterol, triglyceride, HDL and LDL concentrations (mg/dl) were 91.24±20.91 (59.97-128.67), 31.02±10.71 (19.54-47.82), 19.20±4.45 (13.97-25.70) and 65.833 ± 19.34 (40.77-102.90) respectively. On contrary to what was reported in Saanen goats (Elitok, 2012), a higher serum total cholesterol, HDL, LDL and lower triglyceride concentrations were found in studied goats. Serum calcium, sodium and potassium concentrations (mmol/L) were 2.031±0.25 (1.67-2.42), 129.032±24.33 (89.25-152.13) and 3.616±0.74 (2.77-4.91) respectively and obtained values are in agreement with those of female Baladi goats (Azab and Abdel-Maksoud, 1999). On contrary to what was reported in West African Dwarf (Wad) goats (Opara *et al.,* 2010), higher serum cholesterol and lower potassium concentrations were found in studied goats, whereas, sodium concentration remained same. The serum calcium concentration of studied goats was similar to that of Kilis does (Iriadam, 2007). Serum LDH, CK and CK-MB activities (U/L) were 202.535±74.14 (43.33-308.19), 75.221±54.16 (7.74-149.76) and 25.124±20.98 (3.19-61.66), respectively. Reference serum biochemical values determined in Black Bengal goats of the present study correspond to those described goats by other researchers (Mbassa and Poulsen 1991). In conclusion, an electrocardiographic and serum biochemical reference values were established for healthy, adult, female, Black Bengal goats (*Capra hircus*).

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Molecular Epidemiology of *Eimeria* infection in poultry farms located in Udham Singh Nagar district of Uttarakhand

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Abstract

The present study was carried out to determine the prevalence of coccidiosis in broiler and layer farms located in the district Udham Singh Nagar, Uttarakhand, India from January to December 2015. Total 36 poultry farms (22 broiler and 14 layer farms) with history of poor weight gain, anaemia, mortality and passage of blood stained droppings were examined. Of these, 13 broiler and 7 layer farms were found positive for *Eimeria* species. The prevalence of disease was 59.09% in broiler farms and 50.0% in layer farms. Coccidiosis was higher in small flock strength of broiler and layer farms (76.92% and 57.14%, respectively) and lowest in large farms. The prevalence was high in young (3 to 6 weeks) broiler and layer birds and low in adult (more than 6 weeks) broiler and layer birds. The overall prevalence was high in monsoon (55.0%) as compared to winter (25%) and summer (20%). ITS-1 based nested PCR identified six *Eimeria* species, i.e. *E. tenella*, *E. necatrix*, *E. mitis*, *E. acervulina*, *E. brunetti* and *E. praecox* in present study and overall prevalence percentage were 95, 65, 45, 45, 20 and 35 respectively.

Keywords: *Eimeria,* Coccidiosis, Oocysts, Prevalence, PCR.

Introduction

Coccidiosis is one of the most common and economically important enteric disease of poultry caused by *Eimeria* spp. The parasite is generally host specific and each *Eimeria* sp. infects specific part of the intestine. Of 9 species of poultry *Eimeria*, 7 are economically important, viz. *E. tenella*, *E. necatrix*, *E. acervulina*, *E. brunette*, *E. praecox*, *E. mitis* and *E. maxima.* The parasites *E. tenella* and *E. necatrix* are most pathogenic, *E. acervulina, E. maxima* and *E. mivati* are common and moderately pathogenic, *E. brunetti* is uncommon but pathogenic, while *E. mitis*, *E. praecox* and *E. hagani* are relatively non-pathogenic species (Lillehoj and Trout, 1993). Losses due to coccidiosis on yearly basis, including prophylaxis as well as therapy, exceed 2 billion Euros (Dalloul and Lillehoj, 2005) which accounts for approximately 10% of the total investment made in poultry industry. Broiler industry is the major sufferer of coccidiosis estimating a total economic loss of 95.61% (Bera *et al*., 2010). Factors contributing to clinical outbreaks of coccidiosis include damp litter, which favours sporulation of oocysts, contaminated feeder and drinkers, poor ventilation, high stock density, immunosuppressive disease such as chicken infection anaemia, Marek's disease and Infectious bursal disease etc. Coccidiosis in poultry becomes more prevalent in farm due to development of resistance against available anticoccidial drug.

Eimeria species is conventionally identified on the basis of location of lesion in the host intestine, gross appearance of lesions, size of oocysts, sporulation time, prepatent period, schizont size and location of development, location of parasite on the host intestinal epithelium and cross immunization test. The infection caused by *E. tenella* and *E. necatrix* is diagnosed easily on the basis of lesions but infections of other species are difficult to diagnose. Of late biochemical and molecular diagnostic techniques have been developed for diagnosis of *Eimeria* species. Primers specific ITS sequence information on chicken *Eimeria* are now beginning to emerge from the field isolates of Australia and Taiwan (Su *et al*., 2003 and Lien *et al*., 2007). Despite the enormity of the problem posed by coccidiosis to the poultry industry in India, the use of PCR based techniques for species identification has only been recently attempted (Bhaskaran *et al*., 2010 and Aarthi *et al*., 2010). The present paper reports prevalence and identification of different *Eimeria* species through morphometric and PCR technique in commercial and layer poultry farms located in Udham Singh Nagar district of Uttarakhand.

Materials and Methods

Study area: Prevalence of coccidiosis was studied in Udham Singh Nagar district of Kumaon Division of Uttarakhand, India. The climate in district varies from sub-tropical and sub-humid with hottest

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months in May and June. The maximum temperature goes up to 42^{0}C during summers and the minimum temperature is between 1 and 4 ^{0}C. The average annual rainfall is 1296.85 mm, about 90% of the rainfall occurring during the monsoon period, and the remaining 10% of the rainfall in non-monsoon period.

Selection of commercial poultry farms: Total 36 poultry farms (22 broiler and 14 layer farms) were screened from January 2015 to December 2015. These farms had history of poor weight gain, anaemia, mortality and passage of blood stained droppings

Sample collection: Fresh faecal droppings were collected randomly from 10 different places of the flock in sample collecting vials (Hi media) to which 5 ml of potassium dichromate (2.5% w/v) was added. Dead birds (carcasses) were collected in sterilized polythene bags. Samples were properly labelled and brought to laboratory and kept in refrigerator at 4^{0}C until further used.

Faecal examination: Faecal droppings were examined for the presence of oocysts of *Eimeria* by wet smears method. The sample was considered to be negative if three slides from the same sample were found negative for oocysts.

Post-mortem examination of the dead birds: Post-mortem examinations of carcasses were carried out and whole intestines were removed and observed minutely for presence of any pathological lesions. Intestine was cut into different portions (upper, middle, and lower intestine, large intestine and caeca) with sterilized scissor. Wet smears were prepared from the mucosa of respective gut portions and examined under microscope for the presence of oocysts (Soulsby, 1982). The samples found positive for coccidia oocysts were processed for isolation of *Eimeria* species as per method described by Holdsworth *et al.* (2004).

Identification of *Eimeria* species: Morphological identification of oocysts was made as per method of Norton and Joyner (1981).

Molecular Identification of Eimeria spp. using ITS-1 based nested PCR

Isolation of genomic DNA form the positive faecal samples were made through DNA extraction kit (Qiagen, USA) as per protocol described in kit. The concentration and purity of isolated DNA was determined by UV/VIS spectrometer (Perkin Elmer).

The nested PCR protocol using ITS-1 primers was adopted for identification of *Eimeria* species of poultry as described previously (Lew *et al*., 2003) with minor modifications. Flanking primers from 5.8S r RNA and 18S r RNA regions were used in the primary/genus-specific PCR to amplify complete ITS-1 region of *Eimeria* species, while species-specific primers from ITS-1 region were used to amplify the individual *Eimeria* species (Table-1).

Secondary/Nested PCR

The amplified product from the primary PCR was used as template in secondary PCR. For each primer pair, a separate reaction mixture was prepared and subjected to PCR. The amplification of primary PCR and secondary PCR products were checked by gel electrophoresis by using 2% agarose and the gel is visualized by using gel documentation system (Syngene).

Results and Discussion

Of 36 poultry farms, 20 were found positive for *Eimeria* species and overall prevalence was 55.55%. Depending on the type of poultry production system, management practice followed, age of birds, flock strength and climatic conditions, variable prevalence rates of coccidiosis in poultry have been reported (Bachaya *et al.*, 2012, Sharma *et al*., 2013, Kala *et al.,* 2013). Total 13 broiler and 7 layer farms were found positive for coccidiosis. The prevalence was higher in broiler farms (59.09%) as compared to layer farms (50.00%). In broiler farms 100% birds were reared in deep litter system while in layer farms 57.14% birds were reared in cage system and 42.85% deep litter system. Higher prevalence of coccidiosis was in broiler farms was due to the fact that the birds were reared under deep litter system of management while layers were reared in cage as well as deep litter systems. In cage system, chances of contaminations of food and water with faecal droppings are less hence overall low prevalence was observed in layers. The deep litter system provides more conducive environment for the propagation of *Eimeria* species. Nematollahi *et al*. (2009) found 55.96% prevalence while Kala *et al*. (2013) reported higher prevalence in broilers (21.38%) as compared to the layers (11.27%).

Coccidiosis was found higher in small flock strength (< 5000) of broiler and layer farms and prevalence percentage was 76.92and 57.14%, respectively. The disease was low in large flock strength (>10,000) of broiler and layer farms (7.69%, and 14.28%, respectively). The percent incidence of coccidiosis in medium flock strength (5000-10,000) broiler and layer farms was 15.38% and 28.57%, respectively. These changes could be associated with poor management practice such as wet litter and caking of litter which favour the oocysts sporulation, contaminated drinkers and feeders and poor ventilation, poor bio security status and poor

Table1. Primer sequence for nested PCR

Primers for nested PCR		Primer Sequence	Annealing temp.
Eimeria spp.	EF1	AAG TTG CGT AAA TAG AGC CCT C	55^0 C
	ER1	AGA CAT CCA TTG CTG AAA G	
E. acervulina	EAF	GGC TTG GAT GAT GTT TGC TG	71^0 C
	EAR	CGA ACG CAA TAA CAC ACG CT	
E. brunette	EBF	GAT CAG TTT GAG CAA ACC TTC G	71^0 C
	EBR	TGG TCT TCC GTA CGT CGG AT	
E. maxima	EMRA1	GTG AT/AT CGT TC/TG G/AG/AA GTT TGC	71^0 C
	EMFA1	CT/AC ACC ACT CAC AAT GAG GCA C	
E. mitis	EMi1FA	GGG TTT ATT TCC TGT CC/GT CGT CTC	58^0 C
	EMi1RA	GCA AGA GAG AAT CGG AAT GCC	
E. necatrix	ENF	TAC ATC CCA ATC TTT GAA TCG	61^0 C
	ENR	GGC ATA CTA GCT TCG AGC AAC	
E. praecox	EPRA	CCA AGC GAT TTC ATC ATT/C GG GGA/G	61^0 C
	EPFA	AAA A/GCA A/CAG CGA TTC AAG	
E. tenella	ETF	AAT TTA GTC CAT CGC AAC CCT	65^0 C
	ETR	CGA GCG CTC TGC ATA CGA CA	

Table 2. Identification and prevalence of *Eimeria* spp. by ITS -1 based nested PCR in poultry, farm located in Tarai region of Uttarakhand, India

S.N.	Code of poultry farm	Type of bird	*Eimeria* spp. Species identified					
			tn.	nc.	mi.	ac.	br.	pr.
1.	KPF1	B	+	+	+	+	-	-
2.	KPF2	B	+	+	-	-	-	-
3.	LPF	B	+	+	+	-	+	-
4.	VPF	B	+	+	+	-	-	+
5.	KPF3	B	+	+	+	+	-	-
6.	PPF	B	+	-	-	+	-	+
7.	KPF	B	+	-	-	+	-	-
8.	HGPF	B	+	-	-	-	+	-
9.	KPF5	B	+	-	-	-	-	+
10.	CPF	B	+	+	-	-	-	-
11.	FPF	B	+	-	+	-	+	-
12.	NKPF	B	+	+	-	-	-	-
13.	NPF	B	+	+	-	-	-	-
	Prevalence percentage in broiler farm		13/13 (100.00)	8/13 (53.84)	5/13 (38.46)	4/13 (30.76)	3/13 (23.07)	3/13 (23.07)
14.	CKPF	L	-	+	+	-	-	+
15.	NPF	L	+	+	+	+	-	-
16.	RPF3	L	+	-	-	+	-	-
17.	NPF	L	+	+	-	+	-	+
18.	IPF1	L	+	-	-	+	-	+
19.	BPF	L	+	+	+	+	-	+
20.	IPF2	L	+	+	+	-	+	-
	Prevalence percentage in layer farm		6/7(85.71)	5/7(71.42)	4/7(57.14)	5/7(71.42)	1/7(14.28)	4/7(57.14)
	Over all prevalence percentage		19/20(95)	13/20(65)	9/20(45)	9/20(45)	4/20(20)	7/20(35)

(tn- *E. tenella*, nc -*E. necatrix*, mi- *E. mitis*, ac-*E. acervulina*, br- *E. brunetti*, pr-*E. praecox*)

health care practices. Adamu (2015) also reported high incidence of coccidiosis (63.64%) in small flock strength poultry farms.

The prevalence of coccidiosis was higher in birds of 3 to 6 weeks of age (young birds) in both broiler (92.30 %) and layer birds (71.42 %). Its prevalence in more than 6 weeks of age (adult birds) was 7.69% in broiler and 28.57% in layer. Nematollahi *et al*. (2009) and Adamu **(**2015) also found higher prevalence of coccidiosis in chickens aged 5 to 6 weeks. Bachaya *et al*. (2015) found all age groups suspected for coccidiosis, but usually it resolves itself around 6 to 8 weeks of age. Coccidiosis occurs mainly in young birds because immunity quickly develops after exposure and gives protection against later disease outbreak. Unfortunately, there is no cross protective immunity against different *Eimeria* species. Thus, several outbreaks of coccidiosis are possible in the same flock, with different species (Permin and Hansen, 1998).

Season wise, prevalence was high in monsoon (55.0%) as compared to winter (25%) and summer (20%). In broiler farms it was high (61.53%) in monsoon followed by winter (23.07%) and summer (15.38%). In layer farms also it was high (42.85%) in monsoon but similar prevalence (28.57%) was recorded in winter and summer. High prevalence in monsoon (mid June to Sept.) might be due to heavy rain fall followed by high temperature that enhances the sporulation and survivability of oocysts. Low prevalence in summer could be due to high ambient temperature and low humidity that were made unfavourable for sporulation of oocysts. Amin *et al*. (2014) also reported high prevalence of coccidiosis in broilers during monsoon.

Morphometric identification showed that isolated oocysts were from mixed infection. The calculated oocysts index indicated that measured Oocysts belonged to be *E. tenella*, *E. necatrix*, *E. mitis*, *E. acervulina, E. brunetti* and *E. praecox* and overall prevalence of these species were 90, 50, 25, 45, 15 and 25%, respectively. In broilers *E. tenella* (100%), *E. necatrix* (30.46%), *E. mitis* (23.07%), *E. acervulina* (30.76%), *E. brunetti* (15.38%) and *E. praecox* (23.07%) were recorded, while in layers *E. tenella* (85.71%), *E. necatrix* (71.42%), *E. mitis* (28.57%), *E. acervulina* (71.42%), *E. brunetti* (14.28%) and *E. praecox* (28.57%) were recorded. The morphomertric i.e. conventional method is very subjective, tedious, time consuming, needs expertise hence poses difficult in diagnosis and it is suitable for preliminary screening identification of small number of samples only.

The molecular identification of different *Eimeria* species was done trough nested Polymerase chain reaction (PCR) using ITS-1 primers. In primary PCR, sample found positive for *Eimeria* species showed a clear amplification ranging from 400-600 bp. In nested PCR, samples were found positive for *E. tenella, E. necatrix, E. mitis*, *E. acervulina, E. brunetti* and *E. praecox* showed clear amplification on 278 bp, 383 bp, 328 bp, 321 bp, 311 bp and116 bp. Results of ITS-1 based nested PCR showed the entire faecal sample positive to more than one *Eimeria* species and most prevalent species was *E. tenella* in both broiler and layer farms. Total six *Eimeria* species *E. tenella*, *E. necatrix*, *E. mitis*, *E. acervulina, E. brunetti* and *E. praecox* were identified and over all prevalence of these species were 95, 65, 45, 45, 20 and 35%, respectively. In broilers, all sample found positive for *E. tenella* followed by *E. necatrix* (53.84%), *E. mitis* (38.46%), *E. acervulina* (30.76%), *E. brunette* (23.07%) and *E. praecox* (23.07%) while in layers, *E. tenella* (85.71%), *E. necatrix* (57.14%), *E. mitis* (71.42%), *E. acervulina* (71.42%), *E. brunette* (14.28%) and *E. praecox* (57.14%) were recorded (Table 2). High sensitivity of ITS-1 based nested PCR protocol can be attributed to fact that multiple copies of ITS-1 region are presented in the DNA and also inhibitors of PCR are diluted in nested PCR. Molecular identification through ITS-1 based nested PCR methods are much faster, highly sensitive, accurate and technically easier to use in comparison to the morphomertric, i.e. conventional method. Molecular technique may also contribute to the development of new vaccines and selection of new anticoccidal drugs to be used in its control programme. Bhaskaran *et al*. (2010) reported multiple species infections of *Eimeria* in poultry and most prevalent species was *E. tenella* and 80% samples found positive for *E. tenella* followed by *E. mitis* (53%), *E. acervulina* (42%), *E. brunetti* and *E. maxima* (23%) and lowest (15% samples) were found positive for *E. necatrix*. While none of the sample found positive for *E. praecox*. Aarthi *et al*. (2010) identified seven species of *Eimeria* in poultry through ITS-1 based nested PCR. In broiler, all of the samples were positive for *E. necatrix* and more than 50% samples were positive for *E. brunetti, E. tenella, E. maxima* and *E. acervulina* while, 11% and 16% samples were positive for *E. praecox* and *E. mitis,* respectively.

Conclusions

From the present study, it was concluded that coccidiosis is one of the major enteric disease of poultry causing great economic loss to the poultry in spite of using anticoccidial agents. Its prevalence was high in broiler farms of small flock strength in 3 to 6 weeks age group. Prevalence was high in monsoon season. It is recommended that regular examination of faecal dropping will help in detecting subclinical coccidiosis and prevent the economic losses. Prompt and accurate diagnosis of different species *Eimeria* is essential for the prevention, surveillance, control of coccidiosis and prevent from wide spread resistance of anticoccidial drugs. ITS-1 based nested PCR is most sensitive for identification of *Eimeria* species. Six *Eimeria* species in present study namely *E. tenella*, *E. necatrix*, *E. mitis*, *E. acervulina*, *E. brunetti* and *E. praecox* were identified in broiler and layer farms located in Udham Singh Nagar district of Uttarakhand.

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Short Communication

Bovine tropical theileriosis in cattle reared in and around Bhubaneswar

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Abstract

Present work was undertaken to study the incidence of bovine tropical theileriosis in and around Bhubaneswar, Odisha for a period of three years. Out of 5237 blood samples examined, 3876 (74%) were found positive. The prevalence of the disease is highest in animals between 1 to 5 years age (58.15%) followed by 5 to 10 years age (37.64%). Post partum cows are more (76%) affected by the disease than cows in later lactation (14%) and pregnancy (10%). The incidence of the disease is much higher in crossbred (94%) than indigenous cows (6%). Similarly the incidence of the disease is more in rainy season (45.51%) followed by summer (34.16%) and lowest in winter season (20.33%). The study shows that theileriosis is highly prevalent in this area due to hot humid climate favouring tick breeding and densely populated crossbred cattle.

***Keywords**:* Bhubaneswar, Cattle, Prevalence, Theileriosis

Tropical theileriosis is one of the fatal diseases present in the entire Indian subcontinent including India, China, Pakistan, Iraq and Turkey and is endemic in nature. Indigenous cattle and buffaloes have an inherent resistance to this disease and harbour theilerial piroplasms in their erythrocytes as symptomless carriers (Singh, 1990). They act as reservoirs of infection for uninfected animals particularly the exotic and their crosses. The disease has emerged as one of the fatal diseases of crossbred cattle born due to cross-breeding programmes (Singh, 2002). Therefore the present investigation was undertaken to study the occurrence of the disease in different age groups, sex, season, breed and stage of production of cattle in and around Bhubaneswar.

Materials and Methods

The study was conducted between March 2012 and February 2015. A total of 5237 suspected blood samples collected from field veterinarians and also received at Department of Veterinary Pathology and Teaching Veterinary Clinical Complex referred by Veterinary Clinicians were screened for blood protozoa. Theileriosis cases were diagnosed on the basis of clinical signs and demonstrating piroplasms in Giemsa stained blood smear and in few cases Koch's blue bodies inside lymphocytes (Soulsby 1982). Patient data regarding age, sex, stage of lactation, parturition history, clinical signs etc. were also recorded.

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Results and Discussion

Out of 5237 suspected blood samples screened between March 2012 and February 2015, 3876 (74%) cases were found positive by light microscopic examination indicating a high prevalence of the disease in this region. Singh (1990), Ananda *et al.* (2009) and Panda *et al.* (2011) have reported on the prevalence of the disease from different parts of the country. In the present study, blood samples from cattle suspected for haemoprotozoan diseases were referred for diagnosis. Thus the incidence of theileriosis appeared to be high. The disease occurrence pattern varied in different age groups. Highest incidence of theileriosis was in the age group of >1 year to <5 years 2254 (58.15%) followed by >5 year to <10 years 1459 (37.64%), >10 years 94 (2.43%), >1 month to <1 year 48 (1.78%) and < 1 month age 21 (0.54%) (Table 1). This age group of 1 to 10 years is a very important productive period to be vigilant for affection due to theileriosis in cows. Higher incidence of the disease in adult animals has been reported by Roy *et al.* (2004) and Ananda *et al.* (2009). Our study also revealed juvenile theileriosis in 21 cases of less than 1 month age which gives a new dimension in monitoring the disease of new born calves in this region. However there is scanty report of affection in neonatal stage. Gautam, (1981) reported that young indigenous calves were susceptible and suffered severely. Ananda *et al.* (2009) screened 132 crossbred cattle by Giemsa stain and found 41(31.06%) animals positive for T. annulata. They further reported that the prevalence of

Table 1: Prevalence of theileriosis in cattle in and around Bhubaneswar during 2012-2015

Sl. No.	Criteria	Classification	No. of affected cases	Percentage within the group
1	Age	<1 month	21	0.54
		>1 m to <1yr	48	1.24
		>1 yr to <5yr	2254	58.15
		>5 yr to <10yr	1459	37.64
		>10yrs	94	2.43
2	Sex	Female	3713	95.79
		Male	162	4.18
3	Breed	Crossbred	3659	94.40
		Non-descript	216	5.57
4	Stage of production	Postparturient period	351	9.06
		Later lactation period	67	1.73
		Pregnancy period	44	1.14
5	Season	Summer	1338	34.52
		Rainy	1807	46.62
		Winter	728	18.78

theileriosis was highest in older animals than the young animals.

In our study, 3714 (96%) and 162 (4%) positive cases were observed in females and males respectively which is obvious as the population of female are high in dairy farming. Farmers also do not prefer to rear males for their less utility and also there is mechanization of farming and thus males are no more required for drafting or pulling bullock carts. Sahoo (1991) and Panda *et al.* (2011) observed that the infection of bovine tropical theileriosis in Bhubaneswar was higher in females than the males.

The incidence of theileriosis was higher in crossbred animals 94% (3660) than non-descript animals 6% (216) (Table 1). It was noticed that the number of blood samples referred for disease diagnosis of local non-descript breeds were less. The crossbred animals were crossbred Jersey, crossbred Holstein, graded Haryana and graded Red Sindhi animals. Among crossbred animals, highest number of positive cases of theileriosis was in cross bred Jersey (3769), followed by cross bred Holstein (86), Red Sindhi (23) and Haryana (13) breeds of cattle. Crossbred animals are more prone to diseases than indigenous which may be a fact for higher incidence of theileriosis in crossbred animals. Our finding is in agreement with workers like Singh (2002), Zahid *et al.* (2005), Ananda *et al.* (2009) and Panda *et al.* (2011).

Lactation and pregnancy history was available for 462 number of positive cows which revealed higher incidence (351, 76%) in cows within 2 months of postpartum period followed by later lactation period (67,14%) and pregnancy (44,10%) (Table 1). Stress in cows occurs during parturition and early lactational period due to hormonal changes, physiological condition and physical activity along with conditions like anaemia, milk fever, postparturient hemoglobinuria, ketosis, displaced abomasum, metritis, endometritis, pyometra etc. which predispose and aggravate the disease. More (2008) have observed that postpartum stress adds to theileriosis infection in dairy cows.

Seasonwise, the incidence of theileriosis was highest in rainy season 1808(45.51%) followed by summer 1339(34.16%) and winter 729(20.33%) (Table 1). The occurrence of tropical theileriosis is seasonal and coincides with the incidence of ticks on the host in hot and humid climate which also favors the development of the parasite inside the ticks. The findings of the present study were in accordance with the observations made by Radostits *et al.* (1994); Roy *et al.* (2004), Ananda *et al.* (2009) and Panda *et al.* (2011). However, Durrani, (2007) reported highest incidence of theileriosis in summer (62.7%) followed by spring (8%) winter (5.33%) and autumn (5.33%), in Pakistan.

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Incidence of dermatological problems in dogs of Mizoram

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Abstract

The study was carried out to find out the incidence of various dermatological problems in dogs presented at Department of Veterinary Medicine, Selesih, Aizawl and State Veterinary Hospital, Khatla, Aizawl. Skin scrapings were collected from all the dogs with dermatological problems. The overall incidence of dermatological problems were recorded as 17.5% of the total canine cases where as State Veterinary Hospital, Khatla recorded much lower incidence rate of 8.32 %. Among various skin diseases, Sarcoptic mange (36.36%) is the most common disease followed by Fungal (20.13%), Bacterial (14.94%) and Demodectic mange (11.69%). Incidence of various skin diseases were more during summer months. Dogs under one year of age were more frequently affected. The incidence is highest among mongrels, followed by Alsatian, Rottweiler, Doberman .

Keywords: Dermatological problems, Incidences, Dog, Mizoram

Skin serves as a "mirror" reflecting the functional integrity of internal organ systems. Dermatologic problems are reported to be the most commonly encountered and hardest to resolve problem by Veterinarians in small animal medicine (Deboer *et al.,* 2001). In small animal clinical dermatological disorders constitute a majority of cases ranging from 12 to 75 percent. Skin disorders are not usually fatal, but are aesthetically disagreeable to the owner and cause discomfort both to the dog and the owner. Mange is a contagious skin disease of dogs and has zoonotic importance (Kumar *et al.* 2002). It has also been observed during clinical evaluation of mange infestations in mammalian biosystem that they produce significant haemato-biochemical changes upon the impact of stress caused by mites (Sarma *et al.* 2005). Dogs can be infected with fleas, mites, fungus, etc. leading to skin disorders. Skin disorders of dogs vary from acute, self-limiting problems to chronic or long lasting problems requiring lifelong treatment. Like any other places around the world, skin diseases represent one of the commonest single ailments in small animal practices in Mizoram. The present work envisaged to determine the incidence and intensity of different forms of skin diseases in dogs, along with their clinical.

Materials and Methods

The present investigation was undertaken at Department of Veterinary Medicine, C.V.Sc & A.H., Selesih, Aizawl, from the month of 1st August, 2009 to 31st July, 2010. A total of 154 clinical cares with the history of itching irritability, erythema, keratinization etc were served. Information with regard to age, breed, sex was recorded and skin scraping were examined for mites, fungus and bacteria.

Each skin scraping material was divided into two parts; one part was immediately examined under light microscopy at the laboratory of Department of Veterinary Medicine for diagnosis of mites and other ectoparasitic infestations. The other part was inoculated in Sabauraud's Dextrose Agar and Nutrient Agar for mycological and Bacteriological analysis.

The data were analyzed as per the methodology of Snedecor and Cochran (1994).

Results and Discussion

In the present study the overall prevalence of canine dermatological cases in Aizawl was observed as 17.5% (Table 1). Among various skin diseases, the percent prevalence of Sarcoptic mange, Demodicosis and other skin problems (comprising of Bacterial, Fungal, Flea and lice infestation, allergy, etc.) were 36.36%, 11.69% and 51.59% respectively (Table 2), which is in line with Sarma *et al.,* (2005). Month wise occurrence pattern was shown in Table 1. Highest prevalence percentage of various canine dermatological cases were recorded during the month of August to November (20.64%), followed by the period of April to July (17.26%) and then December to March (16.23%).

The age & sex wise prevalence data is shown in Table 2. Young dogs under one year of age were mostly affected (49.35%), followed by older dogs over three years of age (29.87%). Dogs between one to three years

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are least affected (20.77%). The prevalence of skin diseases in relation to sex were nearly equally affected (51.29% and 48.70%, respectively in male and female).

The breed wise prevalence data (Table 2) shows that skin diseases are most common in local breeds of dogs (44.15%) followed by other cross bred dogs (33.12%), Alsatian (14.28%), Rottweiler (4.54%), Doberman (2.59%) and Great Dane (1.29%).

Incidence of canine dermatological cases in Aizawl was observed as 17.5% of the total canine cases, might be due to that most of the dermatological cases were mild and are not always live threatening which then failed to attract owner's attention to bring his animal to the clinics. Percent prevalence of sarcoptic mange, demodicosis and other skin problems (comprising of bacterial, fungal, Flea and lice infestation, allergy, etc.) were 36.36%, 11.69% and 51.59% respectively), which is in line with Sarma *et al.,* (2005). Highest prevalence percentage of various canine dermatological cases were recorded during the month of August to November (20.64%), followed by the period of April to July (17.26%) and then December to March (16.23%), which is in agreement with Khuresh *et al.* (2006). Symptoms exhibited by the dogs with dermatoses irrespective of aetiology involved are pruritis, alopecia and crusting. Similar clinical symptoms were also being recorded by Hill *et al.* (2006).

Dogs under one year of age were mostly affected (49.35%), followed by older dogs over three years of age (29.87%). Dogs which are in the age group between one to three years are least affected (20.77%) which agree with the findings of Sarma *et al.* (2013). Dogs under one year of age could be as a result of the fact that dogs within the age group still have juvenile immune system which is unable to produce specific and sufficient antibodies to protect skin infection from different factors (Ardeth, 2002) and might be attributed to constant exposure to the carrier mothers (Nayak *et al.*, 1997). Dogs of either sex were nearly equally affected (51.29% and 48.70% respectively in male and female) this finding was in line with Brilhante *et al.* (2003) who also reported that there was no sex predisposition in the incidence of skin diseases. Both sexes have the same ability to transmit genetic predisposition to demodicosis, scabies and fungal dermatitis (Morris *et al.*, 1936). However, sex susceptibility is to be analyzed in light of observation that people in the region prefer male dogs over females because of their masculine look, better vigor and no fuss of unwanted pregnancies.

Skin diseases are most common in local breeds of dogs (44.15%) followed by other cross bred dogs (33.12%), Alsatian (14.28%), Rottweiler (4.54%), Doberman (2.59%) and Great Dane (1.29%). Breed composition of canine population in a particular region and popularity of individual breeds can affect the results of breed predilection to dermatitis in the examined area (Pocta and Svoboda, 2007).

In the conclusion, it was observed that Dermatoses are one of the commonest single ailments

Table 2: Age, sex, breed and etiology wise prevalence of skin diseases

Factors		Number of Dogs positive	Percentage (%)
Age	Below one year	76	49.35
	Between one to three year	32	20.77
	Above three year	46	29.87
Sex	Male	79	51.29
	Female	75	48.70
Breed	Mongral	68	44.15
	Alsatian	22	14.28
	Doberman	4	2.59
	Great dane	2	1.29
	Rottweiler	7	4.54
	Others	51	33.12
Etiology	Sarcoptes	56	36.36
	Demodex	18	11.69
	Bacterial	23	14.94
	Fungal	31	20.13
	Others	26	16.88
Factor wise	Unifactorial	129	83.77
	Multifactorial	25	16.23

Table1. Incidence of skin diseases in dog during August, 2009 to July' 2010 in Mizoram

Month	Total No. cases	No of skin infected cases	Percentage
August-November' 2009	155	32	20.64
December, 2009-March, 2010	308	50	16.23
April-July, 2010	417	72	17.26
Overall	**880**		**17.5**

among dogs of Mizoram with more incidences in young age group and local animals during warmer months.

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Genotoxic effect of cadmium and lead in Wistar rat

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Abstract

The present study was conducted to study genotoxic effect of excessive exposure to cadmium, lead and their mixture in Wistar rats. Sixty colony bred albino Wistar rats were divided randomly into five groups. The rats of group I received only deionised water and served as negative control while, group II, III and IV were orally gavaged with cadmium chloride @ 100ppm; lead acetate @ 500 ppm; and mixture of cadmium chloride @ 100ppm & lead acetate @ 500ppm respectively for 28 days. Group V was kept as a positive control, and was given cycophosphamide (20 mg/kg body weight i.p. 24 hours prior to terminal sacrifice). All the three treatment groups showed significant ($P<0.05$) increase in micronuclei as compared to negative control. The findings of the micronuclei study suggested that cadmium and lead when administered alone were equally genotoxic to that of mixture of both the metals at the given dose level however their genotoxic potential was less than the positive control.

Keywords: Cadmium, Lead, Micronuclei

Environmental pollution is a global problem and it has become major threat to existence of mankind on the planet. Cadmium (Cd) is a naturally occurring metallic element that is used for electroplating and galvanization processes, in the production of pigments, in batteries, as a chemical reagent, and in miscellaneous industrial processes (Toxicological profile for cadmium ATSDR, 2012). Lead is second in the ATSDR's (Agency for Toxic Substances and Disease Registry) top 20 hazardous substances. In veterinary practice, lead poisoning is most common in dogs and cattle. Alterations in genetic material are undoubtedly significant in the etiology of cancer and congenital malformations. In nature there are more chances of exposure by a mixture of heavy metals rather by single heavy metal. Present study was therefore, planned to know the genotoxic effect of cadmium chloride and lead acetate by gavaging single as well as mixture of both metal salts in Wistar rat.

Materials and Methods

The sub acute toxicity of cadmium chloride and lead acetate and their interaction was evaluated on 30 male and 30 female rats. All the 60 rats were randomly divided into 5 different groups. Each group consisted of 6 male and 6 female rats. The groups were numbered as group I to V. The group I received only deionised water and served as negative control while, group II, III and IV were orally gavaged with cadmium chloride @ 100PPM; lead acetate @ 500PPM; and mixture of cadmium chloride @ 100PPM & lead acetate @ 500PPM respectively for 28 days. The group V served as a positive control group and was given cycophosphamide intra peritoneally 24 hours prior to sacrifice i.e. on 27th day for genotoxicity study. At the end of 28 days all the rats were sacrifice. Bone marrow cells from both the femurs were collected for evaluation of genotoxicity and micronuclei assay was carried out in bone marrow cells by the method suggested by Zhong and Siegel (2000). Rats belonging to all experimental groups were sacrificed by cervical dislocation and both the femurs were cut at the both ends with bone snips. The bone marrow was aspirated from the body of femurs in the Hank's balance salt solution (HBSS) containing ethylene diamine tetra acetic acid and Bovine serum albumin (pH 7.2). Bone marrow cell suspension was centrifuged for 10 min at 1000 rpm. Supernatant was discarded and the cells in the sediment were mixed carefully. A drop of cell suspension was taken and smeared on clean slides. The smears were fixed with methanol for 5 min and stained with a combination of May Grunewald and Giemsa stain in succession. The fixed smears were stained with undiluted May Grunewald stain for 5 min and diluted May Grunewald stain 1:1(v/v in distilled water) for 5 min. Slides were washed in distilled water and stained with 5% Giemsa for 10 min. Slides were air dried after washing with distilled water. Two thousand polychromatic erythrocytes (PCEs) (Fig. A,B) per animal were scored to determine micronuclei (Fig. C,D,E,F) frequencies and 200 erythrocytes were examined to calculate the ratio

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Table 1: Bone marrow micronuclei assay and PCE/TE ratio in rats exposed to mixture of Lead and Cadmium for 28 days

Parameter	Group I	Group II	Group III	Group IV	Group V
Micronuclei/2000PCE	1.30^{a}±0.362	3.30^{b}±0.362	3.20^{b}±0.361	3.80^{b}±0.345	14.20^{c}±0.735
PCE/TE	117.4^{c}±0.321	107.2^{b}± 1.162	106.8^{b}±1.243	102.2^{b}±1.652	78.0^{a} ±1.931

Superscript should be read row wise for mean comparison.
Mean with similar superscripts in row do not differ significantly (P<0.05)

of Polychromatic erythrocytes (PCEs) to total erythrocytes (TE). The data generated during experiment were subjected to statistical analysis by using standard statistical procedures (Snedecor and Cochran, 1980). Statistical analysis was done by completely randomized design (CRD). One Way Analysis of Variance (ANOVA) was used at the P< 0.05 significant level.

Results and Discussion

The important cytological end point that is routinely used in the genotoxicity evaluation of the chemicals are formation of micronuclei (Agarwal *et al.* 1994). Micronuclei are thought to arise from chromatid or chromosome fragments detached from a chromosome after breakage. This micronucleus does not integrate into the daughter nuclei and remains as a separate entity. Micronuclei have been used to -evaluate the toxicity of a chemical to the erythroid cell population in the bone marrow.

The mean number of micronuclei/2000 polychromatic erythrocytes (PCE) found in groups I, II, III, IV and V were 1.30 ± 0.362, 3.30 ± 0.362, 3.20 ± 0.371, 3.80± 0.345 and 14.20 ± 0. 735 respectively (Table 1).

The mean number of micronuclei/2000 polychromatic erythrocytes (PCE) found in group V (positive control) was significantly higher than group I, II, III and IV. The mean number of micronuclei/2000 polychromatic erythrocytes (PCE) from group II, group III and group IV rats revealed significant increase as compared to group I (negative control). Among different treatment groups there was no significant difference in micronuclei on 28th day post treatment.

PCE/TE ratio was lowest in positive control (group V) and highest in negative control rats (group I). All the three treatment groups (II, III, and IV) showed significantly decreased value of PCE/TE ratio as compared to negative control indicative of decreased haemopoietic activity of bone marrow by both the heavy metals.

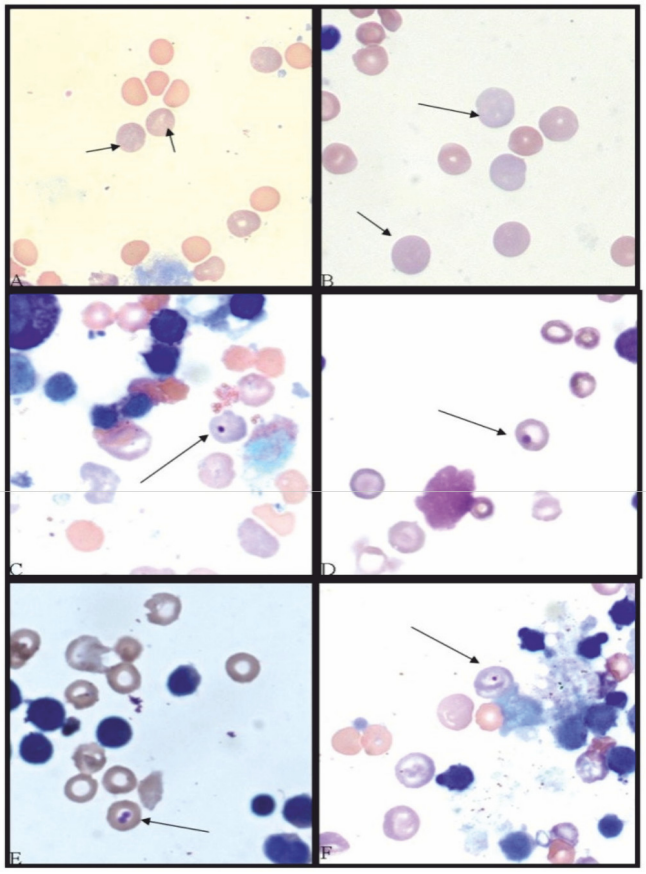

Fig. : Bone marrow cells of rats treated with cadmium, lead, mixture of cadmium &lead and cyclophosphamideshowing:- a) and b) polychromatic cells. c), d), e) and f) polychromatic cells with microneucleus (arrow).

This genotoxic effect of $CdCl_2$ could be attributable to it's direct as well as indirect effect on genome. The direct genotoxic effect of cadmium includes its capacity to form tight covalent bonds with positively charged proteins and DNA and form of DNA adducts. The indirect mechanism of cadmium induced genotoxicity involved the DNA damage through induction of free radical generation as opined by Singh and Chauhan (2003). Lead can substitute for zinc in some enzymes and in zinc-finger proteins, which coordinate one or more zinc cations as cofactors. The substitution of lead for zinc in zinc-finger proteins can have significant effects on de novo expression of the

bound proteins and in any genes transcriptionally regulated by a particular protein. Lead has been found to alter the binding of zinc-finger transcriptional regulator Sp1 to its specific DNA sequences (Toxicological profile for lead, ATSDR, 2007). Fahmy and Aly (2000) found that CdCl2 (1.9, 5.7 and > 6 mg/kg body weight.single i.p. treatment) induced significant and dose dependent increase in micronuclei. Consistent to present study Oyeronke *et al.* (2007) also reported presence of micronucleated polychromatic erythrocytes in the bone marrow cells of the rats treated with mixture of sodium arsenite and lead acetate. Jadhav (2005) also found increase in micronuclei with mixture of eight different metals inclusive of lead and cadmium in different graded doses in drinking water.

The findings of the micronuclei study suggested that cadmium and lead when administered alone were equally genotoxic to that of mixture of both the metals.

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Infectious Bronchitis in poultry birds

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Abstract

Infectious Bronchitis (IB) is highly contagious viral disease involving respiratory and urogenital tracts of both broiler and layers below 6 weeks of age. Aim of this study was to standardized the simple serological test for diagnosis of IB. In field cases IB was detected on the basis of anamnesis, postmortem examination, virus isolation and identification serological. The results of the present study indicated that HA and HI tests are more superior, less expensive, sensitive and rapid for identification of IB Virus (IBV) as compared to AGPT.

Keywords: Infectious bronchitis, Sero diagnosis, Poultry

Infectious Bronchitis (IB) is known to be one of the highly contagious viral disease affecting both broiler and layers (King and Cavanagh, 1991) causing mortality, poor body weight gain in broilers and reduce egg production in layers. It is caused by Infectious Bronchitis Virus (IBV), a member of the group III of genus Corona virus a single stranded RNA virus. Initially it was believed that all the isolates belongs to single prototypes termed as Massachusetts serotype, subsequently other serotypes were isolated from different parts of the world with antigenic and pathogenic difference (Gelb *et al*., 1991).

The upper respiratory tract is primary site of IBV replication followed by viremia and dissemination of virus to other tissues. The ability of virus to undergo continuous genomic shift and drift has lead to emergence of several new serotypes especially in area of intensive poultry farming (Zanalla *et al.,* 2003). Most isolates replicate well in developing chicken embryo following inoculation of allantoic cavity. Diagnosis of IBV is confirmed by isolation of the virus using either chick embryonated egg (ECE) or Tracheal organ culture (TOC) and detection by reverse transcriptase polymerase chain reaction (RT-PCR) (Cavanagh and Naqi, 2003). The group specific Agar gel preparation test (AGPT) can be used for detection of antigen as it is cheap, fast and requires few laboratory facilities. But has an image of poor sensitivity. IBV may be detected directly in tissue of the infected birds by means of immunochemistry (Chen *et al*., 1996) or in situ hybridization (Collison, 1990). The RT-PCR has proved useful in the detection of several RNA viruses (Cavanagh *et al*., 1990). The wide spread use of live and inactive vaccine infectious bronchitis vaccine complicate the disease diagnosis by serological tests. Hence, isolation and demonstration of virus appears to be confirmatory of the disease (OIE manual, 2013). In view of above facts, the present study was planned to isolate and identification of virus from field cases of IB.

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Materials and Methods

IB was suspected in flock based on history of mortality at an early age, clinical signs (respiratory, renal and reproductive dysfunction), vaccination history and gross pathological lesions (serous exudates and haemorrhage in trachea, air sacculitis, visceral and articulate gout) of dead birds. The tissue samples of lungs, trachea, kidney and cloacal swab were collected from the suspected birds. These organs were washed in normal saline and preserved in 50% glycerol saline until the virus isolation was done (Jose *et al.*, 2000).Firstly tissues sample were thoroughly washed in HBBS (Hank's Balanced Salt Solution) after that these samples were homogenized in HBBS@10% w/v, supplemented with penicillin @ 1000 IU/ml and streptomycin at the dose rate of 1.0 mg/ml in sterile pestle mortar with sterile sand. Tissue suspension was freezed and thawed twice, subsequently centrifuged in refrigerated centrifuge at 4000 rpm for 20 minutes. Supernatant was filtered with 0.22 μm syringe filter kept at -20^{0}C until used (Jose *et al*., 2000). Supernatant fluid @ 0.2 ml per egg was inoculated via intra allantoic route in 10 days old chicken's embryonated eggs. Eggs were incubated at 37^{0}C and checked twice in a day according to method described by Gelb and Jackwood (1998). Embryo that died within 24 hrs after inoculation was discarded. Mortality of embryos occurs between 2 to 7 days post inoculation were considered to virus specific. Eggs were

removed from the incubator 3-7 days after post inoculation, and placed at 4^0C for 24 hrs and allantoic fluid of the embryos was collected aseptically for next passage. Harvested fluid was five times blindly passaged in chicken embroynated eggs prior to being considered negative for IBV isolation. Virus concentration (allantoic fluid volume-10 ml) was filled in dialysis begs and kept in Petridis and covered with PEG-6000. When about 1 ml fluid was remained, bag was removed, washed and concentrated virus was kept at -20 ^{0}C. Agrose1% was prepared in 0.9% normal saline and 5-10 ml of agrose was poured on a slide. Well of 3 mm diameter were punched at distance of 2-4 mm after solidification of agar. Wells were sealed with 1% agrose. The wells were filled with antigen and known specific antibody against IBV. Slide was incubated at 37^0C for 24-72 hrs and then at 4^0C, if needed. IBV gives constant positive HA test after treatment of virus with 2% trypsin for 30 minute and then the plate was kept at 37^0C for 30 minute. Prepared two fold serial dilution of virus with normal saline in perplex plate, then mixed it gently and 1% RBCs were add in each well. One well of micro titer was kept as control. Perplex plate was kept at 4^0C for 1 hr and then read the pattern of settlings of cells at an interval of 30, 45 and 60 minute (Mahmood *et al*., 2004). Four HA unit virus was taken and treated with 2 trypsin, then 50µl of normal saline was added in perplex plate and 50 µl of diluted serum (1:5) was added and prepared two fold dilutions of diluted serum. Then 50 µl 4 HA unit virus was added in each well , kept it in incubator for 1 hr then 1% RBC (50 µl) was added in each well and then kept it at 4 ^{0}C similar to HA test and serum end point was recorded (Alexander *et al.,* 1983)

Results and Discussion

IBV was isolated in chicken embryos inoculated with tissues samples from all infected cases. The virus was isolated following inoculation of tissue samples (trachea, lung, cloaca swab and kidney) by allantoic cavity route. After that eggs were incubated for 7 days for virus isolation and 72 hrs for haemagglutination (HA) test. Out of 10 samples, 7 showed embryonic changes. In first and second passage, none of the sample showed any lesion in embryos. After third passage, stunting, mild curling, cutaneous hemorrhage and thickened chorioallantoic membrane but absence of renal damage and very less embryo mortality occurred. The changes in embryos mortality are in accordance to the findings of Susan *et al*. (2010) except embryonic mortality and renal damage. They revealed the ability of the isolates to cause mortalities of SPF embryos by third passage after 72 hrs post-inoculation and stunted growth with severe renal damage and deposition of urates within the ureters and urinary bladder. Hu *et al*. (1996) also recorded comparable result of stunted growth and death in IBV inoculated embryos after two or five serial passage. Less embryo mortality in present study indicated that all field IBV strains were mildly pathogenic. The harvested embryo did not show the lesion of urolithiasis, which could be due to the fact that all IBV are not nephropathogenic or all nephropathogenic difference IB isolates need not to induce renal damage in embryo (Wang *et al*., 1996). HA, HI and AGPT were conducted to identify the virus. Of total 10 samples, 6 gave positive AGPT result with standard IBV antiserum after incubation at 37^0C for 72 hrs followed by incubation at 4^0C 18 hrs. The observations were in accordance to Chandramohan *et al*. (1995). For HA test, none of sample directly agglutinated the chicken RBCs but treatment of virus with 2% trypsin induced the HA activity of virus. The result of HA test was clear and consistent and seen within 35 min for HI test, 4 HA unit virus was used. All samples were found positive for IBV through HA and HI tests. Positive HI test with the known specific antiserum of IBV confirmed that these samples were having IBV.Chen *et al*. (1997) also found induced HA activity after treatment with 1% trypsin. These results were closely comparable with the findings of Mahmood *et al*., (2004) who revealed HA activity of IBV within 5-10 min after treatment with 2% trypsin and concludes that trypsin induce HA (92% sensitivity) is superior to AGPT (76% sensitivity). They also detected the IBV through trypsin induced haemagglutination test (THA) showed 28 - 100% sensitivity test. Present study also indicated that HA and HI tests are more superior, less expensive, sensitive and rapid as compared to AGPT. Similar findings were also reported by De Wit *et al*. (1997) and De Wit (2000). IBV contains α 2, 3 linked nuraminic acid which hinder the HA activity (Schultze *et al*., 1992). HA activity can be induced by treating the virus suspension with phospholipase-c and neuraminidase enzymes, but use of trypsin to induce HA activity is efficient, economic and sensitive.

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Serum biochemical changes in cows suffering from ketosis

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Abstract

The aim of this study the biochemical changes in naturally occurring cases ketosis in cows. Forty ketotic cows and ten healthy cows were included for the study. The ketone bodies were estimated in urine following Rothera's test. Hypoglycemia significantly decreased level of serum insulin and serum cortisol level were recorded in cows with ketosis .

Keywords: Cortisol, Cows, Insuline, Ketones, Ketosis

Introduction

Incidence of ketosis in dairy cattle has been reported from India and abroad (Geishauser *et al*., 1998). The frequency of clinical cases has increased sharply in the recent past because of steep increase in milk production of individual cows.The pathogenesis of the condition has been a subject of controversy (Baird, 1982) and has not been fully elucidated. Ketosis was described as being a disorder of carbohydrate metabolism (Kreb, 1966), deficiency of cobalt and vitamin B_{12} (Hungerford, 1990), impaired metabolism of volatile fatty acids (Sarode *et al*., 1981), high protein diet, over feeding in late pregnancy (Baird *et al*., 1974), excessive intake of butyrate in silage (Mills *et al*., 1986), hepatic insufficiency (Radostitis *et al.,* 2000), dysfunction of adrenal and thyroid gland (Huszenicza *et al*., 2006). The disease itself is being secondary to other diseases (Han and Kim, 2005).There is no systematic study of ketosis in this region of rajasthan , therefore we desired to study the effect of ketosis on some serum biochemical parameters in cows from Bikaner region of Rajasthan

Materials and Methods

Urine samples of 350 post-parturient cows belonging to the college dairy farm, outdoor patients brought for treatment at medicine clinic of College of Veterinary and Animal Science, Bikaner and individual animals shown by owners at their holdings in and around Bikaner were examined for ketosis during 2010-2011.

Forty cows showing clinical signs of ketosis and the urine of which was positive for Modified Rothera's test and urine Diastix were included for biochemical studies (Before and after treatment). For comparison of biochemical status of clinically positive ketotic cows, a total of ten apparently healthy lactating cows were included in this study.

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Results and Discussion

There was highly significant decrease ($P<0.01$) in blood glucose level (Table 1, 2 and 3) in ketotic cows in group A, B and C (35.46±1.22, 36.85±1.05 and 42.48±4.48 mg/dl, respectively) as compared to healthy cows (55.47±1.25 mg/dl). Similar findings were recorded by several workers including Radostitis *et al.* (2007) and Sarkar *et al.* (2011).

The Mean ± SE value of serum glucose after treatment in group A, B and C, are presented in Table 1, 2 and 3. It was 51.71±0.58, 52.99±0.64 and 50.08±1.10 mg/dl, respectively. There was highly significant increase ($P<0.01$) in serum glucose as compared to its pre-treatment level 35.46±1.22, 36.85±1.05 and 42.48±4.48 mg/dl, respectively in group A, B and C.The Mean ± SE value of serum glucose after treatment in group A was highly significantly lower ($P<0.01$) as compared to healthy control. While this value in group B and C had no significant difference as compared to healthy control, but the value attained in group B were more nearer to the healthy control with respect to other groups indicating better recovery than others.According to Bergman (1996) dietary carbohydrates are fermented in the rumen to form volatile fatty acids (VFAs). Acetic acid, propionic acids and butyric acid are the most important VFAs, but propionic acid is the only VFA that can be converted in glucose.

There was highly significant increase ($P<0.01$) in serum ketone levels (Table 1, 2 and 3) in ketotic cows

of group A, B and C (10.42±0.41, 10.87±0.61 and 10.65±0.47 mg/dl, respectively) as compared to that in healthy cows (1.28±0.08 mg/dl). The present study is in agreement with Biswal *et al.* (2009), and Youssef *et al.* (2010).The Mean ± SE value of serum ketone after treatment in group A, B and C, are presented in Table 1, 2 and 3. It was 1.51±0.06, 1.34±0.07 and 1.25±0.06 mg/dl, respectively. There was significant decrease ($P<0.01$) in serum ketone as compared to its pre-treatment level 10.42±0.41, 10.87±0.61 and 10.65±0.47 mg/dl, respectively in group A, B and C.The Mean ± SE value of serum ketone after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group C were more nearer to the healthy control with respect to other groups indicating better recovery than others.

Ketosis is caused by Negative Energy Balance (NEB) characterized by relatively high concentration of ketone bodies with a concurrent decrease in blood glucose level (Dann *et al.* 2005). A dramatic increase in energy requirement during late pregnancy and early lactation makes dairy cows highly susceptible to NEB

Table 1: Effect of Intravenous Glucose therapy on serum biochemical parameters in ketotic cows

Parameters	Healthy Cows(n=10)	Ketotic Cows	
		Before treatment(n=15)	After treatment(N=15)
Serum Glucose (mg/dl)	55.47±1.25^{c}	35.46±1.22^{a}	51.71±0.58^{b}
Serum Ketones (mg/dl)	1.28±0.08^{a}	10.42±0.41^{b}	1.51±0.06^{a}
Urine Ketones (mg/dl)	1.65±0.14^{a}	25.43±1.14^{b}	2.37±0.12^{a}
Total Protein (g/dl)	7.74±0.19^{a}	10.35±0.27^{b}	7.90±0.17^{a}
Albumin(g/dl)	3.68±0.12^{a}	4.33±0.18^{b}	3.84±0.11^{a}
Glbulin(g/dl)	4.06±0.25^{a}	6.02±0.24^{b}	4.05±0.23^{a}
Insulin(µIu/ml)	11.60±0.60^{a}	10.72±0.44^{a}	11.29±0.32^{a}
Cortisol (ng/ml)	5.39±0.27^{c}	3.74±0.15^{a}	4.68±0.14^{b}

Means with different superscripted letters in the same row differ significantly.

Table 2: Effect of Decadurabolin therapy on biochemical parameters in ketotic cows

Parameters	Healthy Cows(n=10)	Ketotic Cows	
		Before treatment(n=15)	After treatment(N=15)
Serum Glucose (mg/dl)	55.47±1.25^{b}	36.85±1.05^{a}	52.99±0.64^{b}
Serum Ketones (mg/dl)	1.28±0.08^{a}	10.87±0.61^{b}	1.34±0.07^{a}
Urine Ketones (mg/dl)	1.65±0.14^{a}	26.55±1.13^{b}	1.88±0.05^{a}
Total Protein (g/dl)	7.74±0.19^{a}	11.56±0.29^{b}	7.95±0.19^{a}
Albumin(g/dl)	3.68±0.12^{a}	4.65±0.23^{b}	3.63±0.12^{a}
Glbulin(g/dl)	4.06±0.25^{a}	6.91±0.34^{b}	4.33±0.25^{a}
Insulin(µIu/ml)	11.60±0.60^{a}	10.95±0.42^{a}	11.78±0.33^{a}
Cortisol (ng/ml)	5.39±0.27^{b}	3.98±0.14^{a}	5.07±0.16^{b}

Means with different superscripted letters in the same row differ significantly.

Table 3: Effect of Insulin therapy on biochemical parameters in ketotic cows

Parameters	Healthy Cows(n=10)	Ketotic Cows	
		Before treatment(n=15)	After treatment(N=15)
Serum Glucose (mg/dl)	55.47±1.25^{b}	42.48±4.48^{a}	50.08±1.10^{b}
Serum Ketones (mg/dl)	1.28±0.08^{a}	10.65±0.47^{b}	1.25±0.06^{a}
Urine Ketones (mg/dl)	1.65±0.14^{a}	26.69±1.68^{b}	2.04±0.08^{a}
Total Protein (g/dl)	7.74±0.19^{a}	11.34±0.33^{c}	8.39±0.14^{b}
Albumin(g/dl)	3.68±0.12^{a}	4.93±0.27^{b}	3.58±0.26^{a}
Glbulin(g/dl)	4.06±0.25^{a}	6.41±0.18^{c}	4.81±0.21^{b}
Insulin(µIu/ml)	11.60±0.60^{b}	5.02±0.46^{a}	10.58±0.43^{b}
Cortisol (ng/ml)	5.39±0.27^{b}	4.69±0.19^{a}	4.97±0.20^{ab}

Means with different superscripted letters in the same row differ significantly.

(Turk *et al.* 2008). Acetyl-CoA production from fatty acids exceeds its removal by the citric acid cycle or lipogenesis, and hence Acetyl-CoA tends to accumulate and contributes to enhanced ketone bodies synthesis. At the onset of lactation, there is a tremendous increase in energy demand by the mammary gland for milk production and causes partly by fat mobilization from adipose tissue. However, excessive fat mobilization can induce an imbalance in hepatic carbohydrate and fat metabolism, characterized by elevated concentrations of ketone bodies (Haelst *et al*., 2008).

There was highly significant increase (P<0.01) in urine ketone levels (Table 1, 2 and 3) in ketotic cows of group A, B and C (25.43±1.14, 26.55±1.13 and 26.69±1.68 mg/dl, respectively) as compared to that in healthy cows (1.65±0.14 mg/dl). The present study is in agreement with Elitok *et al.* (2010).The Mean ± SE value of urine ketone after treatment in group A, B and C, are presented in Table (1, 2 and 3). It was 2.37±0.12, 1.88±0.05 and 2.04±0.08 mg/dl, respectively. There was significant decrease (P<0.01) in urine ketone as compared to its pre-treatment level 25.43±1.14, 26.55±1.13 and 26.69±1.68 mg/dl, respectively in group A, B and C.The Mean ± SE value of urine ketone after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group B were more nearer to the healthy control with respect to other groups indicating better recovery than others.

There was highly significant increase (P<0.01) in serum total protein and Globulin (Table 1, 2 and 3) of ketotic cows of group A, B and C (10.35±0.27, 11.56±0.29 and 11.34±0.33 g/dl) and (6.02±0.24, 6.91±0.34 and 6.41±0.18 g/dl) respectively, as compared to that in healthy cows (7.74±0.19 g/dl) and (4.06±0.25g/dl) respectively, and there was significant increase (P<0.05) in Albumin level (Table 5), in ketotic cows in group A (4.33±0.18 g/dl) but highly significant increase (P<0.01) in group B and C (Table 6 and 7), (4.65±0.23 and 4.93±0.27 g/dl, respectively) as compared to that in healthy cows 3.68±0.12 g/dl. Similar observations were also recorded by Elitok *et al.* (2010). Whereas Simenove *et al.,* (1984) observed lower concentration of total serum protein in ketotic cows.

The Mean ± SE value of serum total protein after treatment in group A, B and C, are presented in Table (1, 2 and 3). It was 7.90±0.17, 7.95±0.19 and 8.39±0.14 g/dl, respectively. There was highly significant decrease (P<0.01) in serum total protein as compared to its pre-treatment level 10.35±0.27, 11.56±0.29 and 11.34±0.33 g/dl, respectively in group A, B and C. The Mean ± SE value of serum total protein after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group A were more nearer to the healthy control with respect to other groups indicating better recovery than others.

The Mean ± SE value of serum albumin after treatment in group A, B and C, are presented in Table (1, 2 and 3). It was 3.84±0.11, 3.63±0.12 and 3.58±0.26 g/dl, respectively. There was significant decrease (P<0.01) in group A but highly significant decrease in serum albumin in group B and C as compared to its pre-treatment level 4.33±0.18, 4.65±0.23 and 4.93±0.27 g/dl, respectively in group A, B and C. The Mean ± SE value of serum albumin after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group C were more nearer to the healthy control with respect to other groups indicating better recovery than others.

The Mean ± SE value of serum globulin after treatment in group A, B and C, are presented in Table (1, 2 and 3). It was 4.05±0.23, 4.33±0.25 and 4.81±0.21 g/dl, respectively. There was highly significant decrease (P<0.01) in serum globulin as compared to its pre-treatment level 6.02±0.24, 6.91±0.34 and 6.41±0.18 g/dl, respectively in group A, B and C. The Mean ± SE value of serum globulin after treatment in group A and B had no significant difference but there was significant increase in group C as compared to healthy control, but the value attained in group A were more nearer to the healthy control with respect to other groups indicating better recovery than others.

Hyperproteinemia along with increased albumin and globulin accompanied by hypoglycemia might be due to energy deficient and protein rich ration given to the high yielders. According to Hibbit (1979) high protein intake exacerbate an energy deficit because of energy losses resulting from its metabolism and excretion. This energy deficit was responsible for development of ketosis.

There was no significant difference in levels of serum insulin (Table 1 and 2) in group A and B of ketotic

cows (10.72±0.44 and 10.95±0.42 µIu/ml), but there was highly significant decrease (P<0.01) in group C (Table 3), (5.02±0.46 µIu/ml), as compared to healthy level (11.60±0.60 µIu/ml). This present study is in agreement with Teli and Ali (2007). Contrary to this high level of insulin in ketotic cows was observed by Kronfeld (1971) and no changes in insulin concentration were seen by and Deboer *et al.,* (1986). The Mean ± SE value of serum insulin after treatment in group A, B and C, are presented in Table (1, 2 and 3). It was 11.29±0.32, 11.78±0.33 and 10.58±0.43 µIu/ml, respectively. There was no significant increase in serum insulin in group A and B, but there was highly significant increase (P<0.01) in serum insulin in group C, as compared to its pre-treatment level 10.72±0.44, 10.95±0.42 and 5.02±0.46 µIu/ml, respectively in group A, B and C.

The Mean ± SE value of serum insulin after treatment in group A, B and C had no significant difference as compared to healthy control, but the value attained in group B were more nearer to the healthy control with respect to other groups indicating better recovery than others.

Low level of insulin may be due to decreased pancreatic β-cell secretary activity (Sakai *et al.* 1996). Insulin deficiency may be associated with hypocalcaemia, and diminished activity of β-cells of endocrine pancreas to synthesize and release insulin (Hove, 1978). According to Aiello (1998) in primary ketosis there is acute involution of the pancreas and this might result in decreased production of insulin.

There was highly significant decrease (P<0.01) in serum cortisol level (Table 1 and 2) of ketotic cows of both A and B group (3.74±0.15 and 3.98±0.14 ng/ml), but non significant decrease was seen in group C (Table 3), (4.69±0.19ng/ml), as compared to healthy cows (5.39±0.27 ng/ml). The findings of the present study are in agreement with Sahinduran *et al*. (2010). Contrary to this high level of cortisol in ketotic cows was observed by Sharma (2006).

The Mean ± SE value of serum cortisol after treatment in group A, B and C, are presented in Table (1, 2 and 3). It was 4.68±0.14, 5.07±0.16 and 4.97±0.20 ng/ml, respectively. There was highly significant increase (P<0.01) in serum cortisol in group A and B, but non significant increase in serum cortisol in group C, as compared to its pre-treatment level 3.74±0.15, 3.98±0.14 and 4.69±0.19 ng/ml, respectively in group A, B and C.The Mean ± SE value of serum cortisol after treatment in group B and C had no significant difference but highly significant difference was observed for group A, as compared to healthy cows. The value attained in group B was more nearer to the healthy control with respect to other groups indicating better recovery than others.

Thyroxin has an important role on the carbohydrate metabolism due to increase glucose turn over and absorption (Kaneko, 1997). According to Rijnberk and Mol (1990) glucocorticoids like cortisol, supply glucose to the organism by the transformation of proteins. Thus reduction in the concentration of thyroxin and cortisol. Adrenocorticotropin is the pituitary hormone that regulates adrenal secretion of cortisol and due to the low concentration of ACTH in high milk yielding cows, there is significant low concentration of cortisol.

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Changes in haematology of dogs with thoracic affections

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Abstract

A random study was carried out to diagnose thoracic affections in dogs. Dogs were examined clinically for thoracic respiratory diseases and blood samples were used to ascertain haemato-biochemical changes in respiratory affections. Results obtained were non-specific however haematological changes provided useful information for management of the disease.

Keywords: Respiratory disease, Thoracic affection, Tirupati

Introduction

Respiratory diseases are important cause of animal morbidity and an early intervention may reduce mortality among affected dogs. Diagnosis and management of respiratory diseases may require continuous monitoring and laboratory aids. Affections of respiratory system exhibits symptoms such as increased respiratory effort, goose honking cough in case of tracheal collapse (Tappin, 2016), moist rales in bronchopneumonia (Amrute *et al.,* 2009), systolic murmur in pulmonary alveolar interstitial disease (Louvet *et al.,* 2008) Patients with compromised respiratory function require to evaluate with a combination of signalment, history and physical examination findings in order to reach a tentative diagnosis and therapeutic plan (Silverstein and Drobatz, 2010). Haemato-biochemical observations aid in diagnosis, hence this study was undertaken.

Materials and Methods

Study was conducted on 106 dogs with a history and clinical signs of any respiratory distress presented to college over a period of one year. History regarding depressed appetite, nasal discharge, epsitaxis, haemoptysis, duration of illness, previous treatment etc. were recorded. Suspected animals were subjected to detailed clinical examination. Physical examination included auscultation of heart and abnormal lung sound, thoracic percussion and palpation of chest area. Venous blood was collected for haematological and biochemical evaluation. Haematological parameters including haemoglobin (Hb) (gm%), total erythrocyte counts (TEC) (millions/mm^3), packed cell volume (PCV) (%), total leukocyte counts (TLC) (thousand/mm^3), differential leukocyte counts (DLC) (%) and biochemical parameters including total protein (gm/ dL) and serum albumin (gm/ dL) were estimated as per procedures described by Coles (1986).

Data obtained were subjected to analysis of variance (ANOVA) by using General Linear Model procedures of statistical software package SPSS 17.0 and means were separated by applying Tukey's test considering statistically difference at $P<0.05$ (Snecdecor and Cochran, 1989).

Results and Discussion

Cases diagnosed as pneumonia exhibited non specific weight loss along with signs of chronic or acute cough, tachypnoea, difficult respiration accompanying nasal discharge. Animals with tracheobronchitis exhibited harsh productive cough, and was observed in middle aged dogs. Auscultation revealed inspiratory crackles at the bronchial area. Respiratory distress, tachypnoea, cough, abnormal heart sounds, cyanosis and pain while sitting on sternal recumbency were recorded with the cases of pulmonary oedema. Cases diagnosed as pleural effusion exhibited dyspnoea, forceful inspiration, delayed expiration, reluctant to lie down on the floor, tachypnoea, open mouth breathing, cyanosis, muffled lung sounds and extended head and neck. Cases presented with the history of automobile accident or severe trauma and external injury exhibited acute dysponea, shallow rapid respiration, cyanosis and auscultation revealed muffled sounds. Such cases were diagnosed as pneumothorax. The animals with diaphragmatic herniation exhibited dysponea, open mouth breathing exercise intolerance, anorexia, depression, vomiting, weight loss, pain on sitting on

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Table 1. Haematological and serum biochemical changes in dogs with respiratory disorders

Parameters	Normal	Affected	
		Before treatment	After treatment
Haemoglobin (gm %)	13.1±0.20[a]	12.0±0.07[b]	12.46±0.05[b]
TEC (million/mm^3)	6.56±0.10[a]	5.96±0.04[b]	6.39±0.02[a]
PCV (%)	38.6±0.50	37.6±0.20	38.16±0.23
TLC (Thousand/mm^3)	11.3±0.30	13.2±0.16	12.1±0.71
DLC (%)			
Neutrophil	69.3±0.88	72.9±0.45	70.7±0.70
Eosinophil	0.83±0.31	1.03±0.07	0.86±0.07
Lymphocyte	29.2±1.13[a]	25.3±0.42[b]	29.2±0.7[a]
Monocyte	3.36±0.14	2.54±0.04	2.90±0.28
Total Protein (gm/dL)	6.30±0.24[a]	4.97±0.10[b]	5.7±0.71[a]
Albumin (gm/dL)	3.36±0.14[a]	2.54±0.04[c]	2.90±0.28[b]

Values with different superscript differ significantly (P<0.05).

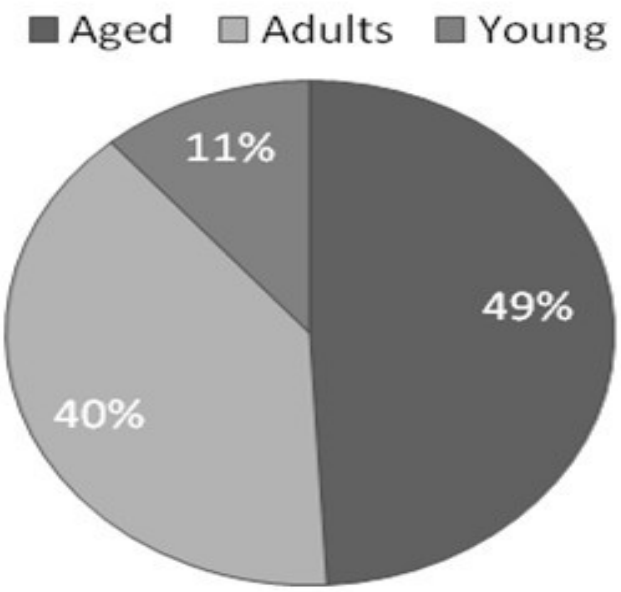

Fig 1: Age wise incidence of respiratory affections in dogs

floor.

Changes in haematological and biochemical parameters are presented in the Table 1. Hb, TEC and lymphocyte count were significantly lower (P<0.05) in respiratory affected dogs as compared to normal dogs and dogs after treatment. However, PCV, TLC and DLC (neutrophil, eosinophil and monocyte) concentration of blood in dogs were similar in healthy, affected and treated dogs. Serum total protein concentration was lower (P<0.05) in respiratory affected dogs .The incidence of respiratory affections based on age and breed in dogs are presented in the Figure 1 and 2, respectively. Highest (P<0.05) in old aged dogs followed by adults and was lowest (P<0.05) in young animals.

Amrute *et al.* (2009) and Liptak *et al.* (2008) observed changes in Hb percent, TEC and lymphopenia

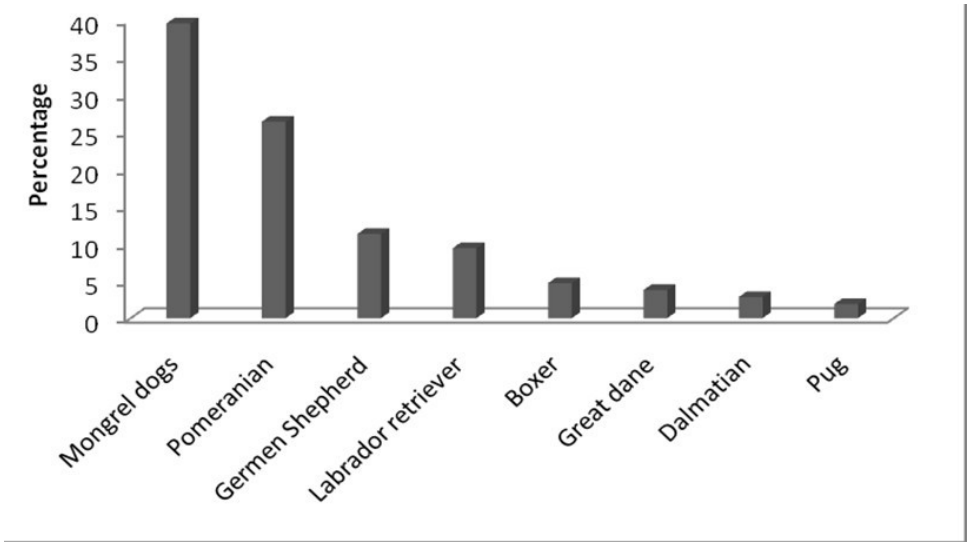

Fig 2: Breed wise incidence of respiratory affections in dogs

in the affected dogs which is in accordance to our observation. Leukocytosis and mild monocytosis were also observed which is similar to the findings of Ricco and Graham 2007. Several studies show lower serum total protein in the respiratory affected dogs (Hunter, 2001; Ricco and Graham 2007). Since fluctuations were within normal physiological range specific reason could not be explained.

Conclusion

Clinical and haemato-biochemical parameters can build a base for monitoring of the disease

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Effect of colibacillosis on haemato-biochemical profile in calves

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Abstract

Aim of this study was to evaluate the effect of colibacillosis on haematology and clinical chemistry in calves. Blood samples of 30 diarrhoeic calves suffering from colibacillosis and 6 apparently healthy calves were collected at 0, 48, 96 and 144 hr following the treatment. Haematological studies in infected animals revealed significant increase ($p<0.05$) in packed cell volume, haemoglobin, total leucocyte count and total erythrocyte count, whereas, the biochemical parameters viz., total protein, albumin, blood glucose, sodium and potassium were significantly decreased. This haemato-biochemical started returning to the normalcy after therapy.

Keywords: Calves, Colibacillosis, Haematology, Clinical Chemistry

Introduction

Colibacillosis is one of the important diseases of neonatal calves and mostly associated with infection with pathogenic serotypes of *Escherichia coli* (Carlton, 1992).Due to profuse diarrhoea and septicemia calves became highly dehydrated that leads to prostration and death. It is a major cause of economic loss in new born calves. The haematology and clinical chemistry provide useful guideline in assessing extent of pathology and also to take appropriate therapy. The present study was undertaken to investigate the pre- and post-treatment status of haemato-biochemical profile in calves suffering from colibacillosis.

Material and methods

Sampling Area: The samples were collected from the district Ganderbal and Srinagar of Kashmir Valley. The Kashmir valley is located between the Karakoram and the Pir Panjal Range in the Indian state of Jammu and Kashmir. It has a moderate climate, which is largely defined by its geographic location. Compared with other plain parts of India, Kashmir valley enjoys a more moderate climate but weather conditions are unpredictable. Study was performed according to the 'Guidelines for Animal Experimentation' approved by the Institutional Animal Care Committee.

For the present study, 30 calves which were diagnosed to be affected with colibacillosis were selected and divided into 5 groups with 6 animals in each. For haematological studies, blood was collected from these calves in clean sterilized glass vials containing EDTA, as anticoagulant. The samples were collected on "0" day and then after 48 hours, 96 hours and 144 hours post treatment. For biochemical study 10 ml of blood was collected in sterile centrifuge tubes and kept in slanting position for about an hour at room temperature for obtaining serum. The blood clot was broken and subsequently centrifuged at 2000 rpm for 30 minutes to obtain serum for biochemical study. Similarly, blood samples were also collected from 6 apparently healthy calves to determine the base values.

The haemoglobin was determined by Sahli's method, packed cell volume (PCV) by Wintrobe method, total erythrocyte count (TEC) and total leucocyte count (TLC) with haemocytometer as per methods of Schalm *et al.* (1986).

Glucose was estimated using reagents supplied by Crest Biosystems, Goa, India, total protein and albumin was determined using the kits supplied by Coral-Clinical System (Crest Biosystems) Goa, India and the serum electrolytes, Sodium and Potassium were estimated using the Flame photometric method (Oser, 1965).

Statistical analysis

The results were subjected to statistical analysis as per the ANOVA method (Snedecor and Cochran, 1994).

Results and Discussion

Haematology of infected animals revealed

Table-1 : Mean±SE of Packed Cell Volume Percentage (PCV%) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	37.72±0.55^{Aa}	37.59±0.54 ^{Aa}	37.91±0.51 ^{Aa}	38.48±0.27 ^{ABa}
Infected untreated	40.27±1.55^{Ba}	42.40±1.76^{Ba}	40.47±1.51 ^{Aa}	39.07±1.42 ^{ABa}
Co-trimoxazole	43.38±1.53^{Ca}	40.63±1.37^{ABab}	39.44±1.72^{Aab}	38.26±1.40^{ABb}
Neomycin	41.29±2.08^{Ba}	39.19±1.64^{Aa}	37.30±1.55 ^{Ab}	36.47±1.62^{Bb}
Gentamicin	41.36±0.69^{Ba}	40.09±0.62^{ABa}	37.78±0.71^{Ab}	36.41±0.88^{Bb}
Ciprofloxacin	44.22±0.86^{Ca}	42.89±0.82^{Bab}	41.25±0.86^{Ab}	40.31±1.05 ^{Ab}

Table-2 : Mean±SE of Haemoglobin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	11.57±0.24^{Aa}	11.98±0.26 ^{ABa}	11.34±0.12 ^{Ba}	11.41±0.09 ^{Ba}
Infected untreated	12.98±0.20 ^{Ba}	13.31±0.36 ^{Ba}	13.40±0.56 ^{Aa}	12.71±0.50 ^{Aa}
Co-trimoxazole	13.38±0.92 ^{Ba}	12.60±0.85 ^{ABab}	11.08±0.76 ^{Bb}	10.79±0.77 ^{Cb}
Neomycin	12.99±0.42 ^{Ba}	11.94±0.36 ^{ABab}	11.30±0.30 ^{Bbc}	10.69±0.26 ^{Cc}
Gentamicin	13.71±0.46 ^{Ba}	12.93±0.42 ^{ABab}	12.00±0.48 ^{ABbc}	11.11±0.45 ^{Bc}
Ciprofloxacin	13.00±0.33 ^{Ba}	12.23±0.29 ^{ABab}	11.44±0.33 ^{Bbc}	10.00±0.37 ^{Cc}

Table-3 : Mean±SE of Total Leukocyte Count (TLC × 10^3/µl) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	7.00±0.27 ^{Aa}	7.27±0.26 ^{Aa}	7.35±0.35 ^{Aa}	7.20±0.31 ^{Aa}
Infected untreated	7.99±0.31 ^{Ba}	7.51±0.27 ^{ABa}	6.99±0.22 ^{Aa}	6.93±0.29 ^{Aa}
Co-trimoxazole	10.67±0.34 ^{Ca}	9.56±0.36 ^{Cb}	8.87±0.42 ^{Bb}	8.68±0.36 ^{Bb}
Neomycin	8.78±0.47 ^{Ba}	8.31±0.41 ^{Bab}	7.66±0.33 ^{Aab}	7.16±0.34 ^{Ab}
Gentamicin	8.38±0.16 ^{Ba}	7.87±0.28 ^{ABab}	7.34±0.34 ^{Abc}	6.74±0.27 ^{Ac}
Ciprofloxacin	8.78±0.16 ^{Ba}	8.45±0.16 ^{Ba}	7.80±0.21 ^{Ab}	7.11±0.21 ^{Ac}

Table-4 : Mean±SE of Total Erythrocyte Count (TEC × 10^6/µl) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	7.39±0.14 ^{Aa}	7.35±0.22 ^{Aa}	7.60±0.20 ^{Aa}	7.56±0.32 ^{ABa}
Infected untreated	8.52±0.40 ^{Ba}	8.71±0.38 ^{Ba}	8.50±0.29 ^{Ba}	8.11±0.70 ^{Ba}
Co-trimoxazole	8.53±0.43^{Ba}	8.14±0.44 ^{ABab}	7.75±0.28 ^{ABab}	7.32±0.26 ^{ABb}
Neomycin	8.02±0.31 ^{ABa}	7.90±0.35 ^{ABa}	7.45±0.33 ^{Aa}	6.97±0.34 ^{Aa}
Gentamicin	8.42±0.34 ^{ABa}	7.87±0.29 ^{ABab}	7.55±0.23 ^{Ab}	7.30±0.21 ^{ABb}
Ciprofloxacin	8.74±0.44 ^{Ba}	8.32±0.42 ^{ABab}	7.83±0.35 ^{ABab}	7.34±0.24 ^{ABb}

Table-5: Mean±SE of total protein (gm/dl) in Calves with clinical colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	6.71±0.19 ^{Ba}	6.25±0.14 ^{Aa}	6.35±0.23 ^{Aa}	6.44±0.20 ^{Ba}
Infected untreated	5.94±0.18 ^{Aa}	5.63±0.18 ^{Aa}	5.59±0.19 ^{Ba}	5.55±0.18 ^{Aa}
Co-trimoxazole	5.91±0.29 ^{Aa}	6.12±0.19 ^{Aa}	6.15±0.17 ^{Aa}	6.29±0.22 ^{Bab}
Neomycin	5.88±0.40 ^{Aa}	6.01±0.28 ^{Aa}	6.35±0.23 ^{Aab}	6.49±0.22 ^{Bab}
Gentamicin	5.63±0.18 ^{Aa}	5.92±0.20 ^{Aa}	6.21±0.19 ^{ABab}	6.52±0.19 ^{Bb}
Ciprofloxacin	5.50±0.30 ^{Aa}	5.99±0.19 ^{Aa}	6.16±0.18 ^{ABab}	6.34±0.18 ^{Bb}

Table-6 : Mean±SE of Serum Albumin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	2.93±0.13 ^{Aa}	2.62±0.13 ^{Aa}	2.72±0.17 ^{Aa}	2.91±0.18 ^{Ba}
Infected untreated	2.26±0.30 ^{Ba}	2.32±0.26 ^{Aa}	2.39±0.19 ^{Aa}	2.40±0.14 ^{Aa}
Co-trimoxazole	2.27±0.20 ^{Ba}	2.53±0.14 ^{Aab}	2.66±0.07 ^{Aab}	2.76±0.09 ^{ABb}
Neomycin	2.32±0.17 ^{Ba}	2.44±0.15 ^{Aa}	2.60±0.13 ^{Aab}	2.80±0.07 ^{ABb}
Gentamicin	2.20±0.21 ^{Ba}	2.43±0.14 ^{Aab}	2.77±0.19 ^{Abc}	2.97±0.12 ^{Bc}
Ciprofloxacin	1.93±0.22 ^{Ba}	2.33±0.14 ^{Aab}	2.51±0.13 ^{Ab}	3.07±0.19 ^{Bc}

Table-7: Mean±SE of Blood Glucose (mg/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	77.58±2.01 ^{Aa}	76.30±2.44 ^{Aa}	75.89±1.50 ^{Aa}	77.20±1.83 ^{Aa}
Infected untreated	59.17±2.57 ^{Ba}	59.56±2.57 ^{Ba}	59.68±2.60 ^{Ba}	62.69±2.14 ^{Ba}
Co-trimoxazole	57.56±6.93 ^{Ba}	63.06±5.72 ^{Bab}	69.39±4.44 ^{Aab}	75.71±3.66 ^{Ab}
Neomycin	69.18±1.93 ^{Ba}	68.72±1.79 ^{ABa}	72.01±2.17 ^{Aab}	76.98±2.27 ^{Ab}
Gentamicin	61.93±4.00 ^{Ba}	66.23±2.57 ^{Bab}	73.21±2.04 ^{Abc}	78.20±2.26 ^{Ac}
Ciprofloxacin	65.10±1.87 ^{Ba}	68.10±2.02 ^{ABab}	72.68±1.84 ^{Abc}	77.14±1.86 ^{Ac}

Table-8: Mean±SE of Serum Sodium (mEq/L) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	139.98±0.94 ^{Aa}	140.91±1.23 ^{Aa}	140.91±1.45 ^{Aa}	140.47±1.24 ^{Aa}
Infected untreated	133.61±1.26 ^{Ba}	132.01±1.72 ^{Ba}	131.65±2.22 ^{Ba}	132.02±2.21 ^{Ba}
Co-trimoxazole	132.59±1.82 ^{Ba}	132.00±2.40 ^{Ba}	133.57±2.36 ^{BCa}	136.29±2.24 ^{ABa}
Neomycin	133.07±2.28 ^{Ba}	135.20±2.39 ^{Bab}	138.12±1.87 ^{ACab}	139.72±1.58 ^{Ab}
Gentamicin	129.46±2.26 ^{BCa}	131.47±2.17 ^{Ba}	134.58±2.09 ^{BCab}	137.15±1.87 ^{Ab}
Ciprofloxacin	126.00±1.52 ^{Ca}	131.20±1.13 ^{Bb}	134.55±0.95 ^{BCc}	139.70±0.74 ^{Ad}

Table-9 : Mean±SE of Serum Potassium (mEq/L) in Calves with Clinical Colibacillosis before and after treatment

Groups	0 hr	48 hr	96 hr	144 hr
Healthy	4.32±0.09 ^{Aa}	4.27±0.12 ^{Aa}	4.25±0.14 ^{ABa}	4.31±0.17 ^{ABa}
Infected untreated	4.09±0.09 ^{ABa}	3.99±0.09 ^{Aa}	3.95±0.09 ^{Aa}	3.96±0.14 ^{Ba}
Co-trimoxazole	4.10±0.20 ^{ABa}	4.18±0.14 ^{Aa}	4.42±0.08 ^{Bab}	4.45±0.07 ^{Ab}
Neomycin	3.95±0.05 ^{Ba}	4.00±0.10 ^{Aa}	4.10±0.19 ^{ABab}	4.29±0.07 ^{ABb}
Gentamicin	4.04±0.02 ^{ABa}	4.13±0.11 ^{Aa}	4.26±0.11 ^{ABab}	4.51±0.13 ^{Ab}
Ciprofloxacin	3.96±0.07 ^{Ba}	4.06±0.08 ^{Aa}	4.21±0.13 ^{ABab}	4.46±0.14 ^{Ab}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly (P<0.05)

significant increase in packed cell volume, haemoglobin, total leucocyte count and total erythrocyte count, whereas, the biochemical parameters viz., total protein, albumin, blood glucose, sodium and potassium were significantly decreased. These hemato-biochemical changes are presented in their respective table1-6.

There was significant increase in packed cell volume which might be due to haemoconcentration confirming the fluid loss from vascular compartment (Table 1). This increase in PCV% is in agreement with the earlier observations (Kumar and Mandial 2002; Kaur *et al.* 2006). Increase in haemoglobin values (Table 2) in diarrhoeic calves might be due to fluid loss from vascular compartment. Similar trend of higher values were also recorded by Kumar *et al.* (2002).

The increase in total leucocyte count might have occurred due to the normal reaction of body defense mechanism against *E. coli* infection and also due to dehydration and haemoconcentration (Table 3). The increase in TLC in the present study was in agreement with the earlier findings of Fernandes *et al.* (2009).

Increase in TEC values in diarrhoeic calves (Table 4) might be due to reduction in the plasma value and haemoconcentration due to loss of extra cellular fluid in diarrhoeic faeces on account *E. coli* induced intestinal epithelial damage. The increase in TEC in the present study is in agreement with the earlier findings (Fernandes *et al.* 2009).

In infected groups of calves suffering from colibacillosis the value of total protein (g/dl) were significantly (P<0.05) lower than healthy control group (Table 5). This lowered protein values may be due to some loss of protein as a result of diarrhoea. Calves affected with diarrhoea may also have maldigestion and malabsorption and may have protein loss from erosive or ulcerative colonic lesions causing protein losing enteropathy. Anaemia due to fecal blood loss might further contribute to protein loss. These findings are in agreement with the earlier findings (Aly *et al.,* 1996). However, increase in total plasma protein in diarrheic calves could be due to dehydration and haemoconcentration and also due to release of intracellular proteins from damaged tissues (Radostitis *et al.,* 1994).

In infected groups the value of serum albumin

(g/dl) was lower as compared to the healthy control group (Table 6). These changes could be due to their loss through inflamed gut epithelium. However, Kaur *et al.* (2006) reported significant increase in plasma albumin level in diarrhoeic calves and ascribed to dehydration during diarrhoea.

Hypoglycemia was an important biochemical finding in diarrheic calves (Table 7). The decreased glucose has been reported earlier (Seifi *et al.,* 2006). The factors responsible for development of hypoglycemia could be anorexia and decreased intestinal absorption of glucose.

The decreased sodium values may be due to increased loss sodium through gastrointestinal tract in diarrhoea. A significant decrease of serum sodium in diarrheic calves (Table 8) has also been reported (Kumar and Mandial 2002; Kaur *et al.* 2006). There was decrease in levels of Potassium (Table 9). Hussain *et al.* (2001) and Chaleva and Encheva (2003) also reported hypokalemia in diarrhoeic calves

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Proteinuria and urine albumin creatinine ratio as indicators of renal failure in dogs

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Abstract

The present study was conducted on 39 dogs suffering from renal failure. Proteinuria was observed in 33 out 39 dogs suffering from renal failure. It was high (3+) in 18, moderate (2+) in 10 and mild (1+) in 5 dogs. The urine albumin creatinine ratio (UAC), the normal value of which is <30 mg albumin per gram of creatinine, was found to be >300 in 15/20 and 30-300 mg in 5/20 dogs. The proteinuria and high urine albumin creatinine ratio in the present studies seem to be the good indicators for screening of the dogs for renal failure.

Keywords: Renal failure, Proteinuria, Urine albumin creatinine ratio

Renal failure is among the most common ailments of dogs and contributes substantially to canine mortality. The serial urinalysis represents the most effective and sensitive clinical diagnostics program for the early detection of renal failure than would serum creatinine or blood urea concentrations (Brown *et al.,* 1985).

Materials and Methods

The present study was conducted on 39 dogs presented to the small animal clinics, Teaching Veterinary Hospital, GADVASU, Ludhiana. Urine of the dogs suffering from renal failure was collected and subjected to the routine physical, chemical, microscopic examination. The urine albumin creatinine ratio was estimated using specific dipsticks with CLINITEK STATUS analyzer, Bayer Healthcare LLC.

Results and Discussion

The dogs suffering from renal failure manifested polyuria and polydipsia as the prominent clinical signs. On the urinalysis of those dogs the urine specific gravity was found to be less than 1.015 in about 50 % of the cases (Table 1). In a normal healthy dog, the urine specific gravity generally varies from 1.015-1.030. Neel and Grindem (2000) observed that in primary renal disease the urine specific gravity approaches the isosthenuric range of 1.008 to 1.012 whereas Alcázar Arroyo (2008) reported that in advanced chronic kidney disease, the range of urine osmolality progressively approaches plasma osmolality and becomes isostenuric. Urine of some dogs suffering from renal failure revealed erythrocytes, pus cells and granular casts (Table 2). In renal failure cases, the presence of erythrocytes and pus cells in the urine indicate renal inflammation, hemorrhage or some infection in any part of urinary tract (Fleming *et al.,* 1989; Sebastian *et al.,* 2007; Acierno and Senior, 2010). The granular casts may be due to presence of some chronic disease process in the kidneys as most of the cases with granular casts were of old age.

Proteinuria is a common disorder in dogs suffering from chronic renal failure even before the onset of azotemia. However, it also indicates the presence of more severe kidney disease after the onset of azotemia (Grauer, 2007). Proteinuria was observed in 33 out 39 dogs suffering from renal failure (Table 2). It was high (3+) in 18, moderate (2+) in 10 and mild (1+) in 5 dogs. Similar observations were made by earlier workers (Grauer, 2007; Acierno and Senior, 2010). Proteinuria observed may be due to the leakage of plasma proteins

Table 1: Gross examination of urine in dogs suffering from renal failure (N=39).

Gross examination of urine (N=39)		Percentage
pH	Acidic	89.74
	Neutral	2.56
	Alkaline	7.69
Colour	Straw coloured	46.15
	Pale yellow	38.46
	Dark yellow	12.82
	Reddish yellow	2.56
Odour	Ammonical	58.97
	Nil	38.46
	Sweet	2.56
Turbidity	Present	12.82
	Absent	87.18
Specific gravity	<1.015	46.15
	1.015-1.030	48.72
	>1.030	5.13

Table 2: Microscopic and chemical analysis of urine (N=39).

Findings	Percentage
Renal epithelial cells	46.15
RBC (5-25/HPF)	15.38
WBC (5-25/HPF)	12.82
Granular casts	25.64
Waxy casts	7.69
Hyaline casts	5.13
Proteins	84.62
Glucose	5.13
Ketones	2.56
Bile pigments and Bile salts	7.69

because of some pathological change in glomerulus. According to Acierno and Senior (2010) the presence of large amounts of protein in absence of bacteria or white blood cells (WBCs) may be due to glomerular disease. According to Littman (2011) the genetic and acquired defects of glomerular permeability may lead to proteinuria and protein-losing nephropathy. Leakage of plasma proteins into the glomerular filtrate can further damage tubular cells and the function of the entire nephron**.**

Urine albumin creatinine (UAC) ratio was randomly estimated in 20 dogs with azotemia. The normal UAC ratio is <30 mg albumin per gram of creatinine. In 15 dogs, UAC ratio was very high (i.e. >300) and in the remaining five dogs it was 30-300 mg albumin per gram of creatinine. Finco *et al.* (1999) reported that progressive increase in UAC ratio may be a marker of an accelerated rate of renal injury. This ratio can be used clinically as one of the basic in-hospital laboratory tests for estimation of proteinuria in cats and dogs as observed by Kuwahar *et al.* (2008).

Hence, the proteinuria and high urine albumin creatinine ratio in the present studies seem to be the good indicators for screening of the dogs for renal failure.

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Incidence of gastrointestinal helminthiaisis in bovines from Jammu region

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Abstract

The present study was aimed to determine the incidence of gastrointestinal helminthiaisis in bovine of Jammu region of India.For this purpose, 750 faecal samples were collected from cattle and buffalo from different areas of Jammu and subjected to parasitological procedures. The overall incidence of helminthiasis was 49.86 % (62.86% in case of cattle and 40.58% in case of buffaloes). Helminthic infection was recorded throughout the year with highest recorded incidence in summer followed by spring. The most prevalent helminth parasites recorded were *Paramphistomum spp*. (28%), *Haemonchus spp*. (12.93%), *Trichurs spp*. (6.53%), *Dicrocillium spp*. (3.87%), *Moniezia spp*. (2.8%) and *Fasciola spp*. (3.46%).

Keywords: Bovine, Gastrointestinal, Helminthiasis, Incidence, Jammu

Introduction

Jammu district of Jammu and Kashmir state has been bestowed with agro-climatic conditions suitable for bovine husbandry and a number of tribal families are traditionally engaged in bovine rearing.Helminthic infection is a major constraint for livestock production and causes great economic losses due to retarded growth, low productivity and increased susceptibility of animals to other diseases. In spite of significant production losses, which may run into millions of rupees (Abunna *et al*., 2010), the problem is persisting because of chronic and insidious nature. Helminthiasis adversely affects ruminants e.g. hematological and biochemical disturbances (Singh *et al*., 2014). In Jammu and Kashmir the incidence has been reported by Yadav *et al*.,(2004) and Kuchay *et al*.,(2011). The present investigation deals with the prevalence of gastrointestinal helminthiaisis of large ruminants of Jammu in order to add more information to already existing data.

Materials and Methods

A total of 750 bovine faecal samples, 272 from cattle and 478 from buffaloes, collected directly from the rectum in plastic containers from different villages of Jammu district from October 2011-September 2012 were examined through direct smear, Sedmentation and floatation methods (Solusby 1982). Identification of eggs was made according to the description given by Solusby (1982).

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Results and Discussion

Among 750 samples collected, 147 (40.27%) were found positive for single mixed helminthic infection. Among various infections, maximum incidence was of trematodes. The most prevalent helminth parasites isolated were *Paramphistomum spp*. (28%), *Haemonchus spp*. (12.93%), *Trichurs spp*. (6.53%), *Dicrocillium spp*. (3.87%), *Moniezia spp*. (2.8%) and *Fasciola spp*. (3.46%) as shown in Table- 1. The overall incidencee was 62.86% in case of cattle and 40.58% in case of buffaloes as shown in Table- 2. The infection was recorded maximum in summer and spring and lowest in autumn and winter as shown in Table 3. The present study showed that the highest prevalence of helminthes was recorded in cattle followed by buffaloes because of higher proportion of time spend on grazing by cattle as compared to buffaloes which are grazed proportionally less and kept mainly in stalls for feeding in the present study area. The highest infection of helminth in case of cattle is in agreement with Mir *et al*. 2013 and Raza *et al*., 2007 who reported 67.15% and 38.72% and 51% and 47% infection in cattle and buffaloes, respectively. The helminthes isolated in the present study are in agreement with the previous findings of Pandit *et al*., 2004; Yadav *et al*., 2004 and Kuchay *et al*., 2011. Wallowing habit, easy dispersion of faeces in water and bulk ingestion of grasses near the water sources increases the risk of amphistomes due to availability of intermediate host (Radostitis *et al*., 1994). Although FAO (1994) recommended strategic dosing against fluke diseases in ruminants in India, non-adaptation of strategic deworming schedule in the region is responsible for high parasitic infection. The higher helminthic infection as observed in summer and spring

Table 1: Incidence of gastro-intestinal helmithiasis

S. No.	Species	Total sample size (N)	Percentage
1	Paramphistome	750	28
2	Dicrocoelium	750	3.87
3	Fasciola	750	3.46
4	Trichuris	750	6.53
5	Moneizia	750	2.8
6	Haemonchus	750	12.93

Table 2: Incidence of gastro-intestinal helminthiasis in cattle and bufalloes.

S. No.	Species	Total positive cattle	Cattle prevalence % (N=272)	Total positive Buffalo	Buffalo prevalence % (N=478)
1	*Paramphistome*	69	25.36	108	22.6
2	*Dicrocoelium*	9	3.30	4	0.83
3	*Fasciola*	9	3.30	17	3.56
4	*Trichuris*	24	8.82	19	3.97
5	*Moneizia*	11	4.04	7	1.46
6	*Haemonchus*	49	18.01	39	8.15
	Total	171	62.86	194	40.58

Table 3: Seasonal incidence of Gastro-intestinal helminthiasis

S. No.	Season	No. of samples	Positive samples	Prevalence percentage
1	Summer	216	151	69.90
2	Autumn	184	39	21.19
3	Winter	180	89	49.44
4	Spring	170	95	55.88
	Total	750	374	49.86

months are in agreement with Sanyal (1998) and Agrawal *et al*. (2002). It can be concluded that there is urgent need for chemotherapeutic and prophylactic strategies for the helminthes control in this region of Jammu & Kashmir State.

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Chronic mastitis in cattle reared in and around Bhubaneswar

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Abstract

The study was undertaken from January 2014 to May 2015. Out of 260 cows with mastitis, 110 numbers of chronic mastitis cases were detected on the basis of history, physical and clinical parameters. Most of the chronic mastitis cases were detected in rainy season in 3-5 years age group. High yielding cross breed jerseys (CBJ) were mostly affected. Hind quarter and single quarter were frequently affected than fore and multiple quarter.

Keywords: Bhubaneswar, Cattle, Chronic mastitis, Incidence

Introduction

Mastitis is the most frequent and costly disease of high yielding crosses breed and exotic cattle. According to NDRI report the overall prevalence of mastitis in India is 45% (NDRI news Vol- 17 No. 1). It is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%){Bhikane and Kawitkar, 2000}. An annual economic loss of over Rs. 6000 crore due to mastitis has been recorded, of this, Rs. 1700 crore are lost due to clinical mastitis and Rs. 4300 crore due to sub clinical mastitis (Financial daily, 2002). Bovine mastitis occurs in subclinical forms and clinical forms. It seems to be of prime importance for early diagnosis of both sub clinical and clinical mastitis as otherwise these may lead to chronic mastitis. Chronic mastitis is the most severe form. It is having a continual inflammation of the mastitis quarter of the udder without any systemic reactions. There is sometimes hardness of udder with fibrosis and/or yellow coloured/watery milk. (Sudan and Sharma, 2010). The present study was envisaged to find out the incidence of chronic mastitis in and around Bhubaneswar which may be helpful in formulation of preventive measures for future course of action.

Materials and Methods

In the present study, 260 cows with mastitis of different districts like Cuttack, Jagatsinghpur, Khurda, Nayagarh and Puri surrounding Bhubaneswar were investigated at the Animal Disease Research Institute (ADRI), Phulnakhara, Cuttack, Odisha on the basis of clinical information related to mastitis and CMT of milk from January 2014 to May 2015, out of which 110 numbers of chronic mastitis cases were detected on the basis of history of mastitis condition for more than 2 months and with history of treatment for 2-3 times, clinical information like decreased milk yield and most of times with scanty milk yield, physical changes in udder like firmness with obstructed teat in some cases, physical changes in milk like watery and flakiness, gel formation in CMT, somatic cell count above 2 lakhs/ml and cytological changes like comparatively more mononuclear cells and clumping of cells. Various information in relation to incidence of chronic mastitis collected like month, age, milk yield, parity, physical changes in udder & milk and quarter affected were collected by questionnaire from the owners of the animals, field veterinarians and data from Animal Disease Research Institute (ADRI), Phulnakhara, Cuttack, Odisha. These data were entered in excel sheet and analysed.

Results and Discussion

In the present study, out of 260 numbers of mastitis cows, 110 numbers (42.31%) of chronic mastitis cases were detected on the basis of screening procedures. District wise incidence of chronic mastitis showed 33, 34, 29, 12 and 2 numbers of cases in Cuttack, Jagatsinghpur, Khurda, Puri and Nayagarh districts respectively with higher incidence in Jagatsinghpur and Cuttack districts.

There was an exclusive study on chronic mastitis by various workers in relation to its incidence. Saikia *et al.* (1989) reported 35 lactating cows affected with chronic mastitis in Indo-Australian Cattle Breeding Project, Barpeta, Assam. Kampa *et al*. (2010) reported

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40% of dairy herds in Muang. Khon Kaen had mastitis at each record and 23 % (44 herds) had the long-term problem with BMSCC >500,000 cells/ml at least 3 consecutive records. Kumar *et al.* (2010) recorded 7% of the mastitis affected quarters ended up in complete fibrosis.

Season wise, the incidence of chronic mastitis revealed 53 (48.18%) in rainy season which was highest and followed by 33 cases (30%) in summer and lowest 24cases in winter (21.82%). Shukla *et al.* (2005) also reported highest number of cases during the rainy seasons. Kumar *et al.* (2010) also reported high incidence clinical mastitis during rainy season, followed by winter and summer. It was in contrast with Zahoor-ul-Haq and Malik (2009) and Ranjan *et al.* (2011) as they reported higher incidence during winter followed by summer. The higher incidence in rainy season may be due to humid climate and unhygienic and unclean environment.

Out of 110 chronic mastitis cases, 105 (95.45 %) cases were in cross breed jersey and 5 (4.55%) cases were in deshi breeds. Cross bred Jersey cattle population was higher in this region of study which might have influenced the result besides the breed susceptibility. Iqbal and Siddique (1999) also reported higher incidence of mastitis in crossbred cows (23.19%) than in non-descript indigenous breeds (7.69%). Shukla *et al.* (2005) reported the prevalence of clinical and subclinical mastitis in crossbred cows was 8.97 and 67.94% respectively. Incidence of clinical mastitis for crossbred cows, indigenous cows and buffaloes were 9.28%, 3.59% and 4.10% respectively as reported by Kumar *et al.* (2010).

Age wise, the highest percentage of population affected by chronic mastitis out of total mastitis cases were in 3-5years age group 58 (52.73%), followed 6-8 years 44 (40%) and e"9years of age group 8 (7.27%). Susceptibility of this age group may be due to productive and reproductive stress. Shukla *et al.* (2005) also reported higher incidence of mastitis in younger age group (4-6 years old) crossbred animals. Zahoor-ul-Haq and Malik (2009) reported higher prevalence of mastitis in 6-8 year age group cows. Sharma *et al.* (2010) observed high prevalence of mastitis in 3 to 6 years old cows (55.10%) than >6 years age and <3 years old animals

Parity wise, the highest population affected with chronic mastitis were 1st-3rd parity 7 (66.36%), followed by 4th- 6th parity 30 (27.27%) and lowest in 7th-12th parity 7 (6.36%). Mustafa *et al.* (2007) reported higher mastitis incidence at fifth pregnancy i.e. 32% in cattle. Sharma *et al.* (2010) observed higher mastitis prevalence in 2nd and 8th parities. Milk yield wise, the highest population affected by chronic mastitis had milk yield 10 litres and more 76 (53.90%) than milk yield below 10 litres 34 (28.57%). The higher milk yielding cows were affected due to more physiological stress and strain of high milk production. Shukla *et al.* (2005) observed mastitis in 69.56% of crossbred cows, each with an average milk yield of 6 litres/day.

Quarter wise, the prevalence of mastitis on quarter basis was 20 (18.18 %) in left fore, right fore 27 (24.55%), left hind 27 (24.55%) and right hind 36 (32.73%) cases. The hind quarters showed highest incidence with 63 (57.27%) than fore quarters with 47 (42.73%) cases. This may be due to more unhygienic condition of hind quarter than fore quarter. Iqbal and Siddique (1999) also observed higher incidence of mastitis in hind quarters than in forequarters in cows. Rajendran *et al.* (2006) reported mastitis prevalence was highest in the left hind quarter (32, 40%), followed by the right hind (21, 26.3%), left fore (15, 18.7%) and right fore quarters (12, 15%) in cows. Mustafa *et al.* (2007) reported higher incidence of mastitis in left hind quarter in cattle. Sharif A and Ahmad T (2007) also reported maximum prevalence in right rear (30.45%) followed by left front, right front and left rear udder quarters with values of 24.50, 23.84 and 21.19%, respectively. Ulemale *et al.* (2007) also observed significant difference ($P<0.05$) of the incidence of clinical mastitis between fore- and hind udder quarters and between right (43%) and left (57%) side quarters. It was also observed that single quarter 72 (65.22%) involvement was more than double quarter 26 (23.91%) and multiple quarter 12 (10.87%) involvement. Ulemale *et al.* (2007) also observed more single teat infected mastitis cases (58%).

Conclusion

It is concluded from the above study that most of the chronic mastitis cases were detected in rainy season. High yielding CBJ of 3-5 years age group were mostly affected. Hind quarter and single quarter were

mostly affected than fore and multiple quarter.

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Alteration in haematological and serum biochemical parameters of cattle with chronic mastitis

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Abstract

Mastitis is one of the wide spread diseases of high yielding exotic and cross bred cattle. In the present study, the aim is to evaluate haematological and serum biochemical parameters of cattle with chronic mastitis. The average values of haemoglobin, TLC, TEC and PCV in chronic mastitis were slightly lower than normal values. The lymphocyte value was slightly higher than normal. The average values of total protein, albumin, globulin, serum urea nitrogen and alkaline phosphatase were found to be within normal range.

Keywords: Cattle, Chronic mastitis, Haematology, Serum biochemistry

Introduction

Mastitis is one of the most frequent, costly and challenging diseases of high yielding cross breed and exotic cattle. The overall prevalence of mastitis in India is 45% on an average (NDRI news Vol-17 No. 1). This condition is responsible for heavy economic loss. Mastitis occurs in subclinical and clinical forms. But sometimes mastitis becomes chronic which is the most severe form of mastitis. It is having a continual inflammation of the mastitis quarter of the udder without any systemic reaction. Sometimes there is hardness of udder with fibrosis and yellow coloured or watery milk (Sudan and Sharma, 2010). When the animals are affected for more than 2 months in chronic mastitis, the milk Somatic Cell Count (SCC) becomes greater than 2 lakhs/ml (Almelo and Tikofsky, 2008). In the present study, 260 numbers of mastitis cows of different districts of Odisha like Cuttack, Jagatsinghpur, Khurda, Nayagarh and Puri were investigated on the basis of clinical information and California Mastitis Test (CMT) of milk from January 2014 to May 2015. Out of which 110 numbers of chronic mastitis cases were detected. From the above 110 cases, 13 numbers were randomly selected for haematobiochemical analysis of blood and serum samples.

Materials and Methods

Total 260 numbers of mastitis cows of different districts surrounding Bhubaneswar were investigated through field veterinarians and Animal Disease Research Institute (ADRI), Phulnakhara, Cuttack, Odisha on the basis of clinical information related to mastitis and CMT of milk from January 2014 to May 2015. Out of which 110 numbers of chronic mastitis cases were detected on the basis of history of mastitis condition, clinical information like decreased milk yield and most of times with scanty milk yield, physical changes in udder like firmness of udder with obstructed teat in some cases, physical changes in milk like wateriness and flakiness, gel formation in CMT, somatic cell count above 2 lakhs/ml for more than 2 months old mastitis cases by Newman lampert stain as per Prescott and Breed method (1910). Blood samples were collected from jugular vein of 13 affected cows after sterilising that area with rectified spirit soaked cotton swab in EDTA vial, labelled and immediately transferred to lab for estimation of haematological parameters like Hb, TLC, TEC, DC, PCV etc. Blood samples were also collected from those 13 affected animals in red topped tubes and kept in room temperature undisturbed for 30 minutes. Then coagulated blood was centrifuged at 3000 rpm for 5 minutes. Serum samples were transferred to clean polypropylene tubes using a pasture pipette and stored at -20^{0}C in deep freeze for further analysis. The various serum biochemical parameters like ALP, total protein, albumin, globulin, calcium, serum urea nitrogen were estimated by using various kits of Coral clinical system, Tulip diagnostic Pvt. Ltd.

Results and Discussion

The haematological values of all parameters

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were estimated. The average haemoglobin value was found to be 9.00 ± 0.45 gm% with a minimum of 6.2gm% to maximum of 11.2gm% indicating decreased Hb value. The average TLC value was found to be 7630 ± 639.35 / cubic mm with a minimum of 3800 to maximum of 10600 per cubic mm. The mean TLC value indicated that there was slight reduction in TLC value. The average TEC value was found to be 4.61 ± 0.24 million/ cubic mm with a minimum of 3.1 million to maximum of 5.6 million per cubic mm showing reduced TEC value. The mean value of PCV was found to be 28.75 ± 1.46 % with a minimum of 19% to maximum of 36% showing a slight decreasing trend. The mean % value of neutrophil was found to be 27.61 ± 1.61 % with a minimum of 18% to maximum of 36%. The mean % value of lymphocyte was found to be 65.42 ± 1.45 % with a minimum of 57% to maximum of 74% showing slightly higher value. The mean % value of monocyte was found to be 3.25 ± 0.76% with a minimum of 1% to maximum of 10%. Blood picture of affected cow showed the mean value of MCV 62.65 ± 1.50 cubic microns with a minimum of 50 cubic microns to a maximum of 67.5 cubic microns. Similarly, MCHC percentage was found to be 31.35 ± 0.33 % with a minimum 29.68% to a maximum of 32.86% showing slight decrease in MCV and MCHC. The lymphocyte value was slightly higher. This may be due to chronicity of infection causing slight anaemia and slight leucopoenia. Son *et al.* (1995) observed increased numbers of monocytes, eosinophils and neutrophils, decreased lymphocytes and unchanged erythrocyte count, the packed cell volume and haemoglobin concentration in clinical mastitis cow as compared with normal mean values. Phiri *et al.* (2007) reported higher value total white blood cell count, eosinophil count and lower value of haemoglobin, packed cell volume and neutrophil count in mastitis cows as compared to healthy cows. Singh *et al.* (2014) revealed significantly ($P<0.05$) higher average values of TLC in sub-clinically ($9.20\times10^3/\mu l$) and clinically ($9.31\times10^3/\mu l$) infected animals than healthy animals ($6.87\times10^3/\mu l$) as well as neutrophilia and lymphopenia in clinical as well as sub-clinical mastitis (SCM).

The serum biochemical values of chronic mastitis cows were also evaluated. The average value of total protein was found to be 6.77 ± 0.30 gm/dl with a minimum of 6.16 gm/dl to maximum of 8.19 gm/dl. The average value of albumin was found to be 3.00 ± 0.16 gm/dl with a minimum of 2.53 gm/dl to maximum of 3.58 gm/dl. The average value of globulin was found to be 3.78 ± 0.30 gm/dl with a minimum of 3.29 gm/dl to maximum of 5.2 gm/dl. The average value of serum urea nitrogen was found to be 11.09 ± 1.47 mg/dl with a minimum of 6.69 mg/dl to a maximum of 17.29 mg/dl. The average value of calcium was found to be 9.47 ± 0.22 mg/dl with a minimum of 8.8 mg/dl to a maximum of 10.2 mg/dl. The average value of ALP was found to be 74.04 ± 12.29 IU/L with a minimum of 51.5 IU/L to a maximum of 129.7 IU/L. The average values of total protein, albumin, globulin, serum urea nitrogen and alkaline phosphatise were found to be within normal range.

The average values of total protein, albumin, globulin, SUN and alkaline phosphatise were found to be within normal range. Phiri *et al.* (2007) reported lower value of plasma calcium and higher value of alkaline phosphatase in mastitis cows as compared to healthy cows. Matei *et al*. (2010) observed higher values of total serum protein (9.14 ± 2.74 g/dl), serum globulin (5.76 ± 1.82 g/dl) and ALP (71.80 ± 50.70 U/l) in subclinical mastitis compared to healthy cows. Singh *et al.* (2014) revealed higher average values of TPP (8.44 g/dl and 8.23 g/dl), albumin (2.447 g/dl and 2.31 g/dl), globulin (5.82 g/dl and 5.93 g/dl) and fibrinogen (0.74 g/dl and 1.21 g/dl) from sub-clinically and clinically infected animals compared with control group (7.44 g/dl, 2.94 g/dl and 4.41 g/dl and 0.60 g/dl, respectively). Mineral estimation revealed significant increase in calcium level in sub-clinical (13.45 mg/dl) and clinical mastitis (13.149 mg/dl) as compared to healthy control (9.44 mg/dl).

Conclusion

The average values of haemoglobin, TLC, TEC and PCV in chronic mastitis were slightly lower than normal. The lymphocyte value was slightly higher. The average values of total protein, albumin, globulin, SUN and alkaline phosphatase were found to be within normal range.

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Leucoderma in buffaloes of Kumarganj Faizabad, Uttar Pradesh

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Abstract

The aim of his study is to record the incidence of leucoderma in buffaloes reared around Faizabad areas of Uttar Pradesh. The study was conducted between January 2013 and March 2014. A total of 3800 buffaloes were screened for skin depigmentation irrespective of age, sex and breed in which 20 were found leukodermic. Among 3800 buffaloes, 3200 were female and 600 were males mainly comprising calves. The cases were diagnosed on the basis of history and clinical signs. Haematology and serum micro minerals status were also evaluated for confirmatory diagnosis. Thus an overall incidence of 0.53% was recorded in buffaloes in Kumarganj Faizabad. The size, extent and pattern of distribution of lesions were recorded.

Keywords: Buffaloes, Incidence, Leukoderma, Lesions

Leucoderma occurs due to abnormality of melanocytes in the epidermis (Radostits *et al*., 2007) like lack of melanocytes or failure of melanocytes to produce melanin or failure of transfer of melanin to epidermal cells. Leucoderma has been recorded in dairy animals, mainly buffaloes (Gill and Gill, 1975, Dhillon *et al.,* 1991). It is not harmful medically and causes no physical pain, but it has aesthetic value because the market value of animal is reduced up to 50 % or more. Till now the exact etiology of leucoderma is unknown and occurs in dark and brown skinned buffaloes (Cockrill, 1974) and it is hypothesized that leucoderma may occur due to autoimmunity, oxidative stress, genetic predisposition, toxic exposure, and copper deficiency. Production of melanin is dependent on copper because copper is a component of tyrosinase. Copper deficiency may, therefore, result in reduced pigmentation (Vegad and Katiyar, 2004). In copper deficiency, rough coat and depigmentation of coat color i.e. red coat becomes reddish yellow, black becomes rusty brown as well as spectacled eyes have been noticed in cattle and buffaloes from Punjab (Randhawa, 1999). Sporadic cases of leucoderma have also been reported in buffaloes from Andhra Pradesh (Panduranga Rao *et al; 2002*).However there is no systematic study from this region hence this investigation was undertaken

Materials and Methods

The research work was conducted in and around the Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.). The present investigation was planned to study the incidence of leucoderma in buffaloes. Screening of leucoderma in buffaloes was conducted since January 2013 to March 2014. This screening was conducted at Teaching Veterinary Clinical Complex, Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Narendra Deva University of Agriculture & TechnologyKumarganj-224229, Faizabad U.P. and 10 villages in surrounding area of Kumarganj namely Pithla, Soraon, Kanji, Chatri, Mukundpur, Gajanpur, Sarai Baggha, Jaraikala, Akma and Chatarganj. A total 3800 buffaloes were screened for the disease in which 20 were found leukodermic. Among 3800 buffaloes, 3200 were female and 600 were males mainly comprising calves. All buffaloes were clinically examined. Respiration, pulse rate, heart rate, rectal temperature, feed intake, rumen motility was in normal range. Urination and defecation was normal. Fecal examination and skin scrapping were performed for the detection of any parasitic eggs/larvae in the feces and detection of demodex, mange and fungal infection respectively**.** Blood samples were collected aseptically for hematological purposes and second set without anticoagulant for serum separation. Diagnosis of leucoderma Incidence and pattern of distributions of lesions of Leucoderma in buffaloes in buffaloes was based on the basis of history, clinical signs, fecal examinations, and skin scrapping, blood and serum micro minerals analysis**.**

Results and Discussion

Out of a total 3800 buffaloes, 20 were found having leucoderma. Thus an overall incidence of 0.53% (Table: 1) was recorded in buffaloes in Kumarganj and

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nearby areas.

Location

A total 2000 buffaloes were presented to our clinic in which 7 buffaloes were found leucoderma with the incidence rate of 0.33%. On tracing back the villages and surrounding areas from where these cases were reported, a total 1700 animals from 10 villages were screened, in which 13 leucoderma cases were found with the incidence of 0.76% (Table:1). Highest incidence was reported in village Pithla which was 2.00 % and no single case was reported from village Akma and Jaraikala.

The results are in agreement with Gapat et al. (2013) who had also recorded an incidence of 0.43% in buffaloes in Latur district of Maharashtra after screening of 4229 buffaloes that is much closer to our sample size. A slightly higher incidence of 0.84% was recorded by Randhawa *et al.* (1993) from Punjab. Dube *et al.* (2014) reported 0.20% incidence in screening of 9732 buffaloes from Latur district of Maharashtra which is much lower than our findings. The higher incidence (0.76%), which is much closer to the finding of Randhawa *et al.* (2009), may be due to trace back of villages from where cases of leucoderma were reported to our TVCC. However the incidence of leucoderma among the buffaloes presented to our TVCC was only 0.33% (Table: 1). Leucoderma is not being a life threatening disease and that may be the reason for poor reporting at TVCC. Randhawa and Randhawa (2002) reported higher incidence of hypocupremia in unorganized farm and lower incidence in organized farm which result in anaemia and leucoderma. In our study also no leucoderma buffalo was reported from university's farm, ILFC.

Table: 1. Area wise incidence of Leucoderma in buffaloes

Name of Village(No of animals)	Incidence
Pithla (150)	3(2.00%)
Soraon (250)	2(0.80%)
Kanji (180)	1(0.55%)
Chatri (93)	1(1.07%)
Mukundpur (140)	2(1.42%)
Gajanpur (220)	1(0.45%)
Sarai Baggha (240)	2(0.83%)
Akma (230)	0(0.00%)
Chatarganj(132)	0(0.00%)
Jaraikala(70)	1(1.42%)
ILFC (100 buffaloes)	0 (0.00%)
TVCC (2000 cases)	7(0.35%)
Grand Total=3800	20(0.53%)

Table: 2. Age wise incidence of Leucoderma in buffaloes

Age Group	Incidence
0- 4 years	3(15%)
4-8 years	11(55%)
8-12 years	6(30%)

Age

Minimum incidence of leucoderma was recorded in 0-4 year age group of animals (15%), followed by 30% in 8-12 year age group and maximum of 55% in 4-8 year age group(Table:2). Hussain (1990) had reported leucoderma in a 7 year old buffalo. Sinha *et al.* (1976) reported leucoderma in a 2 year old heifer and 6 year old buffalo. Higher incidence in 4-8 year age group may be due to the use of animal for production and reproduction, that's why they are more prone to various deficiencies including that of micro minerals. In our study 8-12 year age group of buffalo had 30% incidence which may be due to the senility.

Sex

During the screening of 3800 buffaloes, comprising of 3200 females and 600 males, 20 were found positive for leukoderma, in which 19 were female and 1 was male buffalo calf with the sex specific incidence of 0.59% and 0.17% in female and male respectively. The overall sex wise incidence was 95% and 5% in female buffaloes (Table: 3) and male calf respectively. Randhawa *et al.* (2009) suggested severe leukoderma in female buffaloes covering 80% of the body surface. Males are rarely reared and thus no reports in adult males. Female have higher incidence of

Table: 3. Sex wise incidence of Leucoderma in buffaloes

Sex	Incidence
Female	19(95%)
Male	1(5%)
Total	20(100%)

Table: 4 Distribution of Leucoderma lesion on body part in buffaloes

Body part affected	No. of cases
Ventro-lateral abdomen	7(35%)
Dorsal part trunk	1(5%)
Udder	1(5%)
Muzzle	1(5%)
Brisket	1(5%)
Tail	1(5%)
Perennial region	3(15%)
Multiple area of body	5(20%)

leukoderma because their production and reproduction potential make them more prone for deficiency diseases.

Pattern

The pattern of distribution of skin lesion was recorded. The areas that were affected were muzzle, areas around eyes, brisket, ventral and dorsal abdomen, inner thigh, udder and Incidence and pattern of distributions of lesions of Leucoderma in buffaloes perennial region. In the present study the ventral lateral abdominal area was mostly affected (35%) followed by multiple area, involving neck region, brisket, axilla, lower abdomen, udder, hind quarter, vulva, tail (20%) and perennial region (15%). Depigmentation occur maximum in ventrolateral abdominal because of least exposure of these area to ultraviolet rays of sunlight Singh and Randhawa (2008). According to Chandra and Bharadwaj there is higher pigmentation in dorsal area of body, moderate in lateral area of body and lowest in ventral area of body. So the chances of depigmentation in ventolateral area are higher. In our finding maximum depigmentation is reported in ventrolateral area of body. Mude and Syaama Sundar (2009) reported involvement of multiple body area including neck, around eyes, side of abdomen, flank and thorax. These body areas are mostly of lateral aspect which is moderately pigmented thus there are more chances of depigmentation and occurrence of leukoderma. In our study dorsal part of body/trunk has least incidence; this is due to highest pigmentation in dorsal body area, because of higher exposure of dorsal area to ultraviolet rays.

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Haemato-biochemical profile of dogs suffering from acute renal failure

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Abstract

Five dogs were diagnosed with acute renal failure on the basis of history, clinical presentation and various haemato-biochemical parameters. These dogs had a history of short duration of sickness (<14 days), were young to middle aged, presented with clinical signs of vomiting, melena, mild dehydration and abdominal pain. Mean Hb (13.26 ± 1.04 g%) and PCV (36.06 ± 2.79%) was within normal physiological range in majority dogs with severe neutrophilic leucocytosis (31338±8386.57/µl), high mean serum urea nitrogen (201 ± 42.06 mg/dl), creatinine (18.18 ± 4.98 mg/dl), alkaline phosphatase (186.8 ± 80.04 U/L), triglyceride (84 ± 2.5 mg/dl) and inorganic phosphorus (13.06 ± 0.84 mg/dl), and low calcium (8.24 ± 1.01 mg/dl) and albumin (2.52 ± 0.27 g/dl).

Keywords: Acute renal failure, Creatinine, Dog, Hyperphophatemia, Renal failure, Urea nitrogen.

Renal failure is deterioration of renal function resulting in the accumulation of nitrogenous wastes such as urea and creatinine (Labato, 2001). The two main forms are acute renal failure (ARF)/ acute kidney injury (AKI), which is often reversible with adequate treatment; and chronic kidney failure (CRF)/ chronic kidney disease (CKD) which is often not reversible. Common causes of ARF include antibiotics (aminoglycosides), analgesics, NSAIDs, contrast media or environmental contaminants such as cadmium or mercuric chloride. AKI can result from decreased renal or intra-renal perfusion, a toxic or obstructive insult to the renal tubule, tubulointerstitial inflammation & edema, or primary reduction in the filtering capacity of the glomerulus (Schrier *et al*., 2004). Severe ARF may result in oligouria or even anuria or urine volume may be normal or even increased (Vaidya *et al.*, 2008). The present study describes hematological and biochemistry findings in five dogs with acute renal failure.

Materials and Methods

All the five dogs suffering from acute renal failure presented in this study were less than 6 years of age i.e. young to middle age dogs, out of which four were males and one female. Two dogs were German Shepherd, one each was Labrador retriever, Bull Terrier and non-descript breed. Whole blood sample (2ml) was collected in EDTA coated vials and analyzed for hemoglobin (g%), packed cell volume (PCV %) and total leukocyte count (TLC) using fully automatic Haematology analyser (ADVIA ® 2120 Hematology system, Siemens Healthcare diagnostics Inc., USA). Differential leukocyte count (DLC) was done using giemsa stain as per method described by Jain (1986). For glucose estimation blood was collected in sodium fluoride coated vials. For serum biochemistry, blood samples were collected in vials without any coagulant. Serum was separated and analysed for estimation of serum urea nitrogen (SUN), creatinine, total protein, albumin, total bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, inorganic phosphorus (Pi), cholesterol and triglycerides using Virtos DT-II Chemistry system (Ortho-clinical Diagnostics, Johnson & Johnson Company, USA). Urine was collected in sterile plastic containers after catheterization/cystocentesis/free catch. Specific gravity of the urine was estimated using a refractrometer. Qualitative analysis of urine for proteins, glucose, bile pigments, bile salts, ketone bodies was done by urine dipstick (Multistix® 10 SG Reagent Strips for Urinanalysis, Siemens Healthcare Diagnostics Inc, USA) using CLINTEK STATUS analyzer (Bayer Healthcare, LLC, Germany). For microscopic examination of urine, the samples were centrifuged at 2000 rpm for 5 minutes. The supernatant obtained was discarded and the sediments were then thoroughly re-suspended in the urine. A drop of this reconstituted sediment was transferred on to a glass- slide and a cover slip was placed over it. Initially, the quantity and the type of casts were assessed under low power (10 x) of light microscope followed by high power (40 x) for presence of RBC, pus cells, casts, crystals, microbes, etc. Blood pressure was measured using cuff

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oscillometer (Surgivet) from radial as well as femoral arteries. The hematological parameters were compared with normal physiological levels reported by Jain (1986) and biochemical parameters were compared with normal physiological levels reported by Kaneko *et al.,* (2008).

Results and Discussion

All the dogs had duration of sickness lasting for 2 days to 2 weeks, thus depicting the acuteness of the condition. Vomiting was present in four out of the five dogs, but melena was found in only two. The mean rectal temperature (101.6 ± 0.8ºF) was within normal range. There was mild dehydration (mean value measuring 9.20 ± 2.7%). Mucous membrane of four dogs was congested while the fifth had pink which were in accordance with their hemoglobin levels (10.9-16.6 g%). Four dogs exhibited pain on palpation of lumbar area and had a kyphotic posture. Fleming *et al*., (1989) reported that dog with ARF may show vomiting, diarrhea, dehydration, hypothermia, mucosal injection and pain on palpation of lumbar area as was also seen in this study. The mean heart rate (80 ± 40.3 beats per minute) was within normal range, while the mean respiration rate (53.2 ± 22.6 per minute) was slightly above the normal range, which is also in accordance with the findings of Fleming *et al*., (1989). The mean systolic and diastolic blood pressure in these dogs were 138 ± 18.24 mm Hg and 84.2 ± 9.12 mm Hg, respectively indicating absence of hypertension, a feature more characteristic of CRF and also helps to distinguish ARF from CRF (Ernesto *et al*., 2007).

The mean urine specific gravity was 1.016 ± 0.001 and ranged from 1.015-1.030, which is lower than the normal physiologic specific gravity of urine in dogs (i.e. >1.030). Dipstick reading of +++ proteinuria was recorded in one dog and ++ in three dogs. Adin and Cowgill (2000) observed that dogs with leptospirosis (a common cause of ARF) had urine with specific gravity <1.020, hematuria, proteinuria and granular casts. In the present study, one dog passed reddish urine which on microscopic examination revealed presence of RBCs (>30/hpf), urate and calcium carbonate crystals. One dog had epithelial casts and two had hyaline casts, one dog had cocci in urine.

Table 1: Haematological and Serum biochemical parameters of dogs suffering from Acute Renal Failure

Blood Parameters	Reference range	Mean	Median	Range
Hb (g%)	12.0-18.0	13.26 ± 1.04	12.2	10.9-16.6
PCV (%)	37-55	36.06 ± 2.79	35.2	29-45.1
TEC (× 10^6/ µl)	5.5-8.5	7.52 ± 0.52	7.525	6.7-8.35
TLC (×/µl)	6000-17000	31338 ± 8386.57	26900	18050-63540
Neutrophil (×/µl)	3000-11500	30057.64 ± 8481.44	26362	16051-62269
Lymphocyte (×/µl)	1000-4800	1280.36 ± 325.24	1270	538-2188
Platelet (×10^3/ µl)	200-800	240.2 ± 65.4	313	38-390
SUN (mg/dl)	10.0-28.0	201 ± 42.06	162	93-300
Creatinine (mg/dl)	0.5-1.5	18.18 ± 4.98	13.2	7.7-30.4
Sodium (mmol/l)	141-152	144.8 ± 3.5	144	133-154
Potassium (mmol/l)	4.37-5.35	4.48 ±0.62	4.2	2.7- 4.2
Chloride (mmol/l)	105-115	107 ± 3.86	105	97- 105
Total protein (g/dl)	5.4 -7.1	6.36 ± 0.43	6.8	4.9-7.4
Albumin (g/dl)	2.6-3.3	2.52 ± 0.27	2.4	1.9-3.3
Globulin (g/dl)	2.7-4.4	3.84 ± 0.33	3.8	2.9-4.9
A/G	0.59-1.11	0.58 ± 0.08	0.48	0.38-0.8
ALT (U/L)	21-102	44.4 ±4.86	43	34-60
ALP (U/L)	20-156	186.8 ± 80.04	115	76-503
T. Bilirubin (mg/dl)	0.1-0.5	0.36 ± 0.05	0.4	0.2-0.5
Glucose (mg/dl)	65-118	88.2 ± 4.02	86	80-100
Cholesterol (mg/dl)	135-270	200.4 ± 28.18	214	97-250
Triglyceride (mg/dl)	38.1	84 ± 2.5	64	45-180
Calcium (mg/dl)	9.0-11.3	8.24 ± 1.01	9.1	5.3-10.4
Phosphorus (mg/dl)	2.6-6.2	13.06 ± 0.84	13	10.8-16
Ca/P	2-37	0.654 ± 0.05	0.75	0.33-0.86
Magnesium (mg/dl)	1.8-2.4	1.88 ± 0.27	2	1.2-2.5

The mean hemoglobin and PCV levels were within physiological range (Table 1). Two dogs showed borderline anemia as observed by Davenport *et al*. (1995). The mean TLC (31338 ± 8386.57/ µl) and neutrophil count (30057.64 ± 8481.44/ µl) were above normal physiological range indicating leucocytosis due to neutrophilia. So, these changes may indicate possibility of infectious etiology of ARF in present study. According to De Vriese *et al*. (1997) ARF may be due to sepsis, nephrotoxic injury and severe pre-renal insult.

Mean SUN (201 ± 42.06 mg/dl) and creatinine (18.18 ± 4.98 mg/dl) were very high compared to normal physiological range (Table1). Lee *et al*., (2011) also reported that kidney failure patients with gastrointestinal signs of vomiting or diarrhea have higher levels of SUN as was seen in our study. According to Rule *et al*. (2006), serum creatinine levels do not distinguish ARF from CRF. On the other hand Davenport *et al*. (1993) observed that serum urea was lower in CRF patients than ARF patients, possibly due to low protein uptake by CRF patients.

The mean ALP (186.8 ± 80.04 U/L) and triglyceride (84 ± 2.5 mg/dl) levels were above the normal physiological range, while the mean ALT (44.4 ±4.86 U/L), total bilirubin (0.36 ± 0.05 mg/dl) and cholesterol (200.4 ± 28.18 mg/dl) were within the normal physiological range (Table1). Carr *et al*., (2003) suggested that the liver enzyme activities can be elevated with leptospirosis or with secondary liver involvement.

Mean serum total proteins (6.36 ± 0.43 g/dl) and globulin (3.84 ± 0.33 g/dl) levels were within normal physiological range (Table1). The mean serum albumin level (2.52 ± 0.27 g/dl) was below the normal physiological range. Xenoulis and Steiner (2010) reported that hypoalbuminemia and proteinuria is observed in dogs with acquired glomerular disease.

The mean serum inorganic phosphorus (13.06 ± 0.84 mg/dl), calcium (8.24 ± 1.01 mg/dl) and magnesium (1.88 ± 0.27 mg/dl) levels revealed hyperphosphatemia and hypocalcemia. Ross (2011) stated that serum phosphorus levels are often elevated; however, the degree of elevation may reflect the degree of reduced GFR (Glomerular Filteration Rate) rather than the duration of disease. Vaden *et al*., (1997) conducted a retrospective study of ARF in 99 dogs and found calcium to be less than 8.6 mg/dl.

The mean serum sodium (144.8 ± 3.5 mmol/ L), chloride (107 ± 3.86 mmol/L) and potassium levels (4.48 ±0.62 mmol/L) were within normal physiological range (Table 1)., but two out of five dogs had hyperkalemia with serum potassium measuring 5.4 mmol/L and 6.3 mmol/L (Table 1). Hyperkalemia was also reported by Fleming *et al*. (1989) in dogs suffering from ARF.

Present study indicated that dogs with ARF may be differentiated from CRF patients on the basis of duration of sickness, abdominal pain, blood pressure measurement, hemoglobin, serum urea nitrogen and creatinine.

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Evidence for presence of 3AB nonstructural protein antibody of foot-and-mouth disease virus in randomly sampled pigs of Nagaland

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Abstract

The aim of this study is surveillance of foot-and-mouth disease (FMD) in randomly sampled pigs of the north-eastern state of Nagaland during 2014-2015. A total of 381 serum samples from pigs were collected across six districts of Nagaland and tested in 3AB nonstructural protein (NSP) ELISA, where 39 (10.23%) samples were found positive for NSP-antibodies indicating a mild to moderate level of virus activity. Representative serum samples were also subjected to liquid phase blocking (LPB) ELISA to assess the level of protective antibody against FMD virus serotypes O, A and Asia 1, where none of the pigs were found to have protective $\log_{10}$ antibody titre of e"1.8 against all three serotypes. Moreover, the farmers in the region usually do not vaccinate their pigs routinely against FMD, which correlates with the results of LPB ELISA. As pigs are known to be the amplifier hosts for FMD, they exhale a large quantity of aerosol virus during infection that may initiate infection in other susceptible animals reared nearby. In such circumstances, if pigs are routinely vaccinated against FMD to build up a high level of protective antibody titre, then it can indirectly help to control the disease in the region.

Keywords: Foot-and-mouth disease, Nagaland, Pig

Introduction

Foot-and-mouth disease (FMD) being one of the most infectious and highly transmissible viral diseases of domestic animals and more than 70 wildlife species (Arzt *et al.,* 2010), stands in the way of livestock productivity and the economy of any country especially the developing nations. India is considered endemic for the disease with the prevalence of three serotypes O, A and Asia 1, where serotype O being the most prevalent, accounts for 83-93% of the outbreaks (Pattnaik *et al.,* 2012). The north eastern (NE) region of the country is mostly covered by dense forests and hilly areas (Kumar *et al.,* 2007). In this region especially in Nagaland, rearing of pigs mostly in backyard acts as the most viable and dependable source of earning livelihood for majority of the farming community as it needs very low capital investment and cost for management of animals fed on kitchen wastes and garbage thereby generating significant opportunities for augmentation of household income. Free range or scavenging system is usually not practiced except in some remote areas where human population density is less.

Piggery is accepted by all in Nagaland for domestic consumption, as there is no discrimination between poor and rich or different classes of people in pig rearing. Swine production in Nagaland occupies an important role in rural economy, since pork is the most preferred meat item in the daily food menu of the Naga society. The cold climatic condition of the state also favors consumption of meat by the inhabitants. For the marginal poor farmers, incidence of infectious diseases like FMD creates a major stumbling block for the animal productivity thereby costing a lot for them. As per 19th Livestock Census - 2012 All India Report, the total pigs in Nagaland is 503688, against 10.29 million of total pigs in the whole country. In the present study we intend to obtain primary information on serological status of FMD in pigs reared in some selected districts of Nagaland.

Materials and Methods

A total of 381 pig sera (mostly from backyard piggery) along with the history of vaccination and management practices followed were collected across six districts of Nagaland (30 from the district Peren, 6 from Wokha, 195 from Kohima, 5 from Dimapur, 45 from Zunheboto, 100 from Mokokchung). An indirect ELISA based on 3AB non structural protein (NSP) was used to detect antibodies (Abs) against FMD virus (FMDV) (Mohapatra *et al.,* 2011). Pig serum samples along with the negative and positive control sera were diluted @ 1:20 and anti-pig horse radish peroxidase conjugate was dispensed @ 1:5000. Test sera producing corrected optical density values e"50% of that of the positive control were marked positive.

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Liquid phase blocking (LPB) ELISA was performed as per the procedure followed by Ranabijuli *et al.* (2010) for the measurement of serotype-specific FMDV SP-Ab titre against O, A and Asia 1 to assess the level of protective antibody / overall status of vaccinal immunity (log_{10} titre of e"1.8 against all three prevalent serotypes).

Results and Discussion

In 3AB NSP ELISA, 39 out of 381 (10.23%) sera (20% positivity in Peren, 0% in Wokha, 12.30% in Kohima, 20% in Dimapur, 11.11% in Zunheboto, 3% in Mokokchung) were found positive for NSP-Ab indicating a mild to moderate level of virus activity. The pigs might have been exposed to FMDV during any point of time leading to such seropositivity. There are also reports of FMD outbreaks in some pig farms in the state as in the year 2012, when an outbreak due to FMDV serotype O in pigs was recorded in a government farm at Liere, Kohima. In LPB ELISA none of the pig sera tested was found to have protective log_{10} antibody titre of e"1.8 against all three serotypes indicating absolutely worse herd immunity status, which correlates with the history that FMD vaccination is not followed in pigs in the region. The apparent prevalence of NSP-Ab in bovines in Nagaland state has been reported to be 33.70% (Annual Report, PDFMD, 2014-2015), where several outbreaks were recorded at many places. In the state there is no restriction on animal movement and there is frequent intermixing of FMD-susceptible species by the farming community. In this circumstance, if the disease occurs in any of the species at any place, it easily gets transmitted to other in-contact animals at other places. Moreover, pigs reportedly produce enormous quantity of aerosol virus during the infection phase (Donaldson, 1999) and hence most of the susceptible animals nearby may become infected through respiratory route.

Generally, bovine populations in the country are vaccinated against FMD mostly under FMD control programme and partly under ASCAD (Assistance to States for Control of Animal Diseases) and RKVY (Rashtriya Krishi Vikas Yojona) of the Government of India (Pattnaik *et al.,* 2012), leaving most of the other susceptible species unattended like sheep, goats and pigs. Animal movement has been considered as the predominant cause of FMD in vaccinated zones. Due to the economic value connected with the livestock trade, there is considerable animal movement within the country. The strategy for more effective control of animal movement should be economical and efficient in improving the efficiency of legal animal movement (Pattnaik *et al.,* 2012). When several FMD-susceptible species are reared by the farming community without any control on restriction of their movement, then vaccination only in one species may not help practically to control the disease. Hence, all agriculturally important domestic livestock species including pigs must be considered under the vaccination policy followed by routine surveillance and periodical monitoring of protective antibody status, so that the complicated situation in the disease scenario may be better simplified due to the rising 'herd immunity' level in the susceptible animals. Besides vaccination, effective application of zoo-sanitary measures cannot be ignored. This along with proper biosecurity measures can help in prevention of incursion of the disease and control of virus spread. Many factors responsible for growth in the livestock rearing in NE region have been highlighted by Kumar *et al.* (2007). These factors need to be addressed to accelerate the development of livestock sector in the region, which is an important source of livelihood for million of poor people. Attention should also be paid to improve the disease control measures in the animals in NE region. Very less attention is being paid across NE states for the prophylactic measures towards disease control (Kumar *et al.,* 2007) leading to frequent incidences of diseases. Therefore, a greater emphasis needs to be focused on prophylactic control measures for infectious diseases in the region (Kumar *et al.,* 2007).

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Clinical Article

Notoedric mange in a cat – A case report

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Abstract

A nine months old female cat was brought to Referral Veterinary Polyclinic, Indian Veterinary Research Institute with the history of itching and scratching over the face and ears since a month. On physical examination crusty, alopectic lesions were found on the face and ears and all the physiological parameters were within the normal range. Skin scrapping examination revealed the presence of mites belonging to the *Notoedrus species*. Based on these findings a diagnosis of Notoedric Mange was made. Case was managed with Ivermectin @200 µg/kg, SC, once a week for four weeks. Significant improvement was noticed after two weeks of treatment.

Keywords: Cat, Ivermectin, Notoedres Species, Mange

Notoedric mange is a highly contagious, pruritic disease of kittens, cats , rabbits, dogs and pine civets caused by the psoroptic mite *Notoedres cati* (Scott *et al.,* 2001). The infestation is clinically characterized by extreme pruritus and crusting lesions of the ears, head, neck, back and feet (Kwochka, 1987 and Griffin *et al*., 1993). Because of its contagious nature and zoonotic importance, the treatment and control of notoedric mange remains difficult and challenging (Itoh *et al.,* 2004). We report here the successful medical management of notoedric mange in a cat.

Case Presentation

A nine months old female cat was presented to Referral Veterinary Polyclinic, Indian Veterinary Research Institute with the history of itching and scratching over the face and ears since one month. On clinical examination crusty, alopectic lesions were found on the face and ears. The affected skin was devoid of hair and became thick, leathery and wrinkled. The skin in the affected areas was markedly corrugated and was often covered by large plaques of keratinous crusts resulting from self inflicted trauma induced by pruritus (Fig.1.). Scratching of the affected areas caused the skin to become raw, red and inflamed. Skin scrapings were prepared from the affected areas, cleared by 10% KOH solution and examined under low power of microscope (Soulsby, 1968). Notoedric mite was identified by criteria of Walker (1994). The mite was considerably smaller and more circular than *Sarcoptes scabiei* and the anus was located on the dorsal surface. Projecting scales were not found and on mid dorsal area the striae were broken into scale like pattern (Fig.2).

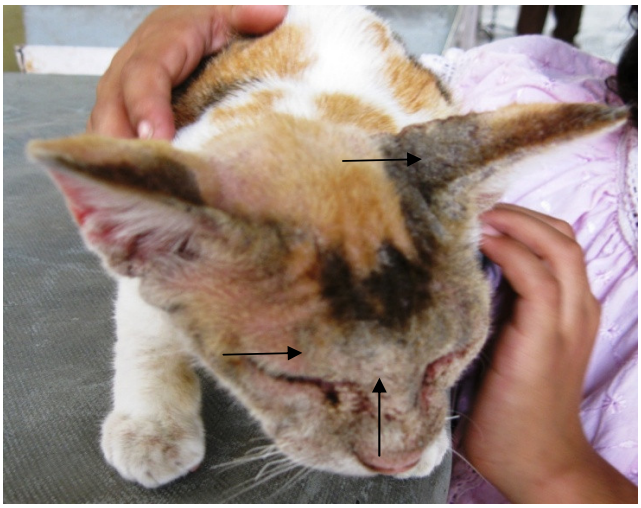

Fig.1: Photograph showing crusty, alopectic lesions on the face and ears

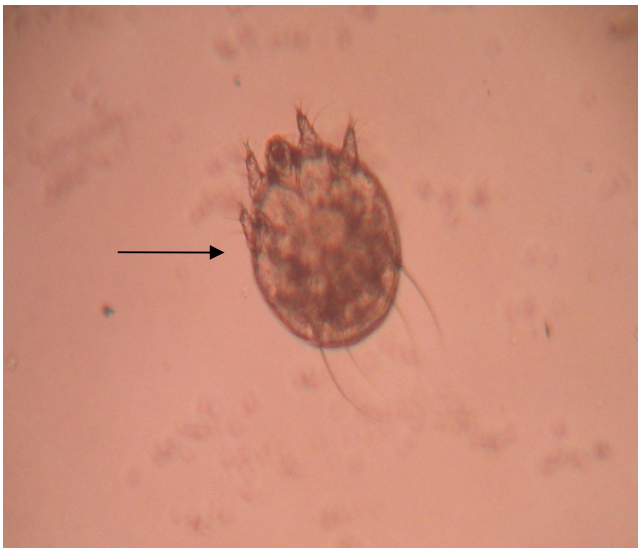

Fig.2: Photograph showing Notoedrus Mite

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Treatment and Discussion

Based on clinical signs and skin scrapping examination confirmatory diagnosis of notoedric mange was made. The case was managed with Ivermectin @200 ¼g/kg, subcutaneously at weekly intervals for a month along with supportive therapy. No adverse reactions were observed after the treatment. On day 7, the pruritus had resolved. There was a significant clinical improvement after 14 days of treatment. Two weeks post treatment, skin scrapings were examined and found negative.

As *Notoedres cati* is communicable to human beings and other animals, it requires immediate and appropriate treatment and the owners must be cautioned when handling the affected cats (Chakrabarti, 1986 and Foil, 2003). Commonly practiced treatments include Selamectin @ 4 mg/kg, as a "spot on" and Ivermectin @ 200 mg/kg, subcutaneously at weekly intervals or fortnightly for a month (Scott *et al*., 2001). In the present study cat was treated with Ivermectin @200 mg/kg, subcutaneously at weekly intervals for 4 occasion along with supportive therapy. The successful recovery in the present study indicated the benefit of Ivermectin therapy in cats for the management of Feline Scabies. This is in accordance with the findings of other workers (Senthil Kumar *et al.,* 2008; Scott *et al*., 2001)

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Ventricular arrhythmias associated with cardiomyopathy in cats

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Abstract

Ventricular arrhythmias were found associated with cardiomyopathy in 05 cats with shortness of breath, marked weakness, lethargy, and fatigue. Cardiomyopathy was confirmed by elevated levels of cardiac Troponin I .Treatment with furosemide, butorphanol, propranolol and nitroglycerine showed improvement in four cats.

Keywords*:* Cardiac troponin-I, Electrocardiography, Ventricular arrhythmia

Diagnosis and treatment of cardiac diseases in cats is a challenging task because of small body and heart size, rapid heart rate, uncooperative nature and abrupt onset of clinical signs. Ventricular arrhythmias are the most common feline arrhythmias and 96% cats with ventricular arrhythmias were found associated with cardiomyopathy (Cote and Jaeger,2008). The present report describes ventricular arrhythmias in cats associated with cardimyopathy alongwith treatment.

Case Presentation

Five cats, brought to hospital in lethargic condition with anorexia for 5-6 days, revealed shortness of breath, pulmonary crackles, marked weakness, lethargy, dyspnoea, tachypnoea,depression,hypotherma(temperature 94.0-95.8 ^{0}F), peripheral vasoconstriction(pale mucus membrane and increased capillary refill time), weak femoral pulse and fatigue .

Electrocardiography at the time of referral (recorded employing hexaxial lead system at 1 mV = 10 mm, with paper speed of 25 mm/s) revealed accelerated idioventricular rhythm (VPC 80-90 per minutes) in two cats (Fig.1); ventricular tachycardia (VPC in run at the rate of 140 per minutes) in two cats(Fig.2) and ventricular asytole in another one cat(Fig.3)

These cats , subjected to qualitative cardiac troponin I estimation (Employing Amicheck-Trop I kit – Zephyr Biomedicals or Diagnos Tropo-I Card – Diagnostic Enterprises Parwanoo, H.P.) to detect myocardial damage, if any, revealed cTn-I levels in between 0.3-1.0 ng/ml on the basis of colour intensity of the band of test samples (Fig.4) .

Cats with ventricular tachycardia and accelerated idioventricular rhythm associated with cardiomyopathy were treated with furosemide(2.0 mg/kg IM daily for 3 days), butorphanol (Butrum- Aristo, 0.3 mg/kg IM dailyfor 4 days), propranolol (2.5 mg per cat PO BID daily) and nitroglycerine (Myovin ointment – Cadila Pharma applied locally in 1.0 cm square area on inner side of ear pinna two times a day initially for 3 days then once daily for 3 days). Clinical signs of shortness of breath, gasping, marked weakness, lethargy, and pulmonary crackles were suggestive of compromised cardiovascular system leading to congestive heart failure. Electrocardiography revealed accelerated idioventricular rhythm (80-90 VPCs per minute) in two cats (Fig.1); ventricular tachycardia (3-4 VPCs in run) in two cat(Fig.2) and ventricular asytole (no ventricular rhythm, occasional P wave of normal configuration with complete AV block) in another one cat(Fig.3) . Ventricular tachycardia may occur during the period of hypoxia and is one of the most serious and potentially life threatening arrhythmias owing to its association with serious heart disease or metabolic derangements. Electrocardiographic features of the cats with cardiomyopathy vary from supraventricular tachycardia, atrial premature complexes, 1st degree AV block, left bubsle branch block , ventricular premature complex to ventricular tachycardia (Jackson, 2001). Semi quantitative estimation of cTn-I revealed elevated levels of troponin-I (0.3-1.0 ng/ml) in these cats indicating myocardial injury or cardiomyopathy (Al-Salmany,2013).

Treatment and Discussion

Treatment of ventricular arrhythmias associated with cardiomyopathy in cats remains challenging for the unproven survival benefit of antiarrhythmic therapy, the palliative nature of treatment, and the unpredictable

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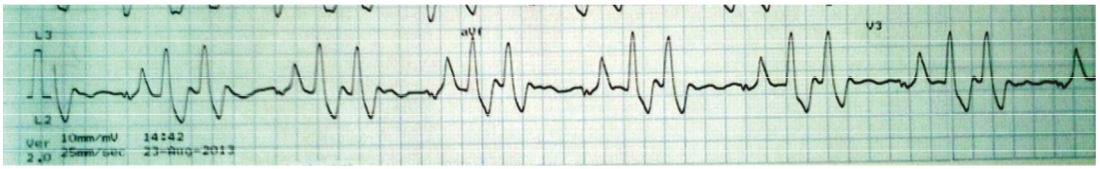

Fig.1. Electrocardiogram of a five year old cat showing VPC at the rate of 90 bpm suggesting accelarated idioventricular rhythm .

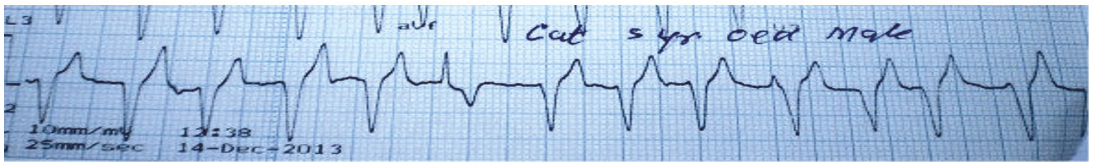

Fig.2. Electrocardiogram of a five year old cat showing ventricular tachycardia at a rate of 140 beats per minutes The sixth complex from the beginning of the strip is a capture complex..

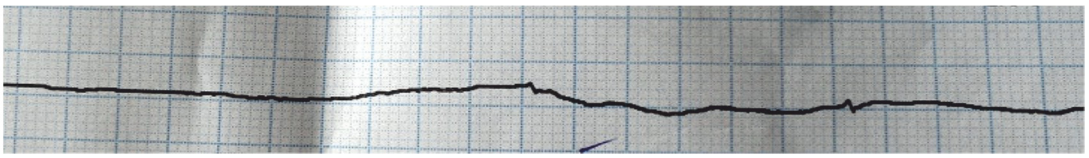

Fig.3. Ventricular asystole in a nine year old cat

Fig.4. Cardiac Troponin I test showing positive reaction band of same intensity as of control indicating cardiac damage.

occurrence of medication intolerance. Lidocaine, a drug of choice in dogs, is not routinely recommended in cats for its neurotoxic effect and causing severe sinus bradycardia and sinus arrest (Tilley and Weitz,1977).Therefore ventricular arrhythmias in these cats were managed with propranolol alongwith butorphanol (an analgesic). Nitroglycerine being a potent direct acting vasodilator facilitated reduction of preload and thus relieved life threatening pulmonary edema. Its action was further potentiated by furesomide .With this line of treatment improvement in clinical condition was observed in four cats at the end of 4th day but therapy was continued and at the end of 30 days four cats were almost normal. One cat with ventricular asystole could not be saved.

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Surgical management of crop fistula in a pigeon

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Abstract

A baby pigeon was reported with complaint of food grain draining through the open wound on lower cervical oesophageal and chest region. Clinical examination revealed crop fistula which was treated by surgical intervention with uneventful recovery.

Keywords: Crop fistula, Pigeon, Surgical management

Avian crop is a vital organ formed by dilatation of cervical oesophagus that precedes the proventriculus and gizzard and has been acts as store house for food. The crop problem occurs most frequently in the neonatal and young birds (Altman *et al.* 1997). Generally these problems referred to as sour crop, crop stasis, crop burns and crop fistula. The crop of the neonates is more fragile and susceptible to injury than the adult ingluvies (Harrison, 1987). The present case deals the surgical management of crop fistula in pigeon.

Case Presentation

A baby pigeon of weight 250 gram was reported with complaint of food grains draining through open wound on lower cervical oesophageal and chest region (Fig.1). Clinical examination revealed traumatic opening of crop with laceration of skin over the affected portion. The food grains swallowed by the pigeon were easily seen at the teared edges of the crop. The bird showed moderate dehydration and gradual weight loss.

Treatment and Discussion

The fistula was repaired under ketamine anaesthesia@30 mg/kgbwt, which was given intramuscularly in the pectoral muscles. The crop was sutured with catgut no. 2/0 suture material in simple continuous suturing pattern and the skin was sutured separately with nylon. Post – operatively, the pigeon was administered with Enrofloxacin @ 5 mg, B-complex @0.25 ml intramuscularly for 5 days and daily dressing of wounds with 5% povidone iodine. Baby pigeon recovered successfully (Fig. 2). Screening through the literature revealed that a variety of etiological factors ranging from feeding of external hot food causing thermal injuries (Billard and Cheek, 2003), food offered at an improper temperature (Mitchell and Tully, 2008) and to overfeeding (Worell, 2000) are responsible for crop fistula in baby birds. But in the present case, the pigeon was lie down on road before presented to clinic, therefore, the actual reason could not be ruled out. However, traumatic injury, feeding of hot grain and trauma caused by beak of crow might be the reason for crop fistula in baby pigeon. In present case, local anaesthesia should be avoided because of

Fig.1 Baby pigeon with crop fisula and dessicated tissues around the crop

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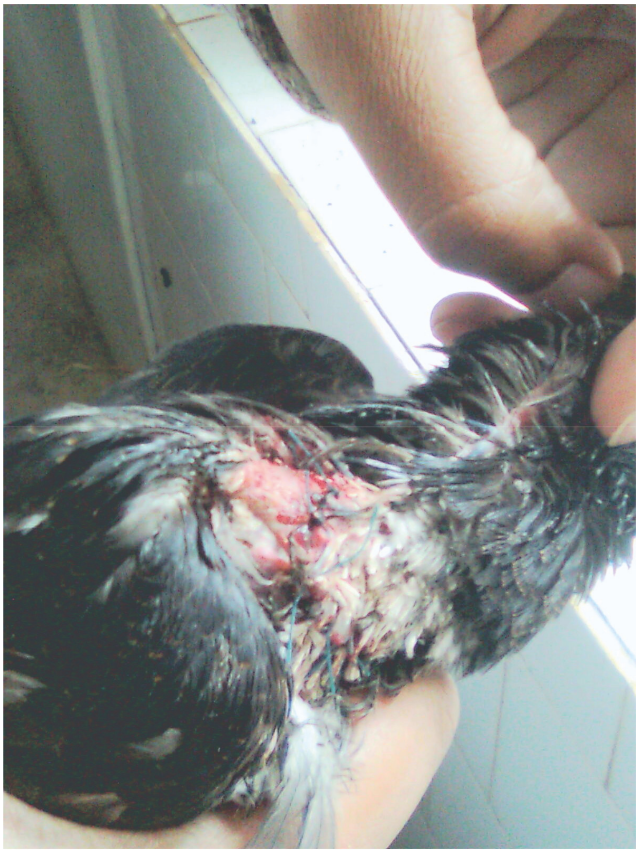

Fig. 2 Baby pigeon after surgery

drug sensitivity (Cooper, 2000). A successful surgical management has also been attempted by Choudhary et al. (2010).

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Generalized dermatitis associated with *Streptococcus Sp*, *Trichophyton Sp* and *Malassezia Sp* infection in a buffalo: A case report

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Abstract

A 4 years old buffalo was presented for generalized dermatitis and mild itching from 15 days is described in the present communication. On clinical examination there was generalized papules covered with scabs, crusts involving face, ear, neck, wither, rump, back, flank, legs, udder, inguinal and tail region. Blood sample was collected for hemogram, skin scrapings for mites and skin biopsy was collected for histopathological examination. Based on clinical and laboratory findings, the case was diagnosed as a dermatitis mix infection with Streptococcal, Trichophyton and *Malassezia*. The animal was treated with Penicillin G @ 10,000 IU/ Kg bwt i/m bid for 7 days, Phenramine maleate @ 10 ml i/m bid for 3 days and 2% ketoconazole shampoo for topical bath for next 15 days.A remarkable recovery was observed after 10 days of treatment . However, the animal recovered completely within a month.

Keywords: Buffalo, Dermatitis, Dermatophytosis, *Malassezia*

Dermatitis is a clinical condition characterized by distinct, elevated plaque of different shape and size. Most of dermatitis have characteristic skin lesions with regional distribution and which may affect productivity and comfort of the animals (Singh *et al.*, 2013). Ringworm, warts, dermatophilosis, photosensitization, drug induced hypersensitivity are common skin diseases in cattle. Few clinical reports on skin diseases are published but there is insufficient data regarding skin problems in buffaloes. The diagnosis is a challenge in bovines because the cases are often presented in chronic stage which makes characteristic skin lesions to change into secondary lesions thereby making diagnosis difficult. The present case of buffalo presented with generalized dermatitis without any classical skin lesions was investigated

Case Presentation

A four year old buffalo from the herd of 11 buffaloes was presented for generalized dermatitis and mild itching since last 15 days. Anamnesis showed no drug hypersensitivity. On clinical examination there was generalized papules covered with scab and crust involving face, ear, neck, wither, rump, back, flank, legs, udder, inguinal region and tail. Blood sample was collected for hemogram, skin scrapping for mites, hair follicles for fungal culture and skin biopsy was collected for histopathological examination. The hemogram showed neutrophilic leukocytosis with left shift (Hb- 9 gm%, TLC- 24270/µl, DLC- neutrophils-80%, lymphocytes-20%). In Serum Biochemistry there is not significant changes seen. Haemprotozoa negative in Blood smear examination. The impression smear of skin showed presence of malassezia yeast on Gram's staining (Fig 1). The culture of skin scrapping showed streptococci and Trichophyton on SDA Agar (Fig 2). The skin biopsy on histopathological examination showed superficial malassezia with deep pyogenic abscess (Fig 3). The observation was diagnosed with dermatitis with mix infection of streptococcal, Trichophyton, and malassezia.

Treatment and Discussion

On the basis of presenting complain, clinical sign, hemogram and impression smear of skin the treatment was started with inj. Penicillin-G @ 10,000 IU/kg. B.Wt I/M bid for 7 days and inj. Phenramine malate 10ml i/m bid for 3 days. There was significant improvement throughout body except on udder and tail. The animal was further treated with 2% ketoconazole shampoo for 15 days. The animal showed complete recovery within a month (Fig 4)

The clinical signs observed in the present case report are not similar to those reported in dermatophytosis in buffalo and different from the cattle. *Trichophyton verrucosum* is a cosmopolitan zoophilic species of fungi that causes ringworm in cattle and other farm animals, from which human can become infected (Weber, 2000). The isolation and morphological identification of T. verrucosum and streptococcus supported the clinical diagnosis. The back and flank

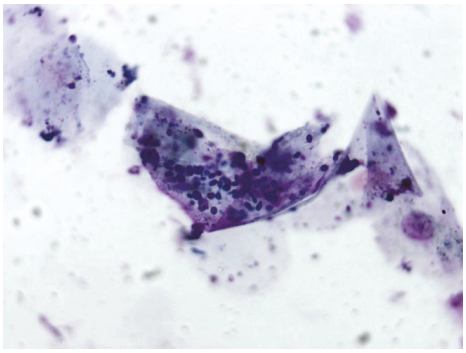

Fig 1: The impression smear of skin lesion -

Fig 2: Culture of T. verrucosum on Malassezia yeast on grams staining SDA agar

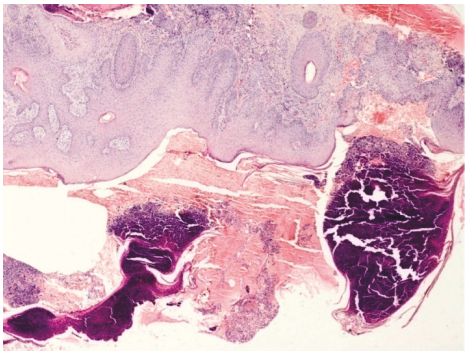

Fig 3: In Photomicrographs of skin biopsy showing focal Pyogenic reaction on H.E. 40X

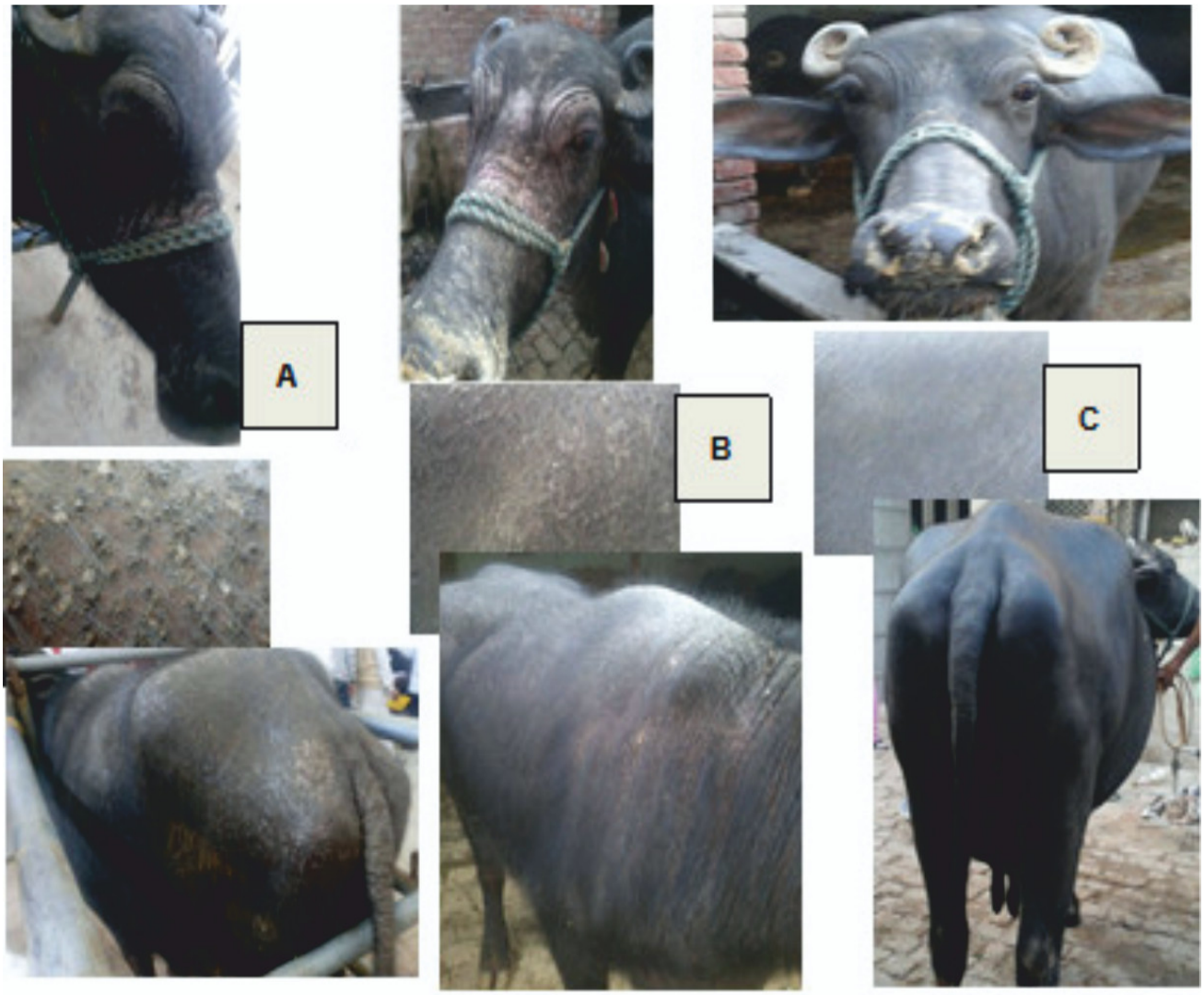

Fig 4: A showing clinical signs when animal came to clinic. B showing improvement after 10 days treatment. C showing complete recovery within a month.

lesions are more exposed than other parts of the body and making these sites more vulnerable for contact and injuries and thus predisposing to infections like *T. verrucosum* and streptococcus. Malassezia was also isolated from the skin lesion but Malassezia have been observed in cattle in significant frequencies, ranging from 16% to 33% in ear and skin samples from healthy cattle. Malassezia are common inhabitant of skin and ear canal of dog and cat (Duarte et al., 2008). There is no literature about malassezia dermatitis in buffalo. Intra-species variations in DNA pattern of *Malassezia* isolates and the presence of specific genetic types in cattle, dogs or humans were observed (Cafarchia et al., 2008). A review of genetic heterogeneity of these yeast in veterinary and human medicine studies is given considering a possible transmission animal to human or human to animal or animal to animal. The close inquiry about of locality of farm where animal were kept reveal presence of dog with skin lesions. Hence buffalo can get malassezia infection from dogs in their contact and can cause dermatitis which can be managed medically.

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Therapeutic managment of pneumonia in Turtle/Tortoise

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Abstract

Thirty one chelonians (turtle and tortoise) with respiratory distress and anorexia for variable period were diagnosed with pneumonia on the basis of clinical symptoms and radiological investigations. Their electrocardiograms revealed sinus tachycardia in almost all cases. In six cases pneumonia was also associated with swollen eye syndrome. Treatment with parenteral enrofloxacin, vitamin-A with /without eye drops (gentamicin with hydroxypropyl methyl cellulose) resulted into 54.83% (17/31) recovery in 5 to 8 days period .Chelonians having heart rate > 80 bpm could not survive despite treatment and succumbed at day 2 to day 4 post therapy.

Keywords: Chelonians, Enrofloxacin, Gasping, Lung opacity, Tachycardia, Vitamin A

Domestication of chelonians (turtle/ tortoise) in urban areas is increasing day by day. Their improper nutrition and poor husbandry practices are leading to many health problems warranting attention of the veterinarians. By virtue of anatomical peculiarities such as lack of diaphragm, 'S' shaped trachea and stress factors in domestication (dehydration, poor nutrition, parasites , and improper husbandry practices), these animals become more prone to respiratory distress. Micro-organisms commonly present in their environment (Pasteurella *testudinis*, Salmonella and Aeromonas present in turtle; Pseudomonas commonly grows in water bowels) get an easy entrance in respiratory tract of turtle/tortoise under stressed conditions leading to upper/lower respiratory tract diseases. Respiratory infections in turtles/tortoises are potentially fatal calling for an early diagnosis. Diagnosis of lower respiratory tract disease (Pneumonia) in chelonians is a difficult task in field practice. It is not known whether pneumonia in chelonians influence cardiac functioning as reported in ruminants (Radostits *et al.,* 2007; Sarita Devi *et al.,* 2015). In India reports on pneumonia in chelonians are lacking. Therefore the present investigation was undertaken to study clinical, radiographic, and electrocardiographic and treatment aspects of pneumonia in turtles.

Case Presentation

Thirty one chelonian (turtle 17, tortoise 14) of sexes (male, 23; female 08), weighing from 15g to 1100 g, with the complaint of anorexia, nasal discharge, sneezing, dullness with or without swollen eyes for 4 to 10 days formed the material for the present study. Ailing turtles were examined clinically and were subjected to ventro-dorsal and dorso-ventral radiography .Their electrocardiogram was obtained employing standard bipolar lead using Magic RX electrocardiographic machine (Maestros Mediline Systems Limited) at 20 mm= 1 mV and speed of 25 mm per second at room temperature (30-32 ^{0}C) as reported elsewhere (Varshney, 2016).

On getting radiographic evidence of pneumonia, the turtles/tortoises were treated with intramuscular enrofloxacin (@ 7.0 mg/kg body weight diluted with saline and given daily) and Vitamin –A (Cod liver oil 1 drop twice per week in food or 5000 units Vitamin-A/ kg per week intramuscularly).Turtles/tortoises with swollen eye were treated additionally with eye drops containing Gentamicin and Hydroxypropyl methyl cellulose eye drops (instilled in the eyes thrice daily). Owners were advised to keep turtles in Luke warm water (30 ^{0}C).

Results and Discussion

Respiratory tract infections in chelonian (turtles/tortoises) are potentially serious and fatal. Thirty one chelonian (turtles 17, tortoise 14) presented at the hospital with the history of anorexia, sneezing, dullness with or without swollen eyes were investigated for disease diagnosis for taking rational therapy. Clinical examination revealed lethargy, dullness, anorexia, neck stretching , open mouth breathing/gasping (Fig.1), tilted swimming /no swimming / hanging in water/, awkwardly swimming round in circles or just generally lopsided in turtles, sneezing, bubbles from nose/ in mouth arousing suspicion of lower respiratory tract infection (Fig 1). Various degree of lung opacities both focal and diffuse in radiographic examination (Fig. 2) confirmed

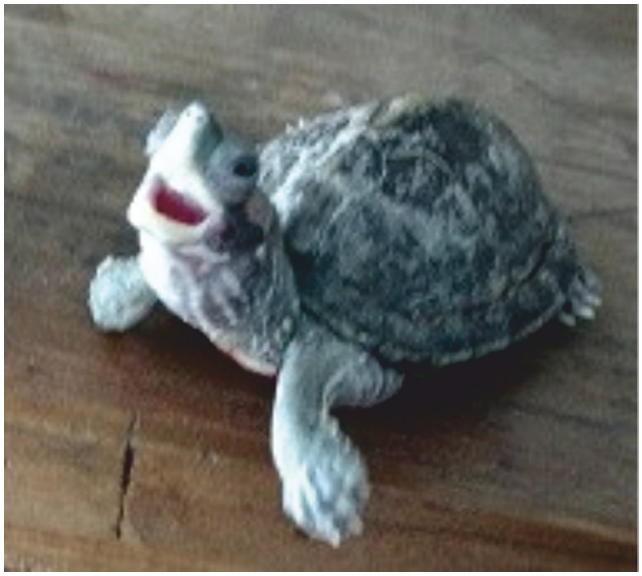

Fig.1. A female turtle showing gasping

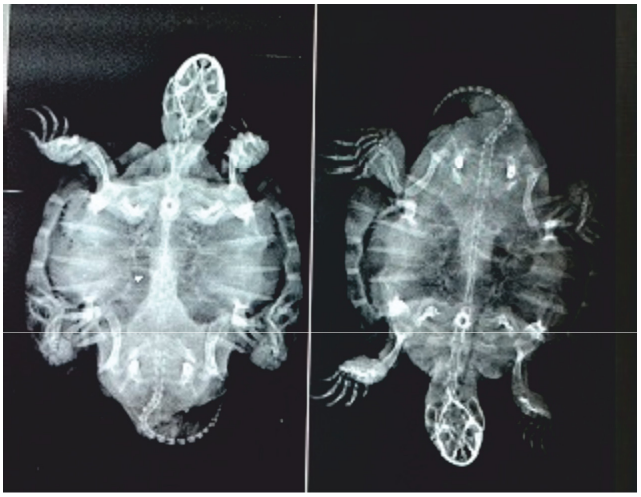

Fig.2. Left-Radiograph of a turtle showing various degree of lung opacity before treatment. Right- Radiograph of the same turtle (left side) 5th day post treatment showing marked improvement in lungs'radio-density.

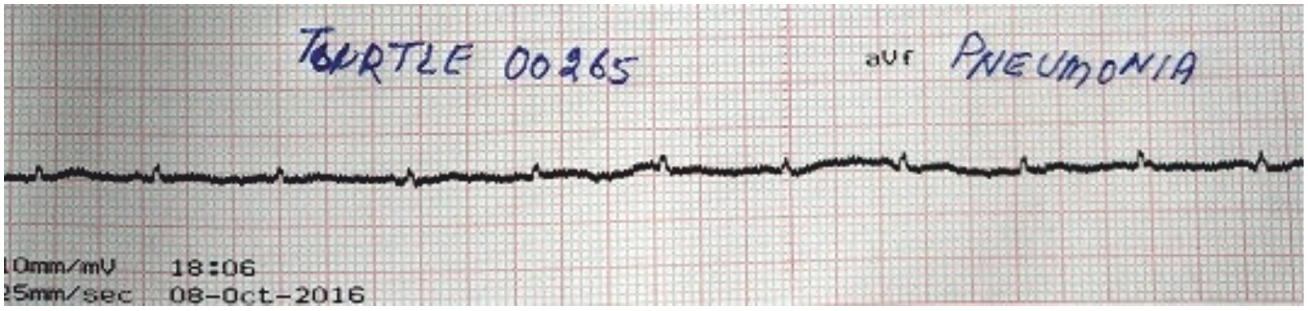

Fig.3. Electrocardiogram of a 15 year old turtle showing sinus tachycardia (heart rate -120 bpm,R wave 0.15 mv, QRS 0.04 sec, 'P' and 'T' waves not appreciable, sinus rhythm). ECG taken at 30 ^{0}C room temperature employing standard bipolar lead system and machine standardized at 10 mm=1 mV with the speed of 25 mm/second.

pneumonic changes. In four cases one lung and in rest both lungs were affected. Increase in radio-opacity of the lungs in chelonians is suggestive of more fluid in lungs, lung consolidation, inflammation or lung infection (Hernandez-Divers, 2006) as it is impossible to differentiate these conditions solely upon a radiograph. Nevertheless, radiographic assessment of the lungs in suspected respiratory problems is very valuable and effective in the disease diagnosis and prognosis. Pneumonia is a common problem in critically ill chelonians (Booner, 2000). Swollen eyes in 6 cases were suggestive of associated ophthalmic infection that complicated clinical picture. Electrocardiographic investigations revealed heart rate varying from 65 to 120 per minute in pneumonic turtles and tortoises (Fig.3) at a room temperature of 30 to 32 ^{0} C as against 32±6 reported in normal tortoise (Kharin and Shamakov, 2009). Tachycardia with sinus rhythm suggested sinus tachycardia. Hear rate of reptiles is dependent on number of variables including body size, metabolic rate, respiration rate and sensory stimulants (Davies, 1981). In ruminants tachycardia was found associated with pneumonia (Radostits *et al.,* 2007; Sarita Devi *et al*., 2015).However, such information is not available for chelonians. It appears that pneumonia accelerated pacemaker activity in the heart, thus increasing heart rate possibly due to hypoxia/anoxia and hypercapnia influencing the vagal tone as reported in dogs and cats (Tilley, 1985).

Out of 31 chelonians, 17 (54.83%) responded well to antibiotic (enrofloxacin) and vitamin-A therapy. Clinical improvement was evident from day third onward with a complete recovery in 5 to 8 days. Most of the respiratory infections in chelonians are caused by bacteria. Therefore enrofloxacin was selected for its broad spectrum anti-microbial activity and ease of one

time administration daily. The aim of supplementing vitamin A was to combat vitamin-A deficiency as respiratory infections and swollen eye syndrome are often secondary to vitamin-A deficiency. Despite the treatment, mortality was to the tune of 45.17%% (14/31).Chelonians who failed to survive had higher heart rate (>80 bpm) and succumbed at day 2 to day 4 post therapy.

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Snake bite in labrador dogs and its management

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Abstract

Three Labrador dogs were presented at the department of Veterinary Surgery & Radiology, OUAT, Bhubaneswar with suspected case of snake bite. One of the dogs was with bleeding at its chin region and another from distal fore limb along with vomition, twitching of muscles, severe salivation, abnormal gait, oedema at the site of bite, lacrimation, heavy panting with bloody stool, anurea and pale mucous membrane. The third dog was having protruded tongue with heavy swelling and cyanotic coloration at the base of the tongue, foamy salivation, pale mucous membrane, pupils dilated, dull, inactive with weak pulse and respiration. All the three dogs were immediately treated with reconstituted polyvalent anti snake venom with normal saline solution, tetanus toxoid, botropase, ceftriaxone along with meloxicam. Subsequent improvement seen after therapy in 2 dogs but the third one died during medication and after 3 days two dogs were completely recovered.

Keywords: Dog, Snake bite, Treatment

Snake bite is quite common in country like India particularly in rainy season. Quite a number of cases of snake bite have been reported in dogs **(**Garg, 2002). The pets get the snake bite while playing in the garden or an accidental encounter with the snakes. In some cases dogs eat the snakes completely or to some body parts. But any encounter with the snakes and envenomation should be considered as an emergency and rapid initiation of proper line of treatment is highly essential for the survival of the animal (Suchitra *et al*, 2010).The severity of snakebite in animals depends on the type of snake, age, size of animal, number of bites and the amount of venom injected (Palanivel *et al*., 2007). The clinical effects are more severe in small animals as compared to large animals. (Mwangi *et al*., 2014)

Most of the cases of snake bites have been reported in dogs and horses (Garg, 2002), but cat is less commonly attacked due to its greater caution and superior agility while hunting (Sukhla, 2009). A snake bite with envenomation should be treated as an emergency condition. Rapid examination, accurate diagnosis and initiation of proper treatment is highly essential in snake bite cases (Vijay, *et al,* 2001) as delayed and inadequate treatment may lead to untoward consequences (Ananda *et al*,2009). This paper reports the prompt and successful management of snake bites in two dogs which could save two precious lives and another more critical patient which could not be saved.

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Case Presentation

A 3 year female Labrador and its 8 month old male young one and another 6 year old Lab were presented in different times at the dept. of Veterinary Surgery and Radiology, OUAT with complaint of bleeding from the chin region, oedema and fluid thrill in the older one. The pup was having fang marks at the right fore limb. Both the dogs were having drooling salivation, drowsiness, gasping, bloody stool, anurea and in-coordination in gait. History revealed, a day before the dogs were seen fighting with a venomous snake in the backyard, at that time owner didn't expect that the dogs were bitten during the infighting. On the next day, dogs were showing the symptoms of snake bite; then they were brought to the department of veterinary surgery. The third one 6 years old male Labrador dog was presented with dull, inactive stage keeping mouth open, protruded and swollen tongue at the base region with cyanotic coloration. Pupils dilated and lying in lateral prostration and presented for treatment.

Physical examination revealed oedema with fang marks at the chin region with slight bleeding. In other one right fore leg site had bitten marks (Fig.1 & 2). The third dog was in more serious condition with inactive, completely dull stage, pale mucous membrane with pupils dilated, mouth open with heavy swelling at the base of tongue with blue colouration and foamy salivation (Fig. 3 & 4). The tongue was at immobile stage. The Collected blood showed without clotting for

long time and the plasma was red (Fig. 5). Based on history, symptoms and physical examination, the cases were suspected for snake bite. Vital parameters like rectal temperature, pulse and respiration of the younger one were 102p F, 48/minutes (min) and 19/min while in female dog these, were 102.8p F, 47/min and 20/min but in third one it was 101ºF, 40/min & 16/min. Further examination showed, presence of cold extremities and decreased body reflexes. Blood samples collected with and without ethylene-diamine-tetra-acetic acid for haematological parameters revealed decreased haemoglobin concentration (10.6g/dl, 11.2g/dl), packed cell volume (35%, 40%), total erythrocyte count (TEC) (3.92M/mm^3, 4.1M/mm^3) and increased total leucocyte count (TLC) (16M/mm^3, 13.5M/mm^3), Alanine aminotransferase (ALT) (84IU/dL, 92IU/dL), creatinine (2.42mg/dL, 1.93mg/dL) in male and female respectively. In the third case there was no time for further investigation of different blood parameters.

Treatment and Discussion

After diagnosis the owner was advised for rest to animals in order to reduce the flow of venom in blood circulation. The sites of bite were properly washed and cleaned with potassium permanganate lotion as per the treatment adapted by (5%) (Suchitra et al., 2010) in 2 dogs only. It was not possible to do such in the third case. The dogs were treated with polyvalent anti snake venom 10ml in 500ml of NSS, along with injection botropase 1ml I/M, cefitriazone 500mg, chlorpheniramine maleate 1ml and tetanus toxoid 1 ample intramuscularly. Only 350 ml of NSS mixed anti snake venom was administered because both of them showed dyspnoa and asphyxia. The animals were kept under observation till the symptoms get subsided. The dogs were provided with milk at night and they took some amount of it. Next morning there was remarkable change with improved condition and both of them urinated and took some amount of milk mixed with glucose. Both of them were simultaneously treated with lyophilised polyvalent anti snake venom 10ml mixed with 500ml NSS. On second day blood collected before antivenom therapy revealed, clotting near about within 45 minutes. There were symptoms like gasping and

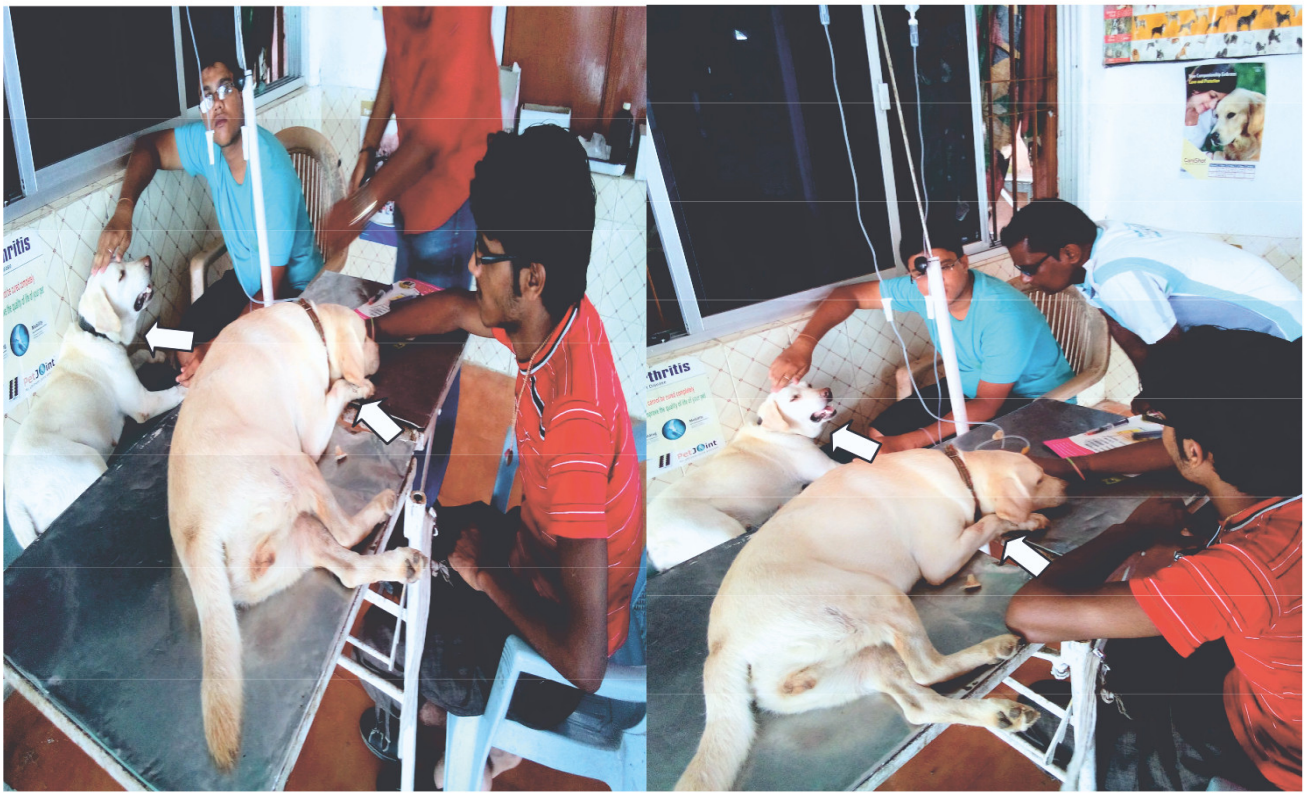

Fig. 1 & 2: showing the 2 labarador dogs one female dog and another its pup taking the NSS mixed Anti Snake Venom serum. (Arrow marks indicating the site of snake bites)

Fig 3 & 4 : Showing swollen base of the tongue along with cyanotic discoloration.

Fig. 5 showing the clotted blood along with red coloured plasma on 2nd day , 45 minutes after withdrawal)

tachycardia seen during anti-venom therapy. Antibiotic, chlorpheniramine maleate and botropase were also continued. After an interval of 3 hours again 500ml NSS mixed with 10 ml anti-venom was administered to each with half of it. On 3rd day, the symptoms were subsided and the animals started taking some amount of liquid diet and defecation with some blood clots. Injection Botropase was continued on 3rd day also. The owner was advised to provide oral iron dextran suspension (Sharkoferol Pet, Alembic) as both of them were anaemic. The alternations in the haematological parameters might be due to damage to the blood cells by snake venom (Ananda. et al.; 2009) which supports to the present report.

Results and Discussion

The increased values of alanine aminotransferase and creatinine may be due to the hepatoxic and nephrotoxic effect of snake venom (O'Shea, 2005). Polyvalent snake anti-venom was the most suitable choice, as it provides protection against venom of four (common cobra, common krait, saw scaled viper and russell's viper) species of snakes. The use of tetanus toxoid provides protection against tetanus spore that might have entered animals body from contaminated snake mouth (Sukhla,2009).The other components of snake venoms are glycoproteins, lipids and biogenic amines such as histamine, serotonin and neurotransmitters (Catecholamines and acetylcholine) (Klassen, 2008). Passing of blood in urine observed in Alsatian dog can be hypothesized to the haemotoxic effect of snake venom which may interfere with many components of the haemostatic system as observed by Wolff, (2006). Such symptoms of haematuria was observed in one of the cases attended which correlates with the report. Moreover, toxins such as the haemorrhagic or haemolysin cause spontaneous bleeding in the gingival sulci, nose, skin, and gastrointestinal tract. However, such bleeding tendencies were not noticed in either of three cases except stool with clotted blood (Haemochezia). Clinical signs such as frothy salivation, dullness, muscular weakness with abnormal gait observed in the present study can be attributed to the

enzymatic and non-enzymatic compounds in snake venom. According to Klassen (2008), the hyalurindase cleaves internal glycoside bonds, in certain acid muco-polysaccharides resulted decreased viscosity of connective tissue allowing other fractions of venom to penetrate tissues. The cyanotic edema observed at the site of bite may be attributed due to enzyme hyalurindase which act as spreading factor.

The clinical features viz.; salivation, haematuria, incordination, pale mucous membrane, cold extremities and vomiting tendencies which were observed in these cases correlates with the observations of other workers (Hungerford, 1990; Palanivel et al., 2007) Clinical signs varies due to different types of snake, their venom composition, depending upon the site of bite and amount of venoms injected etc. (Nicholson, 1995). Intravascular hemolysis may be the cause of hemoglobinurea. Lal et.al., (1998) and Nicholson (1995) have also reported the hemolytic action of venom and hemoglobinurea that simulates to our present report. Moreover, Lysolecithin formed in the tissue by venom may also produce hemolysis. The leukocytosis observed in this study corroborate to the observation of earlier workers (Lal et.al., 1998; Vijay kumar et al., 2001; Singh, 2002). Increased leucocytes count is attributed to systemic infection as snake fangs and oral cavity has bacterial contaminants (Blaylock, 2001). The increased biochemical values like alanine aminotransferase and creatinine may be due to the hepatotoxic and nephrotoxic effect of snake venom (O'Shea, 2005). Since identification of snake was not possible, hence polyvalent snake venom was preferred for the treatment. All the time dogs were treated with polyvalent anti snake venom, corticosteroids, broad-spectrum antibiotics, haemocoagulase and also by fluid therapy. Polyvalent antisnake venom, tetanus toxoid and broad spectrum antibiotics were recommended by Bailey et al, (1992) and Nicholson, (1995). In the present cases, adrenaline along with corticosteroids and antihistamine were used for managing the possible anaphylaxis condition. The use of steroids in snake bites is contraindicated however, their use was only advocated in severe allergic reactions (Lal et.al., 1998). The anaphylactic reactions may be observed occasionally as the antivenom were derived from hyper immunized horse serum contains concentrated purified immunoglobins which may lead to immediate or delayed immune reaction in some cases (Jain, 1986). Antihistamines, hydrocortisone and adrenaline can be given to counter anaphylactic reactions (Lal et.al., 1998). The use of antihistamine for the treatment of snake bite is of general practice but its use is contraindicated as it potentiates the toxic action of venom (Singh, 2002). With the prompt treatment of above therapies to precious lives of two dogs could be saved in the reported paper, but in 3rd case since it was bitten at the tongue which is very vascular and nearer to the brain and presented in more serious condition so it could not be saved.

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ISVM Awards and Rules

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

General rules applicable to all the awards:-

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

1. SHRI RAM LAL AGRAWAL GOLD MEDAL

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

2. INTAS YOUNG SCIENTIST AWARD

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

3. DR. D.C. BLOOD GOLD MEDAL

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

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

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

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

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

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

6. DR. G.N. DUTTA MEMORIAL AWARD

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

7. P. K. DAS GOLD MEDAL

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

8. AWARD OF FELLOWSHIP OF ISVM (FISVM)

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

9. FIELD VETERINARIAN AWARD

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

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

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

this purpose.

10. ISVM MERIT AWARD FOR POST GRADUATE RESEARCH:

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

11. BEST CLINCAL ARTICLE AWARD

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

12. BEST RESEARCH ARTICLE AWARD

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

13. ISVM APPRECIATION AWARD

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

Award Application procedure

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

Remark Note:

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

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

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

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

GENERAL GUIDELINES FOR CONTRIBUTORS

The *Indian J. Vet. Med*. is published twice in a year, June and December. It contains review articles (guest), original/applied research articles, clinical observations, preliminary re-ports of scientific studies and short communications on Veterinary Medicine and Animal Health. In addition, the journal also publishes Letters to the Editor, Tips to Vets and other relevant information's.

The manuscripts are accepted on the basis of scientific importance and suitability for publication on the understanding that they have not been published, submitted or accepted for publication elsewhere wholly or partly in any language. All authors are jointly and severally responsible to the various authorities for the contents of the articles. The Editorial board shall in any case not be held responsible in any manner whatsoever to the contents of the article and the views and interpretations expressed by the authors in the articles.

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Calory	cal	Meter	m
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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

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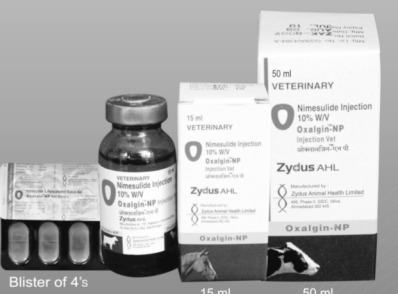

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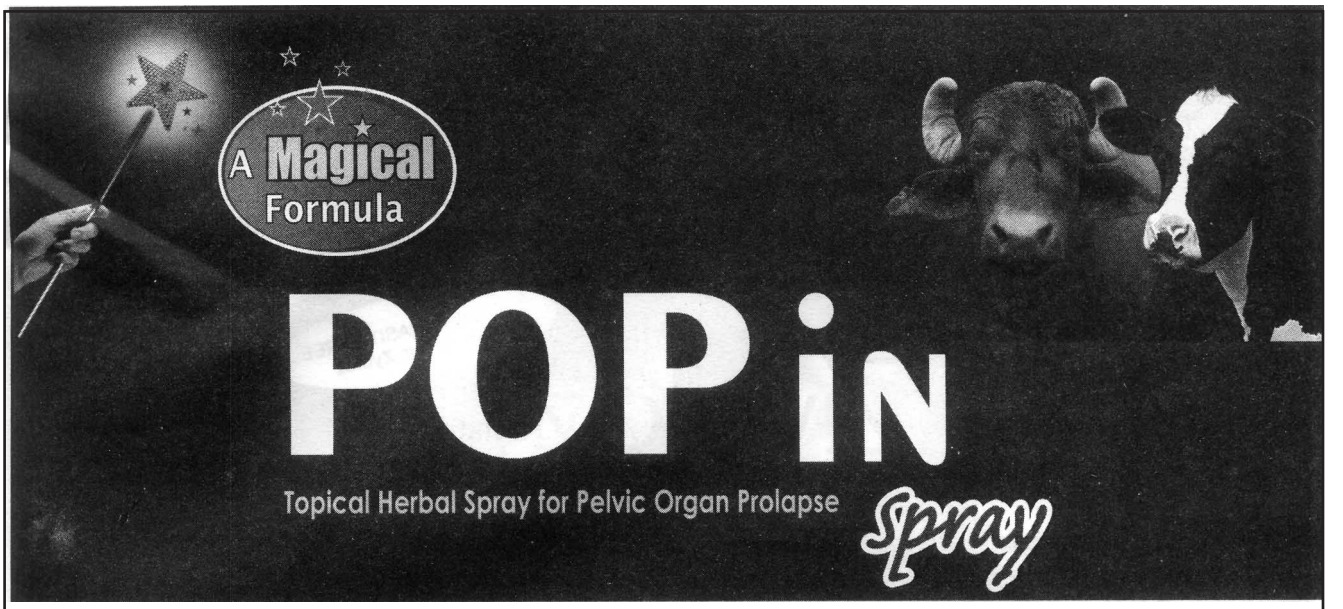

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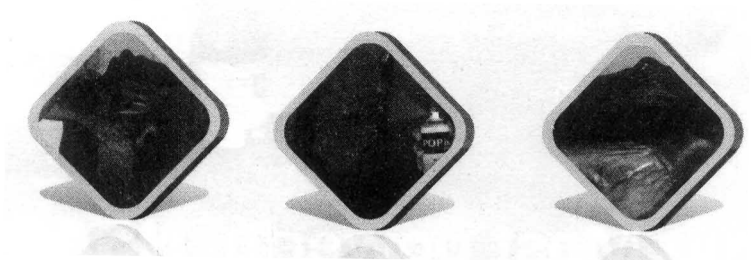

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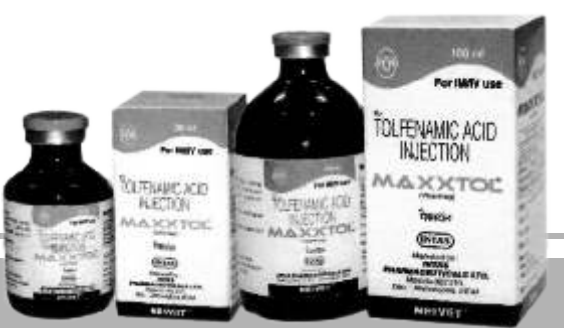

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