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Contents

Contents

Clinical Articles

Indian J. Vet. Med. Vol. 36, No. 2, 2016 pp. 81-86

Research Article

Benzimidazole resistance in sheep flock of an organised farm at Hisar, Haryana

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Abstract

Frequent failure of benzimidazoles (BZ) to control *Haemonchus contortus* in an organised sheep farm at Hisar has led to study the efficacy of drug by egg hatch test (EHT) and allele specific PCR (AS PCR). EHT revealed arithmetic mean and range of ED_{50} value 0.420 µg/ml and 0.213-1.646 µg/ml, respectively. The genotyping of 50 *H. contortus* larvae by AS PCR recorded 38% homozygous resistant (rr), 56% homozygous susceptible (SS) and 6% heterozygous (rS). The gene/allele frequency for 'r' was 0.41 and for 'S' 0.59 in the farm. The results indicated that the benzimidazole resistance level reached alarming level in the farm. For effective management of gastrointestinal nematodiasis in small ruminants regular evaluation of anthelmintic efficacy and monitoring of anthelmintic resistance are needed.

Keywords: Allele specific PCR, Beta-tubulin, Benzimidazole resistance, Egg hatch test, *Haemonchus contortus*.

Introduction

Haemonchus contortus is one of the economically important gastrointestinal nematodes of small ruminants (Sood, 1981; Sykes, 1994) and anthelmintic treatment has been the major mode to control this parasitic infestation. Frequent and improper use of anthelmintics led to emergence of resistant parasites particularly against BZ (Waller, 1997). Benzimidazoles are believed to exert their effect on the parasite by binding with the β-tubulin protein and prevent its polymerization into microtubules (Lacey, 1988). It is proved that the BZ resistance in trichostrongylid nematodes is primarily related with the substitution of phenylalanine (Phe, TTC) by tyrosine (Tyr, TAC) at positions 200 (Kwa *et al.,* 1994, 1995) and 167 (Silvestre and Cabaret, 2002) and a rare mutation at position 198 of the β-tubulin isotype-1 gene where glutamate (GAA) is replaced by alanine (GCA) (Ghisi *et al.,* 2007).

In India, the prevalence of BZ resistance has been reported from many parts of the country, most of them were based on conventional methods and few reports were also based on molecular methods with varying results (Sankar, 2007; Garg and Yadav, 2009; Rialch *et al*., 2014; Chandra *et al.,* 2015). Since using anthelmintics is only option to control nematodes, it is important to maintain the efficacy of currently available anthelmintics where resistance has not emerged and also prevent further selection of resistance where it has already reached alarming level. On this line, the present study was designed to investigate BZ resistance in *H. contortus* from an organised sheep farm at Hisar by *In vitro* egg hatch test (EHT) and allele specific polymerase chain reaction.

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Material and Methods

This study was conducted in an organised sheep farm located at Hisar, Haryana, India. Adequate measures were taken to minimize pain or discomfort to animals while taking samples per-rectally. Third stage larvae (L_3) of *Haemonchus* were obtained from coproculture of sheep faeces and identified as per morphological keys (Van Wyk and Mayhew, 2013).

Genomic DNA extraction from larvae: The protocol for isolation of genomic DNA described by Coles *et al.* (2006) was adopted for larvae with minor modification. Briefly, larvae were exsheathed by incubating in Sodium hypochlorite (aqueous solution, 4% active Chlorine) for 5-20 minutes. The exsheathed larvae were washed in distilled water and single larva was separated into PCR tubes with 2 µl of suspension. DNA was extracted by adding 5 µl digestion buffer (1 mM Tris-HCl, 0.1 mM EDTA and 5mg/ml proteinase K). These larvae were incubated overnight at 41ºC. Proteinase K was inactivated by incubation at 95ºC for 20 minutes. The lysate of single larvae was used as template in primary PCR.

Polymerase Chain Reaction (PCR): The oligonucleotide primers (Table.1) and the preparation of reaction mixture was followed as per Silvestre and Humbert (2000) and Coles *et al.* (2006). The PCR mixture consisted of 5 µl of lysate as template, 10 pmoles

of each primer (P1 and P2), 1.5mM $MgCl_2$, 80µM of each dNTPs and 1 U of Taq DNA polymerase (Promega, USA). The reaction was performed in 20 cycles at 57ºC for annealing. The β-tubulin PCR amplicons were used as templates for nested PCR with same condition as primary PCR and subsequently nested PCR amplicons were digested with the *Rsa*I restriction enzyme for accurate species identification. Allele specific PCR was performed using nested PCR product as templates for diagnosis of BZ resistance using *H. contortus* specific primers in two reactions per larval sample, one for resistant and another for susceptible (Coles *et al*., 2006). The reaction was performed in 33 cycles at 55ºC for annealing. The amplicons were separated by 2.0% agarose gel electrophoresis. The Chi-square test was used to statistically analyse the genotyping of *H. contortus* resistant and susceptible larva.

Egg Hatch Test (EHT) was carried out as described by Le Jambre (1976) with minor modifications (Coles *et al*., 2006). Faecal samples were collected rectally and were stored anaerobically (Hunt and Taylor, 1989). The samples were processed within 7 days of collection. Analytical grade thiabendazole from Sigma, USA was used in the present study. A stock solution of thiabendazole is prepared by 10 mg pure thiabendazole in 10 ml of 3% dimethylsulphoxide (DMSO). The final concentrations of thiabendazole used were 0.0125, 0.02, 0.05, 0.1, 0.2, 0.3, 0.5 and 1 µg/ml for all the samples. The tests were carried out with 3 replicates plus control well and were repeated thrice. Minimum of 200 eggs were added in each well. The samples were incubated at 27ºC for 48 hours. After incubation one drop of Lugole's iodine was added and counted free larvae and eggs under binocular stereomicroscope and the percentage of hatched larvae and ED_{50} value for the eggs was calculated by log probit analysis.

Result

Identification of *Haemonchus* larvae: The length of the larvae was 625 to 750 µm. The intestinal cells were rectangular in shape and usually 16 in number (Figure 1). Based on morphology and PCR-RFLP, it was found that 88% larvae were *H. contortus* followed by *Trichostrongylus* species and *Strongyloides* species. PCR-RFLP of *H. contortus* larvae showed three fragments at 440 bp, 190 bp, and 150 bp (Figure 3).

Amplification of β-tubulin gene of *H. contortus*: Nested PCR amplified approximately 820 bp product (Figure 2). In Allele specific PCR the size of the specific bands were susceptible allele specific gene at 603 bp, resistant allele specific gene at 222 bp and non allele specific gene at 750 bp (Figure 4).

Arithmetic mean and range of ED_{50} value were 0.420 µg/ml and 0.213-1.646 µg/ml, respectively in EHT (Table 3). The present EHT data suggest that *Haemonchus* of this farm is resistant to thiabendazole as ED_{50} was calculated as 0.420 µg/ml, which was well above than the value (0.1µg/ml) recommended by World Association for Advancement of Veterinary Parasitology (WAAVP).

The results of larval genotyping for BZ resistance using AS-PCR are shown in Table 2. A total of 50 *H. contortus* infective larvae were genotyped. The results indicated that 38% of *H. contortus* were homozygous resistant (rr, TAC), 56% homozygous susceptible (SS, TTC) and 6% heterozygous (rS, TAC/TTC) (Fig. 4). The results showed resistant level reached alarming level. The gene/allele frequency for 'r' was 0.41 (41%) and for 'S' 0.59 (59%) in the farm.

Table 1: Oligonucleotide Primers

Primers	Sequence (5 C-3 C)
Primary PCR	
Forward	P_1 – 5' GGC AAA TAT GTC CCA CGT GC 3'
Reverse	P_2 - 5' GAA GCG CGA TAC GCT TGA GC 3'
Nested PCR	
Forward	P_3 - 5' GTG CTG TTC TTG TTG ATC TC 3'
Reverse	P_4 - 5' GAT CAG CAT TCA GCT GTC CA 3'
Allele specific PCR	
Non allele specific forward	5'GGA ACG ATG GAC TCC TTT CG 3'
Non allele specific reverse	5' GGG AAT CGA AGG CAG GTC GT 3'
Susceptible allele	5' ATA CAG AGC TTC GTT GTC AAT ACA GA 3'
Resistant allele	5' CTG GTA GAG AAC ACC GAT GAA ACA TA 3'

Table 2: Genotyping of *H. contortus* larvae

Location	Number of larvae	Genotype Frequency			Allele Frequency	
		Homozygous resistant (rr)	Homozygous susceptible (SS)	Heterozygous (r)	Resistant (S)	Susceptible (rS)
Organised Sheep Farm, Hisar	50	19 (0.38)	28(0.58)	03 (0.06)	0.41	0.59

Table 3: ED_{50} (µg/ml) of thiabendazole in EHT and resistance status

Location	ED_{50}	Lower limit	Upper limit	Resistance status
Organised Sheep Farm, Hisar	0.420	0.213	1.646	R

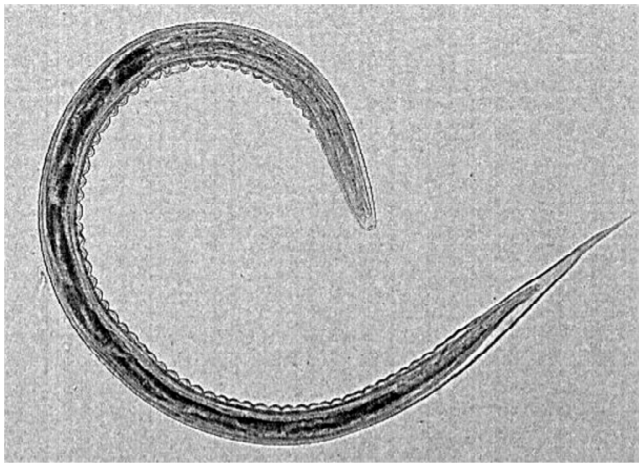

Fig 1: L_3 larvae of *Haemonchus contortus* showing kinked tail and rectangular intestinal cells.

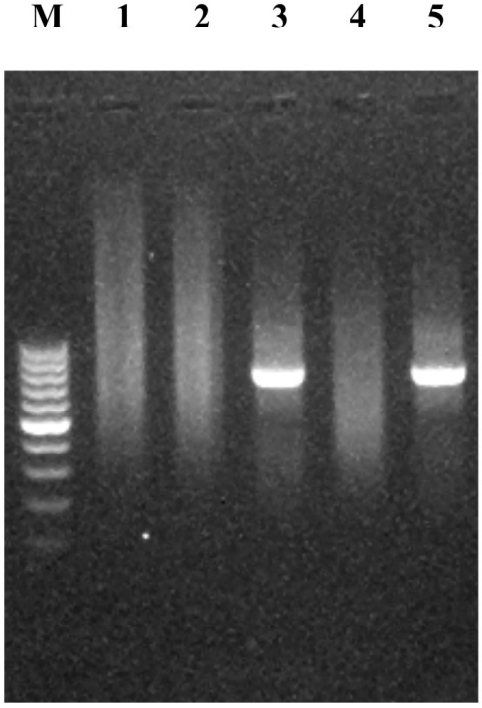

Fig. 2: Nested PCR amplification
Lane M: 100 bp marker, Lanes 3 &5: *H. contortus*

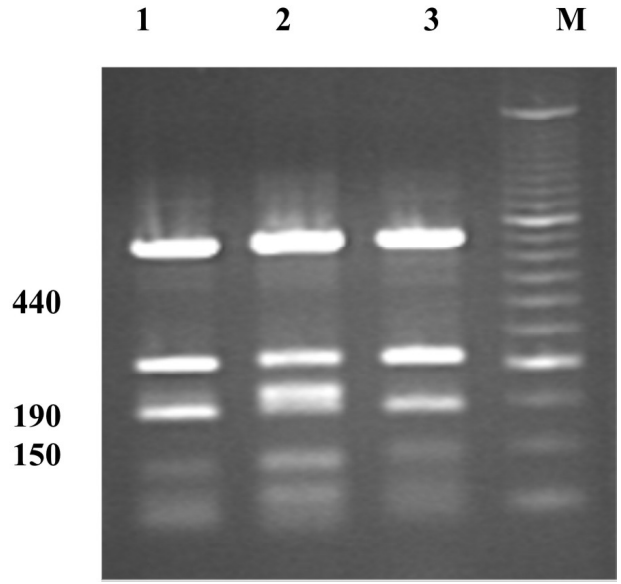

Fig. 3: RFLP with *RsaI* enzyme
Lane M: 50 bp DNA ladder
Lane 1-3: *H. contortus* 440, 190 and 150 bp

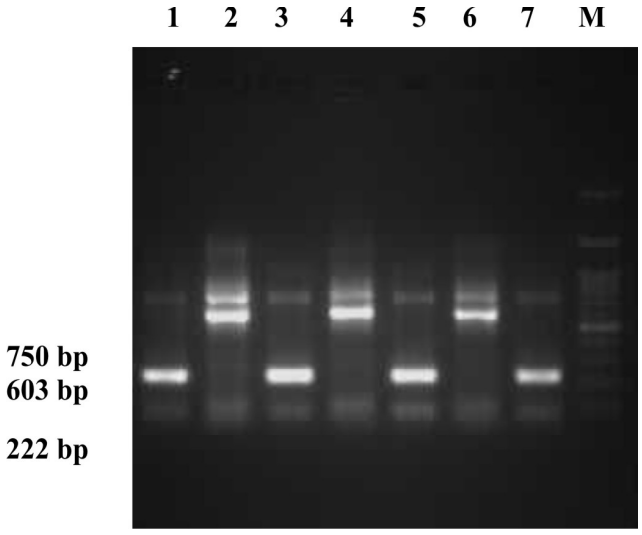

Fig. 4: Allele specific PCR of *Haemonchus contortus*
Lane M: 100 bp marker, Lanes 1, 3, 5 & 7: Resistant
Lanes 2, 4 & 6: Susceptible

Discussion

Resistance to anthelmintics amongst nematode parasites of small ruminats has now reached alarming proportions and threatens profitable small ruminant production in many countries (Fleming *et al*., 2006; Larsen, 2006). Among anthelmintics, benzimidazoles are widely used due to its broad therapeutic ratio and index. BZs have been regularly used in India for controlling GI nematodes in small ruminants (Gill, 1996) since few decades. However, potency of these drugs to provide the expected degree of control. During the present study *H. contortus* larval (L_3) were genotyped based on β-tubulin isotype1 for studying genetic basis of BZ resistance. In nested PCR, the amplified product was of 820 bp. The nested PCR-RFLP results confirmed the larvae were *H. contortus,* which was commensurate with earlier studies (Chandra *et al*., 2015). The results of larval morphology and PCR-RFLP showed that *H. contortus* was predominant species with lesser prevalence of *Trichostrongylus* spp., and *S. papillosus*.

The EHT is recommended by WAAVP (Coles *et al.,* 2006) and is the most widely used *In vitro* method for BZ resistance detection in sheep and goats population (Varady *et al.,* 2006). The EHT is based on the ability of eggs of resistant populations of GIN to embryonate and hatch in higher concentrations of BZ than eggs from susceptible populations of GIN (Coles *et al.,* 2006). The results are interpreted using ED50 or ED99 values (drug concentrations producing 50% and 99% inhibition of hatching in the test, respectively). However, this test detects BZ resistance only if >25% resistant worms are present in a population (Martin *et al.,* 1989). The delineation dose of 0.1µg/ml of TBZ in EHT provides a good estimate of genotypic resistance (Cudekova *et al.,* 2010). BZ resistance was also detected in Uttarakhand by conventional methods (Laha *et al.,* 1999; Rialch *et al.,* 2013, 2014).

AS-PCR results showed susceptibility to BZ as 603 bp and resistance specific gene of 222 bp and non-specific gene at 750 bp. Our results confirmed by these observation on larval population for detection of point mutation involved in BZ resistance (Sankar, 2007; Garg and Yadav, 2009; Chandra *et al*., 2015).

In the present study, BZ resistance was detected by both *In vitro* method, EHT as well as molecular test, AS-PCR. Both these methods revealed presence of BZ resistance in organised sheep farm. It could be due to continuous use of BZ compounds at the farm. Probability rotation of anthelmintic is not being followed at the farm.

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The authors thank Indian Council of Agricultural Research, New Delhi for financial assistance under the project of All India Network Programme on Gastrointestinal Parasitism (AINP on GIP). Thanks are also expressed to the Director and Joint Director (Research), ICAR-IVRI, for providing necessary facilities.

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Evaluation of serum total bile acids and ultrasonographic changes in histologically confirmed cases of chronic hepatitis in dogs

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Abstract

Chronic hepatitis is a frustrating disease with inevitable progression to cirrhosis and thus has a poor prognosis. Definitive diagnosis of this condition invariably requires liver biopsy. The aim of the present study was to determine the total bile acid values and ultrasonographic changes in histologically confirmed cases of chronic hepatitis in dogs. The parameters studied in clinical cases were serum biochemistry including total bile acids, liver biopsy and ultrasonographic changes. The incidence of chronic hepatitis in dogs was 0.25%. Based on the histopathological findings cases were grouped into four categories viz., chronic progressive hepatitis, chronic nonspecific hepatitis, chronic cholangio-hepatitis and liver cirrhosis. Liver enzymes like alanine amino trasferase and Alkaline Phosphatase were significantly (Pd"0.05) higher and Gamma Glutamyltransferase was highly significantly (Pd"0.001) elevated when compare to the healthy control. Total bile acids were elevated without statistical significance (Pd"0.05). On ultrasound examination, nine animals revealed hyper echogenicity, one showed partial hyperechogenicity and another one had irregular border. Our finding proves that ultrasonographic examination cannot be used as a sole method to diagnose chronic hepatitis in dogs; however, it can be used as a reliable tool for preliminary screening. Chronic hepatitis can better diagnosed when ultrasonography is combined with serum biochemical analysis especially the serum total bile acid values and liver biopsy.

Keywords: Bileacids, Chronic progressive hepatitis, Chronic cholangio hepatitis, Liver cirrhosis and Ultrasonography.

Introduction

Canine chronic hepatitis (CH) is characterised histologically by the presence of hepatocellular apoptosis or necrosis, a variable mononuclear or mixed inflammatory infiltrate, regeneration and fibrosis (Van den Ingh *et al.,* 2006).Chronic hepatitis is often perceived as a frustrating disease with inevitable progression to cirrhosis and thus has a poor prognosis (Watson, 2004). The specific cause and pathogenesis are unknown. The diagnosis of chronic hepatitis should be made after a documented rise of alanine amino transferase (ALT) for a minimum of 4 months, with concomitant histological evidence of liver damage (Fuentealba *et al.,* 1997 and Sterczer *et al.,* 2001). The combined measurement of alkaline phosphatase (ALP/ SAP) and serum bile acid (SBA) is very sensitive and specific for detecting liver diseases and bile acid estimation could be used for screening chronic hepatitis (Twedt 1998; Sterczer *et al*., 2001). Measurement of bile acids should be considered the first line test to assess liver function in a non jaundiced dog (Bexfield and Watson, 2006). The liver biopsy is always required for definitive diagnosis (Rutgers and Haywood, 1988; Twedt, 1998; Sterczer *et al.,* 2001). Different nomenclatures have been used to classify chronic hepatitis by different authors on varying time. However, Sevelius (1995) has classified chronic hepatitis into four groups based on histopathological lesions viz., chronic progressive hepatitis, chronic nonspecific hepatitis, chronic cholangio hepatitis and liver cirrhosis. Ultrasonography is an extremely useful tool in the investigation of hepatobiliary disease (Vijayakumar *et al*., 2011). The liver size will be reduced in chronic hepatitis and cirrhosis, appear hypoechoic in nodular hyperplasia and is hyper echoic in hepatitis and fibrosis (Bexfield and Watson, 2006). There are few published reports on ultrasonographic changes and total bile acid values in histologically confirmed cases of dogs. The purpose of this study was to find out the ultrasonographic and total bile acid changes in hoistologically confirmed cases of chronic hepatitis in dogs.

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Materials and Methods

The sick dogs brought to small animal out patient unit at Madras Veterinary College, Chennai with clinical signs suggestive of liver disease were screened by following diagnostic tests: 1. Serum biochemistry – ALT, ALP, GGT, total and direct bilirubin, total protein and albumin, glucose, cholesterol, blood urea nitrogen (BUN), creatinine and total bile acids; 2. Liver biopsy and 3. Ultrasonographic findings. Six animals brought from the Chennai city police for routine health check-up acted as healthy control. The dogs were maintained

under prescribed diet and routine health care activities. The health status was analyzed by their records and physical and haematological examination.

The serum biochemical parameters were analyzed by semi auto analyzer through commercial kits. The total bile acids were analyzed by enzymatic technique using 3 ?-hydroxy bile acids (Randox Laboratories, UK). The procedure was followed as per the manufacturer's guidelines. Blind percutaneous transabdominal technique as described by Center (1996a) was followed and 14G true-cut disposable biopsy needle was used. In few cases, necropsy samples were collected because of owner's inconvenience or failure of biopsy attempts at treatment or owing to the end stage of disease. The samples were collected and stored in 10% formalin and processed routinely. The changes were read under light microscope. Based on the histopathological lesions, animals were classified into four groups as per the guidelines of Sevelius (1995).

Statistical Analysis

One way ANOVA was used to compare the means of serum biochemical changes by SPSS software version 13.

Results and Discussion

During the period of study, a total of 15026 cases were presented to the small animal clinics of Madras Veterinary College Hospital, out of which 39 cases were found to have chronic hepatitis accounting for an incidence of 0.25%. Out of 39 animals, 30 had histopathology report from biopsy and/or necropsy specimens. In rest of the cases, tissue samples could not be collected mostly due to the owner's non compliance for undertaking biopsy or necropsy. The histopathological changes in chronic progressive hepatitis were focal necrosis of hepatocyts with neutrophil infiltration (Fig. 2), degeneration of hepatocytes with fibrous tissue proliferation (Fig. 3) and portal fibrosis (Fig. 4). In chronic cholangio hepatitis, the lesions were periportal fibrosis (Fig. 5) and cholestasis (Fig. 6). The liver cirrhosis had nodular regeneration (Fig. 8) and cirrhosis i.e., regenerative nodules of hepatocytes are surrounded by fibrous connective tissue that bridges between portal tracts (Figs. 8&9). The chronic nonspecific hepatitis had periportal infiltration with mononuclear cells (Fig. 7).

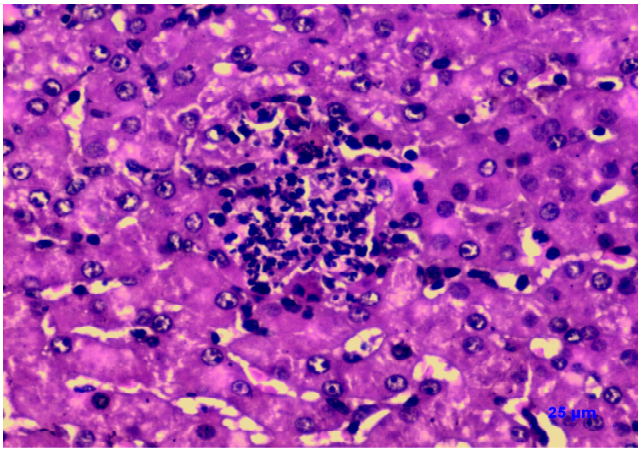

Fig. 2 Section of liver shows focal necrosis with neutrophil infiltration (H&E Bar 25 mcm)

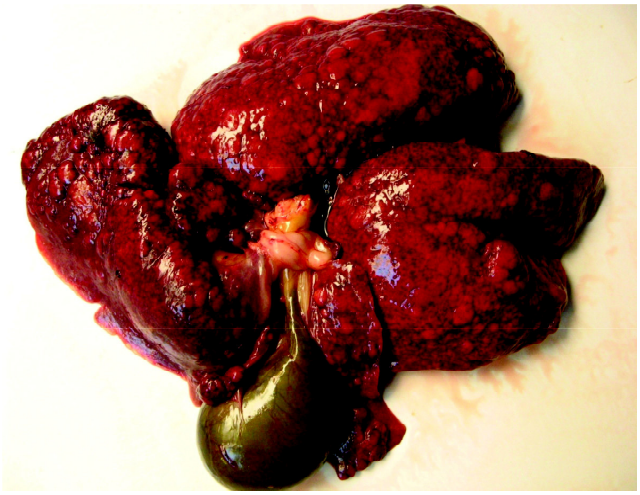

Fig. 1 Cirrhotic liver shows with numerous nodules and shrunken appearance

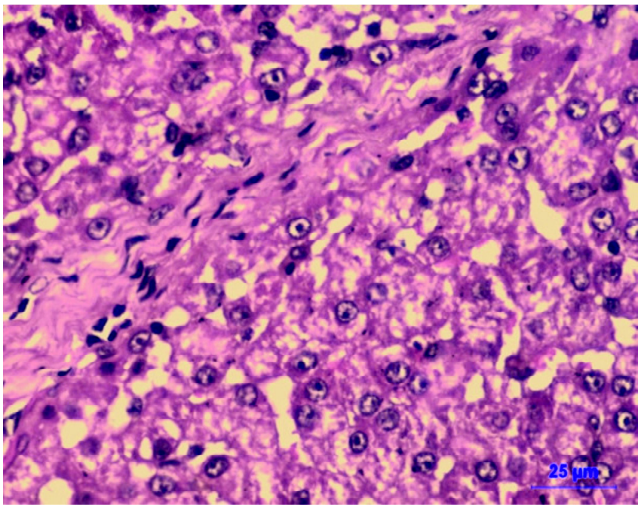

Fig. 3 Section of liver shows degeneration of hepatocytes with fibrosis (H&E Bar 25 mcm)

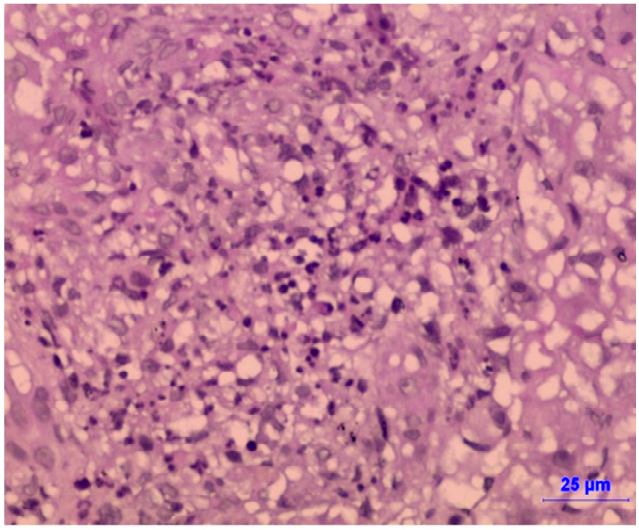

Fig. 4 Histopathological section of liver showing portal fibrosis (H&E Bar 25 mcm)

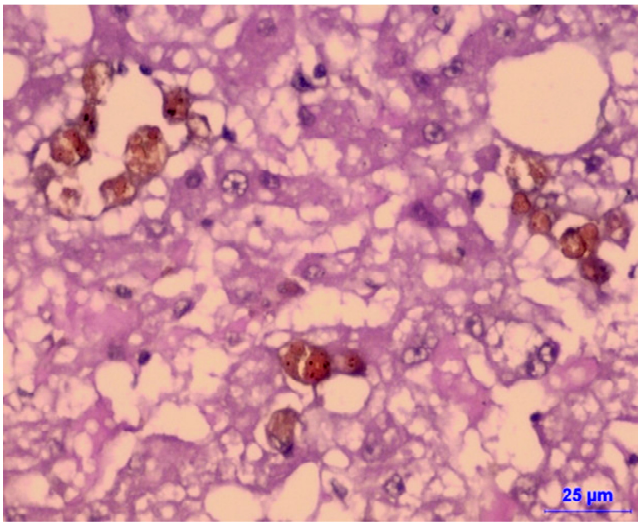

Fig. 6 Histopathological section of liver showing cholestasis (H&E Bar 25 mcm)

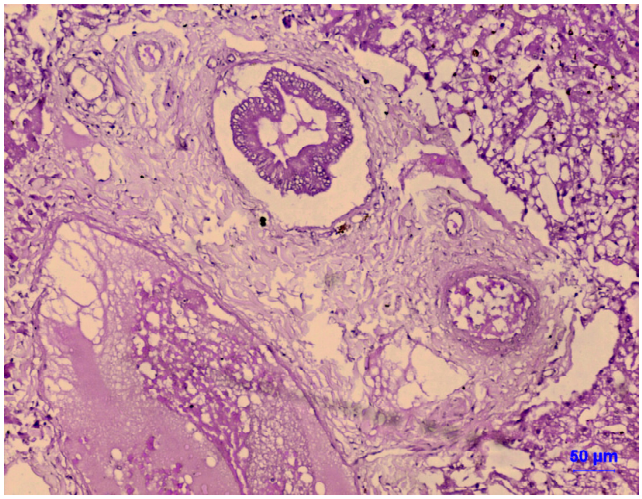

Fig. 5 Histopathological section of liver showing periportal fibrosis (H&E Bar 25 mcm)

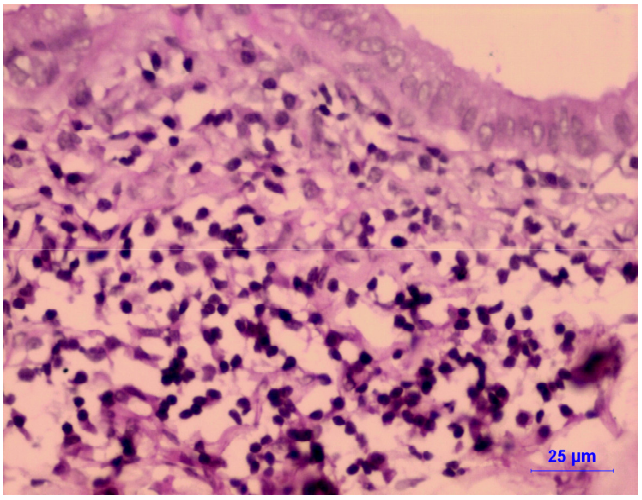

Fig. 7 Histopathological section of liver showing periportal mononuclear infiltration (H&E Bar 25 mcm)

Table 1. Serum biochemical changes (mean ± S.E.) in canine with different types of chronic hepatitis

Parameters	Health, Control (n = 10)	Chronic Progressive hepatitis (n = 13)	Chronic Cholangio hepatitis (n= 7)	Liver Cirrhosis (n=9)	F – Value
ALT (U/L)	$30.77^{a} \pm 3.30$	$152.47^{b} \pm 31.81$	$162.42^{b} \pm 29.4$	$114.56^{b} \pm 15.79$	4.62^{*}
SAP (U/L)	$92.21^{a} \pm 7.53$	$288.93^{b} \pm 62.10$	$471.08^{b} \pm 116.12$	$364.88^{b} \pm 87.35$	4.54^{*}
GGT (U/L)	$3.81^{a} \pm 0.42$	$12.14^{b} \pm 2.35$	$23.31^{c} \pm 4.34$	$15.95^{bc} \pm 3.06$	8.31^{**}
Total Bilirubin (mg/dl)	$0.45^{a} \pm 0.03$	$1.42^{b} \pm 0.21$	$2.38^{c} \pm 0.33$	$1.41^{b} \pm 0.24$	11.15^{**}
Direct Bilirubin (mg/dl)	$0.28^{a} \pm 0.02$	$0.98^{bc} \pm 0.18$	$1.43^{c} \pm 0.23$	$0.85^{b} \pm 0.18$	6.77^{*}
Total Bile acid (µmol/L)	11.25 ± 1.74	26.35 ± 4.89	124.83 ± 63.09	105.59 ± 61.66	2.15
Total Protein (gm/dl)	$7.2^{b} \pm 0.14$	$6.39^{ab} \pm 0.30$	$5.88^{a} \pm 0.25$	$6.06^{a} \pm 0.37$	3.76^{*}
Albumin (gm/dl)	$3.27^{b} \pm 0.10$	$2.15^{a} \pm 0.09$	$2.15^{a} \pm 0.15$	$2.03^{a} \pm 0.18$	19.58^{**}
Globulin (gm/dl)	4.03 ± 0.14	4.15 ± 0.31	3.87 ± 0.28	4.09 ± 0.32	0.172
BUN (mg/dl)	21.13 ± 1.14	35.83 ± 3.97	22.42 ± 5.61	29.02 ± 9.00	1.79
Creatinine (mg/dl)	$0.95^{ab} \pm 0.07$	$1.22^{b} \pm 0.10$	$0.96^{ab} \pm 0.13$	$0.62^{a} \pm 0.16$	4.51^{*}
Glucose (mg/dl)	105.60 ± 3.48	99.31 ± 3.71	105.00 ± 6.16	94.2 ± 3.62	1.55
Cholesterol (mg/dl)	221.42 ± 12.09	229.53 ± 17.74	241.07 ± 32.2	221.21 ± 24.69	0.161

*denotes mean values differ Significantly($p<0.05$); ** denotes mean values differ significantly($p<0.001$); Mean bearing the different superscript in the same row differ significantly.

Based on the histopathological report, the incidence of chronic progressive hepatitis was recorded in 43.34% (13 cases), chronic cholangio- hepatitis 23.33% (7 cases), liver cirrhosis 30% (9 cases) and chronic non specific hepatitis 3.33% (1 case). The mean serum biochemical values in control and chronic hepatitis groups are presented in Table 1. The mean serum ALT, SAP and direct bilirubin values of chronic progressive hepatitis, chronic cholangiohepatitis and liver cirrhosis groups showed significant (P<0.05) increase i.e., 4-5 times elevation when compare to helathy animal control. The enzyme values were comparatively higher in chronic cholangiohepatitis than liver cirrhosis and chronic progressive hepatitis. There was a highly significant (Pd"0.001) increase in the GGT and total bilirubin values of all the three chronic hepaitits groups when compared to control group. The type of bilirubin was direct in all cases.

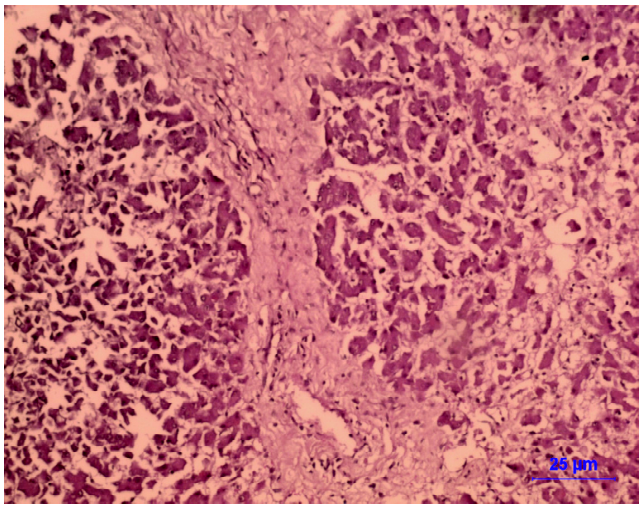

Fig. 8 Section of liver shows nodular hyperplasia of hepatocytes surrounded by fibrous tissue septa (H&E Bar 25 mcm)

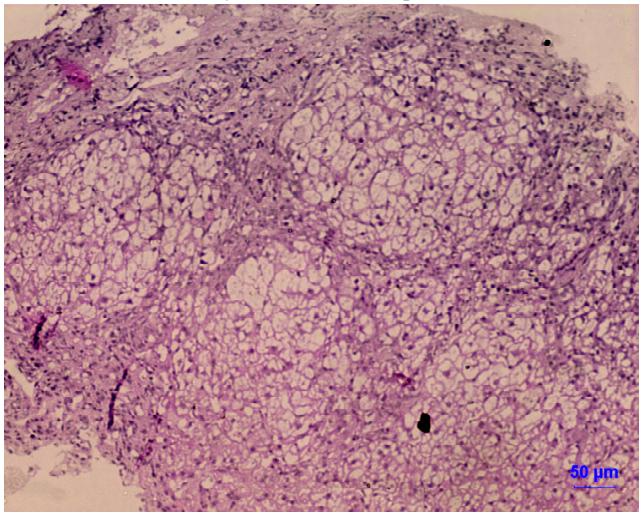

Fig. 9 Section of liver shows regenerative nodules of hepatocytes are surrounded by fibrous connective tissue that bridges between portal tracts (H&E Bar 50 mcm)

Total bile acids concentration in control group of this study was 11.25 ± 1.74 µmol/L. The present observation concurred with Sevelius (1995) who reported the reference value of 12.63 ± 7.38 µmol/L. A non-significant increase in serum total bile acids was present in all types of chronic hepatitis. Significant (Pd"0.05) hypoproteinemia and highly significant (Pd"0.001) decrease in albumin values were noticed in chronic hepatitis when compared to the control. Other biochemical parameters like globulin, BUN, creatinine, glucose and cholesterol showed insignificant differences in comparison to their respective control values.

The ultrasonographic features of control dogs were well in agreement with classical ultraosnographic

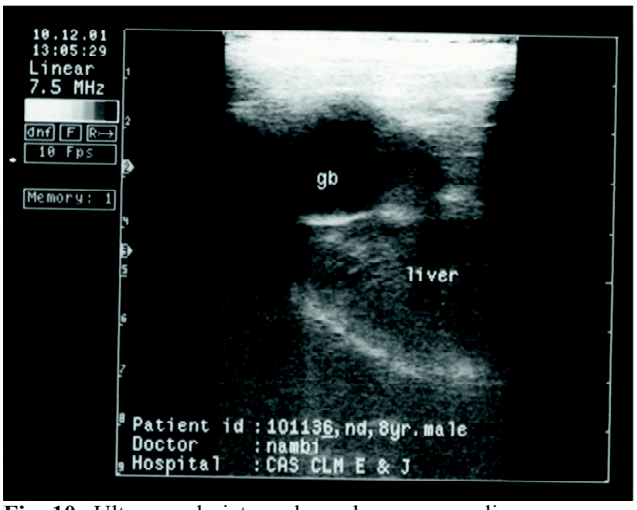

Fig. 10 Ultrasound picture shows homogenous liver

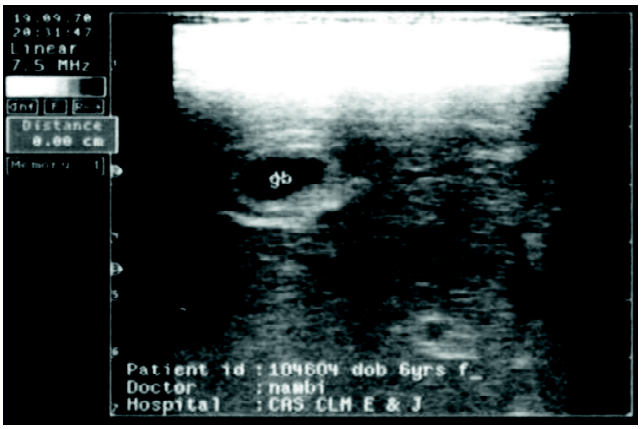

Fig. 11 Ultrasound picture shows double wall appearance of gall bladder

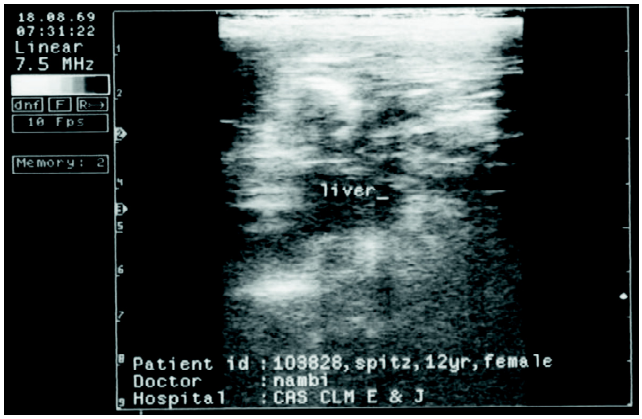

Fig. 12 Ultrasound picture shows diffuse hyperechoic liver

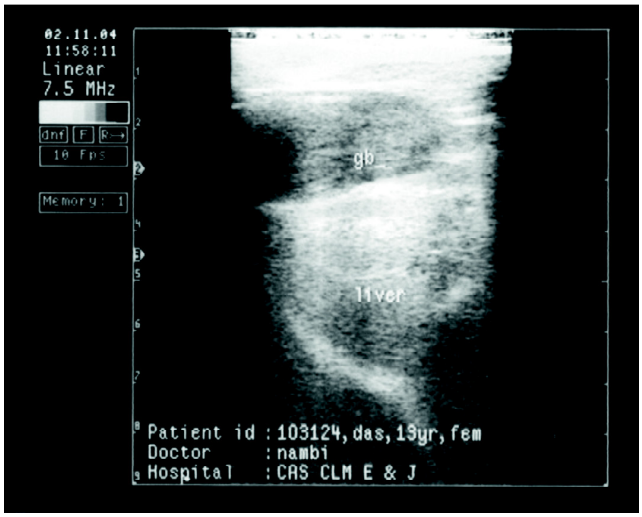

Fig. 13 Ultrasound picture shows partial hyperechoic liver

features of normal liver i.e., uniform coarsely granular echotexture with visible portal vein and pear shaped, anechoic vesicular gall bladder and associated structures as described by Nyland *et al*. (2002). Ultraosnography was done in all the histologically confirmed cases of chronic hepatitis. The predominant ultrasonographic changes noticed in chronic hepatitis cases were homogenous liver in 12 cases (40%) (Fig 10), diffuse hyperechoic liver in 6 cases (20%) (Fig 12) and ascites in 6 cases (20%) (Fig 14). Other changes included thickened gall bladder wall in 2 cases (6.67%), sludge in gall bladder (double wall appear) (Fig 11), partial hyper echogenicity (Fig 13), homogenous liver with ascites (Fig 14) and irregular liver borders in one case each (3.33% each) . Boomkens and coworkers (2004) reported that approximately 1% of referred population of their clinic for companion animals suffered from hepatitis and Nambi (1993) recorded 0.47% incidence of hepatitis in the dogs attending Madras Veterinary

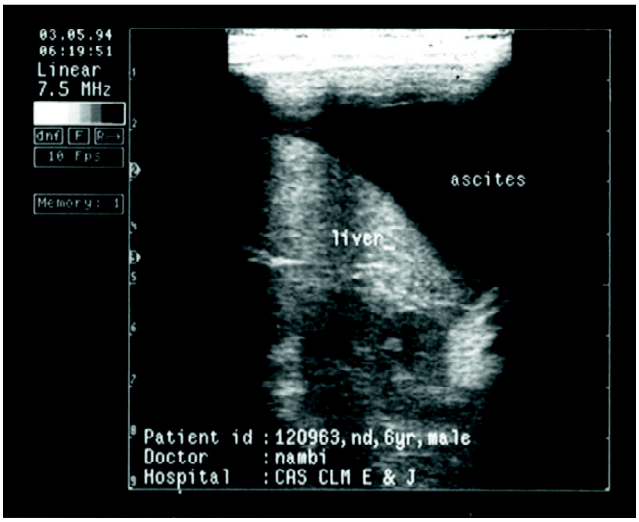

Fig. 14 Ultrasound picture shows homogenous liver with ascites

College hospital through a study spread across three years. Fuentealba et al. (1997) recorded 0.7% of chornic hepatitis in a retrospective study. The incidence in our study was 0.25% which is more or less similar to findings in identical studies.

Serum biochemistry was characterized by elevated alanine aminotransferase (ALT) activity consistent with the ongoing hepatocellular damage (Sterczer *et al*., 2001). Plasma ALP activities increase in clinical patients with hepatic necrosis and chronic hepatitis although not consistently; the increase that develop is usually 3-6 times the upper normal limit (Center, 1996a). Van den Ingh *et al*. (1988) reported the median value of 23 U/L and range 13-42 U/L for GGT in 8 female Doberman dogs with chronic active hepatits. Sevelius (1995) reported that significantly higher ALT, SAP and GGT concentrations in chronic cholangiohepatitis than in dogs with other liver disease viz. chronic progressive hepatitis and liver cirrhosis. The comparatively higher values of enzymes in chronic cholangiohepatitis in this study were comparable to Sevelius (1995) and others. Vijayakumar *et al.* (2011) reported that chronic hepatitis animals had high bilirubin values than other hepatic disorders in dogs. Shih *et al*. (2007) reported that the median elevation of total bilirubin in chronic hepatitis was 0.65 mg/dl with the range of 0.45-27.30 mg/dl. Van den Ingh *et al.* (1988) reported in a study of eight Dobermann dogs that total bilirubin was elevated in all the cases and it was mainly of the direct type (median 80% and range 70-88%).

A non-significant increase in serum total bile acids was present in all types of chronic hepatitis. Two animals, one each in chronic cholangiohepatitis and liver cirrhosis showed high values viz. 395.73 and 584.32 µmol/L, which probably led to high standard error and thus statistical insignificance. The reason might be due to the development of porto-systemic shunt in these animals. Center *et al*. (1991) reported that the range of serum bile acids in dogs with chronic hepatitis was 0 to >250µmol/L. Sevelius (1995) observed that the mean fasting serum bileacid concentrations were elevated in chronic cholangiohepatitis (189.89 ± 143.67 µmol/L) and they were significantly higher than in dogs with other types of chronic inflammatory liver diseases. It has been observed that total bile acids were very much increased in chronic cholangiohepatitis and liver cirrhosis than in chronic progressive hepatitis. Center (1996a) reported that diseases associated with cholestasis resulted in increased serum bile acid concentrations owing to reduced bile acid excretion and regurgitation of bile acids into the systemic circulation. Vijayakumar *et al*. (2011) reported that chronic hepatitis animals had significantly low level of albumin values than other hepatopathies. Our findings are in accordance with earlier report.

Bexfield *et al*. (2011) reported that changes in hepatic parenchymal echogenicity included hypoechogenicity (n=12), hyperechogenicity (n=10) or a combination of hypo- and hyperechogenicity (n=40) when compared to echogenicity of the spleen in a retrospective study on 68 english Springer spaniels. Six dogs had a normal appearance to the liver on ultrasound. An abdominal effusion was present in 27 dogs.Vijayakumar *et al*. (2011) reported that chronic hepatitis dogs had diffuse hyperechogenic parenchyma with less distinct portal vessels associated with peritoneal fluid accumulation. The findings were in concurrence with the reports of Shih *et al*. (2007). In their observations out of 21 dogs changes in hepatic echogenicity were present in 16 dogs and included inhomogenous (8), hypoechoic (5) and hyperechoic (3) changes. Nine dogs had one or more nodules in the liver. Abnormal liver size was noted in 6 dogs and 2 dogs revealed abdominal effusion Our findings are in partial agreement with Shih *et al*. (2007) and Vijayakumar *et al*. (2011), because only six cases showed diffuse hyperechogenicity one with partial hyper echogenicity and another one with irregular border in our study. This may be due to the individual animal's disease stage. Nyland and Mattoon (2002) reported that increase in hepatic echogenicity was due to fibrosis in liver. Further, ultrasonographic diagnosis is not equivalent to histological diagnosis in liver disorders (Bexfield and Watson, 2006).

Conclusion

From the finding of our study it can be concluded that ultrasonographic examination cannot be used as a sole method to diagnose the chronic hepatitis in dogs however it can be used for preliminary screening. The liver enzymes like ALT, SAP and GGT were elevated 4-5 times in comparison to control group in all the types of chronic hepatitis. Elevation of serum total bile acid was recorded in all types of chornic hepatitis without any statistical significance. Chronic hepatitis can be better diagnosed when ultrasonography is combined with serum biochemical analysis, especially serum total bile acid values and liver biopsy.

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Mastitis in cows and buffalo of Kangra valley of Himachal Pradesh: an epidemiological study

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Abstract

Overall incidence of mastitis among cattle and buffaloes was found to be 12.83 per cent in cases presented to Veterinary Teaching hospital and 6.94 per cent in University livestock Farm, Palampur (H.P.). Sub-acute mastitis was most frequent than acute and per-acute form of mastitis. Higher incidence of mastitis was recorded in crossbred cows, high yielders (14 litre/day and above) and in the early stage of lactation. The incidence of mastitis was more in older cattle which were in 4th lactation onwards. In cows, maximum incidence of mastitis was observed in rainy season while in buffalo it was highest in autumn season. The incidence of mastitis was almost same in fore quarters and in hind quarters. Left side quarters were found to be more prone to mastitis as compared top right side quarters.

Keywords: Bovine mastitis, Incidence, Kangra valley of Himachal Pradesh,

Mastitis is a disease of economic importance in bovine and economic losses due to mastitis in India have been reported as high as Rs.28093.20 million per annum of which.76.55 per cent losses are attributable to sub-clinical mastitis. Sub-clinical mastitis is by far the more costly disease in most of the dairy herds. The treatment of mastitis has also become costly due to multiple etiologic agent of mastitis and this is no successful vaccine for Prevention of the disease. Even after treatment, many cases develop fibrosis of the udder leading to permanent loss in milk production. Rearing of such animal become uneconomical and one of the reason for culling of the valuable animals before they complete their full productive lives.

Some of the earlier reports have indicated the higher prevalence rate of sub-clinical mastitis in cows and buffalo (Sharma and Rai, 1977; Singh and Baxi, 1980). Sub-clinical mastitis though remains undetected but causes 10.0 to 25.0 per cent loss in milk production (Radostits *et al.,* 2007). The incidence and causative agent of mastitis varies from region to region. More over emergence of microbial resistance in different parts of the country warrants continuous research and surveillance for harnessing this disease. Therefore, the present study was conducted during January, 2009 to March, 2010 on cows and buffalo suffering from clinical as well sub-clinical mastitis, to ascertain the incidence of bovine mastitis in relation to various factors in Kangra Valley of Himachal Pradesh.

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Materials and Methods

All the lactating animals brought to the College Veterinary Clinics, Palampur were screened for mastitis using different direct and indirect tests as suggested by Radostits *et al.* (2007). The milk samples were streaked on blood agar media for isolation and identification of bacteria (Cruickshank *et al.,* 1975). The cases found positive were included in the study. Detailed history of these animals included age, breed, lactation stage, parity (i.e. number of lactation), milk yield duration of this condition, quarters involved and their sides were undertaken. These animals were further subjected to physical examination and clinical examination i.e. Swelling, pain hotness, integrity, lesions and injuries on teats. Rectal temperature, heart rate, respiration rate and other systemic parameters were also recorded.

Results and Discussion

Incidence : Out of 296 Lactating cows and buffaloes presented to College Veterinary Clinics, 38 (12.83%) were found positive for mastitis & in University Livestock Farm out of 317 lactating cows, 22 (6.94%) cases were of mastitis. A varying incidence of mastitis in and Palampur has been reported by different workers viz., 8.0 per cent (Sharma *et al.,* 1993); 19.9 per cent (Pal *et al.*, 1994) and 6.70 per cent (Sharma and Prasad, 2003). In table 1 the incidence of various types of mastitis is presented. On the basis of

animals affected, the maximum incidence of mastitis was observed in the sub-acute from (46.67%) followed by acute (18.33%), sub-clinical (16.67), chronic (15.00%) , and per-acute (3.34%), whereas on the basis of quarters affected the highest incidence was observed in sub-acute (44.90%) followed by acute (18.33%), Sub-clinical *and* chronic (16.32% each) and per-acute (4.09%). In cases of cows, highest incidence was of sub-acute (50.00%) whereas in case of buffaloes, chronic mastitis was most prevalent (50.00%) followed by acute (33.33%) and sub-acute (16.67%). On the contrary, on quarter basis sub-acute (48.84%) mastitis was higher as compared to sub-clinical (18.60%) in cows, whereas in case of buffaloes, chronic mastitis was most prevalent (50.00%) followed by acute (33.33%) and sub-acute (16.67%). The findings of present study are not in agreement with above mentioned workers. The reasons for difference may be attributed to variation in the number of cases studied, the techniques followed, geographical variations, different husbandry practices and seasonal variation followed, in this part of Himachal Pradesh.

Breed of animal : The Table 2 summarized breed related incidence of mastitis in lactating cows and buffalo. It was observed that cross-breds (75.00%) were the most susceptible to mastitis as compared to zebu/ non-descript (18.33%) and pure-bred (6.67%). All the buffaloes included in this study were zebu/non-descript. The higher susceptibility of crossbred might be due to production stress, as during peak lactation almost 80% of net energy is utilized towards this purpose leading to lower immunity. However, higher incidence of mastitis in case of indigenous animals has been reported by other workers (Sharma, 2001; Peer *et al.*, (2009) which is in contrast to the present findings. This may be attributed to rearing of more number of cross-bred dairy animals in and around Kangra valley as compared to pure bred.

Age : The Table 3 summarizes the detailed relationship between age of the animal and the occurrence of mastitis in cows and buffaloes. The incidence of mastitis was maximum in age group of above 8 years in cows (56.26%) and buffaloes (33.33%) and the overall was incidence was 56.66 per cent. A linear trend of mastitis with increasing age was observed. As the increased, the incidence of mastitis also increased, the incidence of mastitis also increased. The studies of various researchers have revealed that there was loosening of teat sphincter along with increased patency of teat canal. Hence, the chances of mastitis by ascending infection might be increased by manifolds (Smith and Coetzee, 1978; Ghosh *et al.*, 2004).

The increased incidence of mastitis was observed with the increase with the increase in number of lactation up to 4th lactation. However, in the 5th lactation and above if was slightly decreased. In the lactation 4th the overall incidence (28.34%) in 5th lactation and above, 3rd lactation (16.66%), 2nd lactation (11.67%) and 1st lactation (5.00%). The Table 4 revealed the relationship between the occurrence of mastitis and the number of lactation. The incidence of mastitis increased as the lactation progressed and it attained a highest proportion during third and forth lactations when the cows produce more milk than all other lactations. This might be possible that as the number of lactation increases, the udder become more pendulous and more prone to infection (Sexena, 1990). Busato *et. al,*. 2000 has also reported similar trend to the result of present study.

Effect on milk yield : The overall incidence of mastitis in relation to milk yield in the group of maximum milk production (14 litre/day and above) was the highest (30.00%) as compared to low yielders. The detailed results have been shown in Table 5. Miltenburg *et. al*. (1996) also reported that the incidence was more in high yielders. The stress of over producing might have potentiated the onset of mastitis in high milk producing cattle.

Season : Table 6 summarized month/season related incidence of mastitis lactating animals. It was observed that rainy season (40.00%) was more susceptible to mastitis as compared to autumn (26.66), summer (25.00%) and winter (8.34%) (Singh *et al.*, 1996; Mukherjee and Dash, 2003; Singh and Pachauri, 2004). This might be due to the reasons that during these months, the floors of the cow shed also remain wet. The teats also become moist, which favours microbial growth and colonization.

Stage of lactation : Table 7 summarize incidence of mastitis *vis-à-vis* stage of lactation. It revealed that the animals in early lactation (i.e. 1 month to below 3 months) were more prone to mastitis and the incidence was 60.00%) followed by late (i.e. 6 months *&* above and the incidence was 23.33%) and mid (i.e. between 3 months to below 6 months and the incidence

was 16.67%) stage of lactation. The findings of present study are in simulation with earlier findings of other workers (Pal *et al.,* 1994; Ghosh *et al*., 2004; Palanivel *et al.,* 2008; Peer *et al*., 2009). However, Dakshinkar *et al.* (1999) observed higher incidence of mastitis at 4-month stage of lactation (i.e. early stage) followed by 3-month stage of lactation (i.e. early stage) which is contradictory to the result of present study. Oliver and Calvinho (1995) indicated that bovine udder appears to be markedly susceptible to new inflammatory infections during physiologic transition of the mammary gland from lactation to involution. Since milk production remains high in early lactation, chance of transmitting infection to under is also high as milk production remains high as milk is a favourable media for bacterial growth and multiplication. The mammary gland is more susceptible to new infection during early and late dry period, which might be due to the presence of udder

Table 1: Incidence of mastitis in cows and buffalo

S.No.	Type	Cows (n=54)	Quarters affected (n=86)	Buffaloes (n=6)	Quarters affected (n=12)	Total number of cows & buffaloes (n=60)	Total quarter affected (n=98)
1	Per-acute	2(3.72)	4(4.66)	0(0.00)	0(0.00)	2(3.34)	4(4.09)
2	Acute	(9.(16.66)	14(16.27)	2(33.33)	4(33.33)	11(18.33)	18(18.36)
3	Sub-acute	27.(50.00)	42(48.84)	1(16.67)	2(16.67.)	28(46.67)	44(44.90)
4	Chronic	6(11.11)	10(11.63)	3(50.00)	6(50.00)	91(15.00)	16(16.32)
5	Sub-clinical	10(18.51)	16(18.60)	0(0.00)	0(0.00)	10(16.67)	16(16.32)
	Total	4(100.00)	86(100.00)	6(100.00)	12(100.00)	60(100.00)	98(100.00)

Figures in parentheses indicated per cent affected.

Table 2: Incidence of mastitis in relation to breeds

S.No.	Breed	Cows (n=54)	Buffaloes (n=6)	Total (n=60)
1	Pure	4(7.40)	0(0.00)	4(6.67)
2	Crossbred	45(83.34)	0(0.00)	45(75.00)
3	Zebu/ Nondescript	5(9.26)	6(100.00)	11(18.33)
	Total	54(100.00)	6(100.00)	60(100.00)

Figures in parentheses indicate per cent affected.

Table 3: Incidence of mastitis in relation to age

S.No.	Age	Cows (n=54)	Buffaloes (n=6)	Total (n=60)
1	< 5 years	4(7.40)	2(33.34)	6(10.00)
2	5-8 years	18(33.34)	2(33.33)	20(33.34)
3	>8 years	32(59.26)	2(33.33)	34(56.66)
	Total	54(100.00)	6(100.00)	60(100.00)

Figures in parentheses indicate per cent affected.

Table 4: Incidence of mastitis in relation to number of lactation

S.No.	Parity	Cows (n=54)	Buffaloes (n=6)	Total (n=60)
1	1st lactation	2(3.70)	1(16.67)	3(5.00)
2	2nd lactation	5(9.26)	2(33.33)	7(11.67)
3	3rd lactation	10(118.52)	0(0.00)	10(16.66)
4	4th lactation	21(38.89)	2(33.34)	23(38.33)
5	5th lactation and above	16(29.63)	1(16.66)	17(28.34)
	Total	54(100.00)	6(100.00)	60(100.00)

Figures in parentheses indicate per cent affected.

washing and teat dipping, which in turn increase the number of potential pathogens on the skin of the teats (Radostits *et al, .2007)*

Location/side of quarter in relation to incidence of mastitis

The incidence of mastitis was more in fore quarters (51.02%) as compared to hind quarters (48.98%). These findings corroborate with the findings of compared of Dakshinkar *(*1999) and peer *et al.* (2009) who reported the highest incidence of mastitis associated with fore quarters. On the other hand, the results of present study are in contrast with the findings of some other workers where higher involvement of hind quarters 56.64 per cent(Smith and Coetzee, 1978), 57 % and 53.72 % have been reported, The possible reason for the higher incidence of affection in fore quarters might be that cows / buffaloes while sitting extend their fore legs and because of this the hind quarters get paddling of the muscles of hind legs whereas the fore quarters come in direct contact with the floor. Secondly, fore quarters are handled first and are liable for excessive pulling.

Left side quarters (60.20%) were found to be more prone to mastitis as compared to right side quarters (39.80%). In both cows and buffaloes, left sides quarters

Table 5 Incidence of mastitis in relation to milk yield (litre/day)

S.No.	Milk yield	Cows(n=54)	Buffaloes(n=6)	Total(n=60)
1	Below 5 litre / day	3(5.56)	2(33.33)	5(8.34)
2	5 litre /day – below 8 litre /day	8(14.81)	2(33.34)	10(16.66)
3	8 litre /day – below 11 litre /day	12(22.23)	0(0.00)	12(20.00)
4	11 litre /day – below 14 litre /day	14.(25.92)	1(16.67)	15(25.00)
5	14 litre /day and above	17(31.48)	1(16.66)	18(30.00)
	Total	54(100.00)	6(100.00)	60(100.00)

Figures in parentheses indicate per cent affected.

Table 6: Incidence of mastitis in relation to month /season

S.No.	Month/season	Cows(n=54)	Buffaloes(n=6)	Total(n=60)
1	March-May(Summer)	14(25.93)	1(16.66)	15(25.00)
2	June-August(Rainy/Monsoon)	23(42.59)	1(16.67)	24(40.00)
3	September-November(Autumn)	13(24.08)	3(50.00)	16(26.66)
4	December-February(Winter)	4(7.40)	1(16.67)	5(8.34)
	Total	54(100.00)	6(100.00)	60(100.00)

Figures in parentheses indicate per cent affected

Table 7: Incidence of mastitis in relation to stage of lactation

S.No.	Stage of lactation	Cows(n=54)	Buffaloes(n=6)	Total(n=60)
1	1 month -below 3 months (early)	32(59.26)	4(66.67)	36(60.00)
2	3 months-below 6 months (mid)	9(16.67)	1(16.66)	10(16.670
3	6 months and above(late)	13(24.07)	1(16.67)	14(23.33)
	Total	54(100.00)	6(100.00)	60(100.00)

Figures in parentheses indicate per cent affected

Table 8a: Location of quarter in relation to incidence of mastitis

S. No.	Location of quarter	Cows (n=86)	Buffaloes (n=12)	Total (n=98)
1	Fore	46(43.48)	4(33.34)	50(51.02)
2	Hind	40(46.52)	8(66.66)	48(48.98)
	Total	86(100.00)	12(100.00)	98(100.00)

Figures in parentheses indicate per cent affect

Table 8b: Side of quarter in relation to incidence of mastitis

S. No.	Side of quarter	Cows (n=86)	Buffaloes (n=12)	Total (n=98)
1	Right	35(40.70)	4(33.33)	39(39.80)
2	Left	51(59.30)	8(66.67)	59(60.20)
	Total	86(100.00)	12(100.00)	98(100.00)

Figures in parentheses indicate per cent affected.

are were more affected as compared to right side quarters. The left quarters are milked first and this could be the reason (Pal *et al., 1994*). Moreover, under village condition milking is usually done from the left side of the animal and if milker's hands are not cleaned properly organism may transmit from hand to the udder (peer *et al., 2009*). The table 8a & 8b summarize the incidence of mastitis in relation to lactation/side of quarter.

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Babesia gibsoni infection in dogs: an hospital based study

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Abstract

Dogs presented to outpatient department of medicine, IVRI over the period between April 2014 to April 2015 showed that 1.82% infection rate of *Babesia gibsoni* using PCR as diagnostic test. Incidence was higher (2.8%) in summer than winter (< 2%). Dogs over 4 years of age harbor maximum infection (3.04%) where as minimum cases were recorded in dogs less than one year old (0.89%). Among the breeds, maximum number of positive cases were found in Boxer (23.08%), followed by Dachshund (15.79%), Saint Bernard (4.65%), Dalmatian (4%), Spitz (3.85%), Doberman (3.13%), Labrador (2.33%), Rottweiler (3.05%), Mongrel (1.72%), Mastiff (1.72%), Pug (1.40%), German Shepherd (1.36%), Great dane (1.12%) and Pomerarian (0.98%), respectively. Incidence was higher in dogs (1.19%) than in bitches (0.63%).

Keywords: *Babesia gibsoni*, Dog, Polymerase chain reaction

Introduction

Canine babesiosis is a hemoprotozoan infection, caused by *Babesia canis* or *Babesia gibsoni*. *B. canis* (4 to 5 µm) is more common in the United States, especially in the Gulf Coast region. *B. gibsoni* has been recognized as an important pathogen that affects dogs in the Middle East, Africa, Asia, Europe, and many areas of the United States (Taboada and Merchant, 1991; Casapulla *et al.,* 1998). The disease is characterized by a remittent fever, progressive hemolytic anemia, hemoglobinuria, and marked splenomegaly and occasionally death (Yamane *et al.,* 1993.

Climatic conditions in India are quite favorable for the growth and multiplication of arthropods, it act as vector for many diseases of animals including canine species (Jadhav *et al.,* 2011). There is a relative paucity of studies in canine *Babesia, Ehrlichia, Anaplasma, Hepatozoon* and *haemotropic Mycoplasma* infections in India. Most cases of canine tick-borne diseases reported from the Indian subcontinent have been diagnosed by traditional methods using microscopic observation of microorganisms in stained blood smears (Megat Abd Rani *et al.,* 2010). There are sporadic reports of canine babesiosis based on conventional diagnostic methods (Sundar *et al.,* 2004; Singh *et al.,* 2012) in India. In a large study (n = 5,832) conducted in Chennai, *B. gibsoni* was reported with a prevalence of 0.1% (Sundar *et al.,* 2004) in client-owned dogs using bloods smear evaluation only. In another study reports 9% and 22% in dogs from Uttar Pradesh and Assam, respectively, infected with *Babesia,* but the species of piroplasm infecting these dogs was not reported (Chaudhuri, 2006). The pathogenicity of *Babesia* tried to vary in different regions of India and this is due to host factors and/or differences in the species present (Megat Abd Rani *et al.,* 2010). It is likely that both *Babesia vogeli* and *B. gibsoni* are co-endemic in India and the ticks *Rhipicephalus sanguineus* and *Haemaphysalis longicornis* are the putative vectors, respectively (Shaw *et al.,* 2001). True status of canine babesiosis is still not clear barring few reports employing the PCR based assays (Megat Abd Rani *et al.,* 2011; Laha *et al.,* 2014).

Despite substantial recent advances in knowledge regarding biology of *Babesia*, immunopathogenesis, diagnostic testing molecular diagnosis is still not in practice *Babesia* infections (Birkenheuer, 2004). Serological approaches also have their limitations particularly in species-specific diagnosis; both false positive (Homer *et al.,* 2000) and false negative results (Harrus and Waner, 2010) may interpretation. Therefore the present study was undertaken to diagnose *Babesia gibsoni* infection in dogs by microscopic examination and Polymerase chain reaction.

Materials and Methods

The study was carried out in Referral Veterinary Polyclinic (RVP), Indian Veterinary Research Institute (IVRI), Bareilly (UP). The dogs presented to Outpatient department of Medicine Division (OPD-Medicine) with clinical signs of tick borne hemoprotozoan diseases were enrolled for this study. Canine clinical cases showing the symptoms of fever, diarrhoea, lethargy, staggering

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gait, anaemia, debilitated condition or presence of ticks were screened for Babesiosis.

Collection of blood

Blood smear was prepared from the ear margin capillary bed, air-dried and fixed in methanol and then stained with Giemsa stain for microscopical examination of *B. gibsoni* within the RBCs. The smears were examined under 100 optical fields before declaring as negative for *Babesia* organisms.

Polymerase Chain Reaction

The blood samples (2 ml) were collected from sephanous/cephalic vein in clean dry sterilized ethylene diamine tetracetate (EDTA) from ailing dogs for molecular analysis of *Babesia gibsoni*. DNA from EDTA-treated blood (200 µL) was extracted using the Purelink Genomic DNA Mini Kit (Invitrogen).

A primer set including Gib599F (52 -CTCGGCTACTTGCCTTGTC-32) and Gib1270R (52 -GCCGAAACTGAAATAACGGC-32) was used to amplify a 670 bp fragment of the 18S rRNA gene region specific to *B. gibsoni* (Inokuma *et al.,* 2004). The PCR mix consisted of 12.5 pmol of each primer, 100 µM of each dNTP (Invitrogen), 1X PCR reaction buffer, 1.25 U Taq DNA polymerase (Invitrogen) and 5 µL DNA in a final volume of 25 µL. The cycling conditions were as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 90 s, and a final extension step of 72°C for 10 min. A negative sample control (canine blood DNA only) and a negative DNA control (Milli-Q water in a substitute of DNA) were included in the PCR reaction. The PCR products were run on 1.5% agarose gel containing ethidium bromide (Aysul *et al.,* 2013).

Results and Discussion

The ailing dogs presented to RVP, with the history of tick infestation, recurrent fever, prolonged illness and unresponsive to routine treatment were subjected to individual clinical examination. Study was carried out during period of April 2014 to April 2015. During the observation period a total of 4774 dogs were presented. Out of 4774 cases, 132 dogs were suspected on the basis of clinical signs and 87 (01.82%) were found positive for *Babesia gibsoni* by PCR.

Eljadar (2010) examined a total of 951 suspected dog samples from Small Animal Clinics, GADVASU, Ludhiana, and three local private veterinary hospitals for haemoprotozoan infections and reported 3.17% samples to be positive for *B. gibsoni*. Singh *et al.* (2011; 2012) revealed prevalence of *B. gibsoni* in the range of 0.65%–8.26% in the region of Ludhiana. The prevalence of canine babesiosis from various parts of northern India has been ranged from 0.66 to 8.9% (Varshney and Dey, 1998; Chaudhuri, 2006; Singh *et al.,* 2011). Wide variation in climatic conditions prevailing in different parts of India might be responsible for varying percentage of these tick borne infections. As far as the detection of *B. gibsoni* with PCR based assays is concerned, many studies have been carried out worldwide and the prevalence has been ranged from 3.3 to 55% (Macintire *et al.,* 2002; Inokuma *et al.,* 2004; Mokhtar *et al.,* 2013; Laha *et al.,* 2014).

Positive cases were comparatively higher (2-8%) in months where temperature is higher such as February to July while rest of months (August to January) relatively lesser positive cases (< 2%). Details of positive cases among different months are presented in table 1. Month wise analysis of the incidence data revealed highest incidence in the months of April to June and lowest in months of winter season. The disease was most prevalent in warm seasons as compared to winters. The probable reason behind this trend may be correlated to the seasonal activity of the tick, which is abundance in hot and humid period of the year, thus resulting in the higher incidence of haemoprotozoan infections in warm months during warmer seasons (Soulsby, 1982).

Distribution of positive cases was found directly proportional to the age. Dogs over age of 4 years harbor maximum cases (3.04%) where as minimum cases were recorded in less than one year groups (0.89%). Details of positive cases among different age groups are presented in table 1. Contrary to this finding several authors have observed the prevalence of the haemoprotozoan infections to be highest in young dogs (Abdullahi *et al.,* 1990; Samradhni *et al.,* 2005).

Beagle, Bhutia, Bull dog, Chi hua chua, Cocker spanial, Cross bred, Golden retriever, Lhasa apso and Majestic are the breed where no cases of *Bebesia gibsoni* infection were reported. Although this may not be related to genetic resistance but seems to either due unlikely to be exposed to infection or low number of cases included in the study. Boxer were found heavily infected (23.08%)

Table 1: Distribution of *Babesia gibsoni* infection in dogs

Variables		Total cases	Male (%)	Female (%)	Overall (%)
Months					
April	2014	385	13 (03.38)	06 (01.58)	19 (04.93)
May	2014	392	12 (03.06)	06 (01.53)	18 (04.59)
June	2014	344	06 (01.74)	05 (01.45)	11 (03.20)
July	2014	397	03 (00.75)	05 (01.26)	08 (02.01)
August	2014	428	04 (00.49)	01 (00.23)	05 (01.17)
September	2014	406	02 (00.49)	0(0.00)	02 (00.49)
October	2014	333	0(0.00)	01 (00.30)	01 (00.30)
November	2014	294	01(00.34)	0(0.00)	01 (00.34)
December	2014	292	0(0.00)	0(0.00)	0(0.00)
January	2014	276	01 (00.36)	0(0.00)	01 (00.36)
February	2014	318	03 (00.94)	01 (00.31)	04 (01.26)
March	2014	449	06 (01.34)	02 (00.44)	08 (01.78)
April	2015	460	06 (01.30)	03 (00.65)	09 (01.96)
Total		4774	57 (01.19)	30 (00.63)	87 (01.82)
Age					
< 1 yr		2204	13 (00.59)	05 (00.23)	18 (00.89)
1-< 2 yrs		597	10 (01.66)	03 (00.50)	13 (02.18)
2-< 3 yrs		450	06 (01.33)	04 (00.89)	10 (02.22)
3- < 4 yrs		337	07 (02.08)	03 (00.89)	10 (02.97)
e" 4 yrs		1187	21 (01.77)	15 (01.89)	36 (03.04)
Total		4774	57 (01.19)	30 (00.63)	87 (01.82)
Breed					
Beagle		27	0(0.00)	0(0.00)	0(0.00)
Bhutia		8	0(0.00)	0(0.00)	0(0.00)
Boxer		13	3 (23.08)	0(0.00)	3 (23.08)
Bull dog		2	0(0.00)	0(0.00)	0(0.00)
Mastiff [a]		58	1 (1.72)	0(0.00)	1 (1.72)
Chi hua chua		1	0(0.00)	0(0.00)	0(0.00)
Cocker spanial		13	0(0.00)	0(0.00)	0(0.00)
Cross bred [b]		51	0(0.00)	0(0.00)	0(0.00)
Dachshund		19	1 (5.26)	2 (10.53)	3 (15.79)
Dalmatian		25	1 (4.00)	0(0.00)	1 (4.00)
Non-descript breed [c]		871	10 (1.15)	5 (0.57)	15 (1.72)
Doberman		96	2 (2.08)	1 (1.04)	3 (3.13)
Golden retriever		5	0(0.00)	0(0.00)	0(0.00)
Great Dane		89	1 (1.12)	0(0.00)	1 (1.12)
German Shepherd		662	5 (0.76)	4 (0.60)	9 (1.36)
Labrador		1290	21 (1.63)	9 (0.70)	30 (2.33)
Lhasa apso		6	0(0.00)	0(0.00)	0(0.00)
Majestic		1	0(0.00)	0(0.00)	0(0.00)
Pomerarian		1122	8 (0.71)	3 (0.27)	11 (0.98)
Pug		215	1 (0.47)	2 (0.93)	3 (1.40)
Rottweiler		131	1 (0.76)	3 (2.29)	4 (3.05)
Saint Bernard		43	1 (2.33)	1 (2.33)	2 (4.65)
Spitz		26	1 (3.85)	0(0.00)	1 (3.85)
Total		4774	57 (01.19)	30 (00.63)	87 (01.82)

Figures in parenthesis indicate percentage

[a] Mastiff included Bull Mastiff, English Mastiff and Nepolian Mastiff

[b] Cross bred included all breed which is cross product with any well established breed to Indian breed.

[c] Non-descript breed included Indian non-descript breeds, Rampur hound and Mongrel

followed by Dachshund (15.79%), Saint Bernard (4.65%), Dalmatian (4%), Spitz (3.85%), Doberman (3.13%), Labrador (2.33%), Rottweiler (3.05%), Desi (1.72%), Mastiff (1.72%), Pug (1.40%), GSD (1.36%), Great dane (1.12%) and Pomerarian (0.98%), respectively.

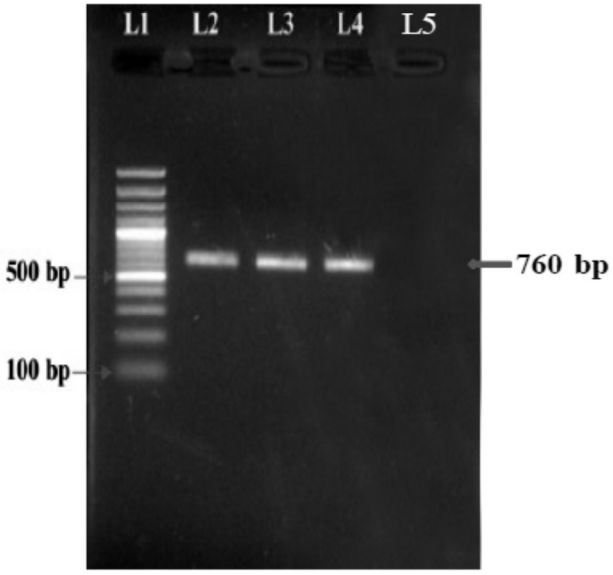

Fig. 1: Amplicons corresponding to 18S rRNA gene of *Babesia gibsoni*. L1: DNA marker; L2: positive DNA control; L3: Test sample; L4: Test sample; L5: Negative control.

A total of 57 (01.19%) male dog were found positive where as 30 (00.63%) female were detected positive for *B. gibsoni* infection over observation period. Among different months of years comparatively more number of male were found positive than female groups except July (05, 01.26%) where higher number of female dogs were found positive than male (03, 00.75%). Details of number and percentage of positive cases in both sexes among different months are presented in table1. Differences in percentage of cases among sex were not significant although owner preference of male for their pet contribute more number of positive cases. Similarly in different age groups and breed higher numbers of positive cases were diagnosed in male in comparison to female. The data obtained in the current study, showed that the assays recorded no statistical significance difference in the prevalence of the disease among males and female dogs. These results are incongruous with Amuta *et al.* (2010) and Singh *et al.* (2011).

Conclusion

PCR detects parasite DNA from earlier stages of infection with a significantly low level of parasitemia than Giemsa-stained thin blood smear. These results suggest that the PCR method has the potential to detect the parasite DNA in very early stages of infection.

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Alterations in leukogram of buffalo calves following oral administration of flubendiamide, lead and their combination

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Abstract

This report documents the toxic effects of Flubendiamide (Flb), a recently introduced pesticide, on mammalian leukogram. The effect of flubendiamide, lead (Pb) and their combined exposure on leukogram in buffalo calves was studied. Flubendiamide (@ 0.024 mg/ kg body weight once daily) and lead acetate (@ 9.2 mg/ kg body weight) were administered orally to for a period of 90 days. Flubendiamide alone exposure resulted into decrease in TLC, but changes in differential leukocyte count did not show any constant pattern. Exposure to lead alone resulted into significant decline in TLC, though neutrophil, eosinophil and basophil percentage did not alter significantly. Lymphocyte percentage showed decreasing trend while monocyte percentage showed increasing trend. Combined exposure of Flb and Pb resulted into decrease in TLC and neutrphil percentage to a degree greater than those observed in Pb alone exposed group suggesting synergistic effects of the two toxicants.

Keywords*:* Buffalo; Flubendiamide; Haematology; Lead; Leukogram; Pesticide; Toxicity

Flubendiamide, an insecticide discovered in 20th century, is highly effective against the insects of order Lepidoptera. It selectively activates ryanodine receptors of insects, leading to massive intracellular release of calcium ions (Tohnishi *et al.,* 2005; Lahm *et al.,* 2009). Its toxic effect in mammals has not been widely investigated so far, though it is believed to be very safe for non-target species. We recently reported alterations in erythrocytic indices in buffalo calves exposed to fluobendiamide (Ranjan *et al,*. 2014). Lead (Pb), a toxic heavy metal widely distributed in the environment as a pollutant, is toxic for both man and animals (Swarup and Dwivedi, 2002; Patra and Swarup, 2000). High levels of lead in feed, fodder, mineral mixture and drinking water intended for consumption by domestic animals has been documented indicating prevalence of Pb toxicity in animals (Bharathidhasan *et al*., 2008; Dey *et al.,* 1996).

Exposure to a cocktail of pollutants including pesticides and heavy metals commonly occurs under natural circumstances. Presence of one pollutant may influence the toxicity of other pollutant (Donder *et al.,* 2011). For example, exposure to a combination of chloryripfos (an organophosphorus pesticide) and lead induces greater biochemical alterations than those induced by either alone (Krishna or Ramachandran, 2009). Therefore, present study aimed to investigate effects on leukogram after flubendiamide, lead and their combined exposure to buffalo calves.

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Materials and Methods

The present study was carried out in 16 healthy, 8 to 12 months old male buffalo calves with body weight in between 120-180 kg. They were dewormed and acclimatized for two weeks in the experimental animal shed of the department. The animals were maintained under identical managemental practices and provided green fodder, wheat straw and drinking water *ad libitum*. The experimental protocol followed the ethical guidelines on the proper care of experimental animals and was approved by Institute's Animal Ethics Committee.

The animals were divided into four equal groups. Group I animals received no treatment to serve as untreated control. Group II animals were drenched flubendiamide (Fame, Bayer Cropscience Limited, Sabarkanta, Gujarat) at the dose rate of 0.024 mg/ kg body weight once daily. Group III animals received lead acetate (Merck Specialties Private Ltd., Mumbai, India) at the dose rate of 9.2 mg/ kg body weight orally once daily. Group IV animals received single oral dosing of both flubendiamde (0.024 mg/ kg) and lead acetate (9.2 mg/ kg). The treatment was continued for 90 days. Blood samples were collected by jugular venipuncture on 0, 30, 60, 90 days of treatment and 30 days post-treatment using disodium EDTA as anticoagulant for estimation of various erythrocytic indices (Benjamin, 1985). Results obtained were analyzed statistically using one way Analysis of Variance (Snedecor and Cocharan, 1994)

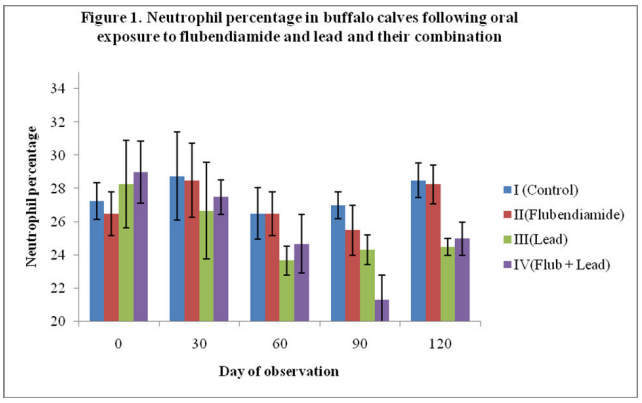
Results and Discussion

Total leukocyte count (TLC) as observed on different observation days is given in table 1. Differential counts of neutrophil, lymphocyte, monocyte, eosinophil and basophil are depicted in figure 1, 2, 3, 4 and 5 respectively.

In flubendimamide exposed animals, TLC decreased on day 60 and 90 to reach a value 23.99 per cent lower than day 0. On day 90, TLC was significantly lower than corresponding values in control group as well as day 0 value within the group. Changes in neutrophil, lymphocyte, monocyte, eosinophil and basophil percentages did not show any constant pattern or significant difference within the group. Results of the present study revealed that flubendiamide exposure leads to decrease in TLC count, but changes in differential count remains inconsistent.

In animals exposed to lead alone, TLC decreased significantly on day 60 and 90 to reach a level 21.11 and 39.44 per cent lower than day 0. On day 30 post-treatment TLC increased, but was still significantly lower than day 0 level. No significant change in neutrophil, eosinophil and basophil percentage were observed on different observation days. The lymphocyte decreased, while monocyte increased significantly on day 90. On the contrary to present findings, Palipoch *et al.* (2011) observed non-significant increase in TLC and lymphocyte count, but decrease in neutrophil and monocyte percentage in *Nile tilapia* after exposure to lead nitrate for 28 days. Leukocytosis due to neutrophilia with a regenerative left shift possibly caused by increased bone marrow myeloid- erythroid ratio is a common finding in lead induced haematological changes (Mitema *et al.,* 1980). Teijon *et al.* (2000) reported decrease in total number of leukocytes following oral administration of lead but leukocytosis was reported after intraperitoneal administration of lead indicating influence of the route of administration of lead on blood leukocyte counts. Epidemiological studies involving occupationally Pb exposed workers showed non-significant increase, in neutrophils (Pinkerton *et al.,* 1998), other study revealed an increase in lymphocyte and decrease in neutrophil count without any effect on the total of leukocyte count (Osfor *et al.,* 1998). These studies point controversies over the changes in peripheral blood leukocyte count in Pb exposed subjects.

Multiple factors like inflammatory conditions metabolic disorders and physiological stress etc. may be responsible for alterations in neutrophil count (Sacher and Mc Pherson, 1992). Kuijpers *et al.* (1999) suggested that lead might be acting like organism that increases phagocytosis. Therefore, the leucocytic alteration in the present study, might be due to the toxic effects of lead on spleen, thymus, bone marrow, lymphnodes and



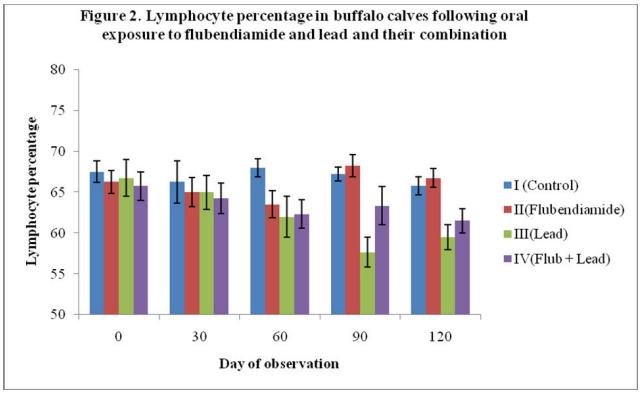


Table 1: Total leukocyte count (X 10^3/ µl) in buffalo calves exposed to flubendiamide and lead and their combination.

Group	Day of observation				
	0	30	60	90	120
I (Control)	10.57 ± 0.30^{aA}	11.19 ± 0.47^{aA}	10.39 ± 0.36^{aB}	9.80 ± 0.53^{aC}	10.69 ± 0.52^{aB}
II (Flubendiamide)	10.15 ± 0.54^{bA}	10.505 ± 0.53^{bA}	9.38 ± 0.55^{bAB}	7.715 ± 0.46^{aB}	7.84 ± 0.44^{aA}
III (Lead)	10.42 ± 0.55^{bA}	10.95 ± 0.54^{bA}	8.22 ± 0.47^{aA}	6.31 ± 0.64^{aAB}	8.30 ± 1.25^{aA}
IV (Flubendiamide and Lead)	10.22 ± 0.42^{cA}	10.78 ± 0.41^{cA}	7.98 ± 0.35^{bA}	5.13 ± 0.20^{aA}	7.60 ± 0.85^{bA}

Note: Superscripts with small letters in a raw and capital letters in a column differ significantly ($P<0.01$)

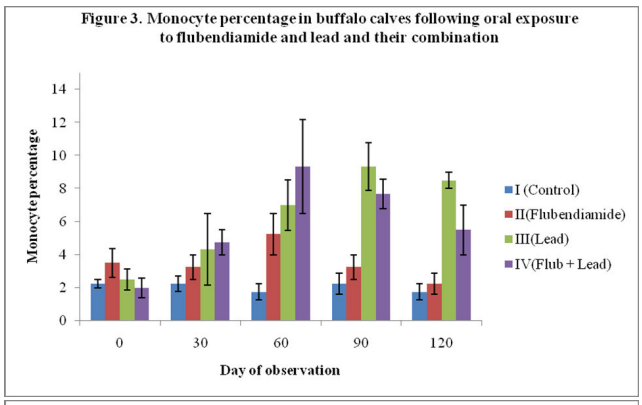


Figure 3. Monocyte percentage in buffalo calves following oral exposure to flubendiamide and lead and their combination

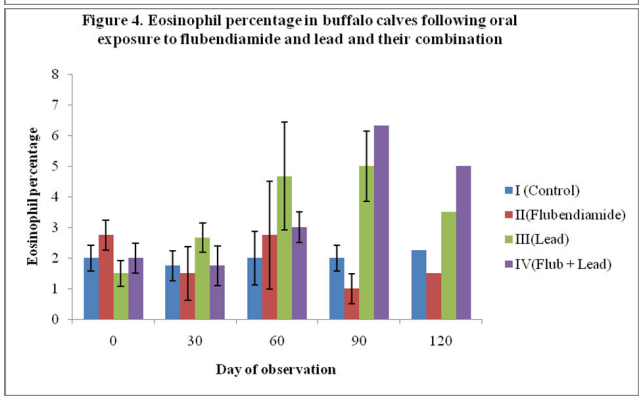


Figure 4. Eosinophil percentage in buffalo calves following oral exposure to flubendiamide and lead and their combination

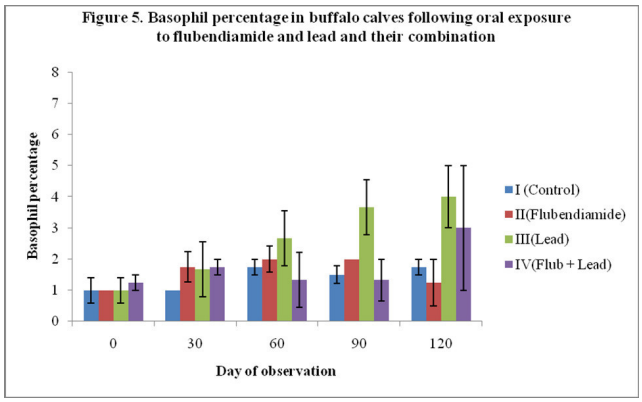


Figure 5. Basophil percentage in buffalo calves following oral exposure to flubendiamide and lead and their combination

Payer's patches, involved in the regulation of peripheral blood leukocyte count. Teijon *et al.* (2003) reported that lead administration by oral route causes histological modification in spleen including increase in number of lymphocytes as well as edema resulting into spleenomegaly. Teijon *et al.* (2000) observed that spleen was clearly sensitive to lead, especially when it is administered intraperitoneally and causes decrease in red blood cell count and alterations in white blood cells.

In animals treated with both flubendiamide and lead, TLC decreased significantly on day 60 and 90 to become 21.92 and 49.80 per cent lower than day 0. On day 60 and 90 the count was lowest among corresponding values observed in other treatment groups and control. On day 30 post-treatment, TLC increased to reach 48.15 per cent higher than day 90 value, but was still significantly lower than day 0. No significant change was observed in lymphocyte and basophil percentage on different observation days. However, monocytes and eosinophils showed an increasing trend while neutrophils showed a decreasing trend during combined flubendiamide and lead exposure. Therefore, it can be concluded that combined exposure to flubendiamide and lead causes decrease in TLC and neutrophil percentage to an extent greater than those observed in Pb alone exposed group. This suggested that the two toxicants may have synergistic effects on alterations in leukogram.

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Blood pressure and haematobiochemical changes in dogs with renal failure

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Abstract

The present study was undertaken to study the hematobiochemical and blood pressure levels in 28 dogs suffering from renal failure. Hematology revealed that dogs were anemic on day of presentation with high TLC and absolute counts. The mean levels of BUN and creatinine in cases of renal failure were 139±14 mg/dl and 9.5±1 mg/dl, respectively with high phosphorous levels of 14.73±1.22 mg/dl. The blood pressure of dogs was measured with oscillometric method revealed mean systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure levels as 137±21, 78±14 and 97±16 mm Hg, respectively. According to Classification of Hypertension Consensus Panel of American College of Veterinary Internal Medicine, out of 28 dogs with renal failure, 75% were having SAP <150 mm Hg (minimal risk), 7.14% were having SAP: 150-159 mm Hg (mild risk) and 17.8% were having SAP: 160-179 mm Hg (moderate risk) and none of the dogs were in severe risk category (SAP >150 mm Hg).

Keywords: Blood pressure, dog, hypertension, renal failure.

Introduction

Systemic hypertension is persistent elevation of systemic blood pressure. Primary hypertension is apparently rare in dogs and cats, systemic hypertension in veterinary practice is usually associated with another disease or condition like kidney disease, hyperthyroidism, hyperparathyroidism, ocular diseases, liver diseases, etc. The organs most commonly affected by hypertension include eye, kidney, brain and heart (Stepien and Rapoport 1999). The clinical signs associated with hypertension in these organs include retinopathy, hyphema, neurologic signs of intracranial hemorrhage, seizures, renal insufficiency and cardiovascular diseases (Brown *et al.,* 2007 and Leblanc *et al.,* 2011). Systemic hypertension has been reported in 50% to 93% of dogs with renal failure (Cowgill and Kallet 1986). To manage this conditions, it is important for monitory to measure BP. for acertaining hypertension and efficacy of anti-hypertensive therapy.

Clinically, noninvasive methods for BP measurement are considered more appropriate for being simple and cause less stress to the patient and minimize hemorrhage and secondary infections (Mishina *et al* 1997), ischemia, thrombosis and embolism, which may arise due to arterial catheterization done in invasive/ direct method. The information about the prevalence of systolic or diastolic hypertension in dogs is scarce, especially in India.

Material and Methods

Study was undertaken in 28 dogs presented with the history of vomiting, diarrhea, chronic anorexia at Small Animal Clinics of Teaching Veterinary Hospital, GADVASU, Ludhiana and later on confirmed to be suffering from renal failure based on hematology and biochemical analysis. Dogs were gently restrained in lateral recumbency for examination. Before measurements of blood pressure, dogs were acclimatized in measurement room for 10-15 minutes. An oscillometric monitor was used to obtain measurements in conjunction with an appropriately sized cuff with width 40% of the circumference of limb. A series of 5 readings were obtained for each animal. Mean systolic, diastolic and mean arterial blood pressure was then calculated after rejection of the first reading and any outliers. Hematological and biochemical analysis were done as per standard methods.

Results and Discussion

In cases of renal failure, the CBC of dogs revealed that majority of dogs were anemic on day of presentation with the mean levels of Hb was 10.03±4.1 g/dL. The mean level of TLC, absolute neutrophil and lymphocyte counts presented in Table 1. As the kidneys fail, they produce less erythropoietin, resulting in decreased production of red blood cells to replace the natural breakdown of old red blood cells. There is direct correlation between the degree of anemia and the extent of chronic renal failure as assessed by serum creatinine concentrations (King *et al.,* 2006). Anaemia occurs in up to 50% of dogs and cats with chronic renal failure. Although anaemia may also be detected in patients with acute renal failure, typically developing as a result of blood loss such as gastrointestinal hemorrhage

(Robertson and Seguin 2013).

The mean levels of alkaline phosphatase and total bilirubin were above the normal physiological range i.e. 215.3±60 U/L and 0.97±0.45 mg/dl, respectively (Table 2). This may be due to septicemia, causing multi organ damage, thus resulting in hepatorenal syndrome, a condition in which there is progressive kidney failure in an individual with of the liver insufficiency. The mean levels of BUN and creatinine in cases of renal failure were 139±14 mg/dl and 9.5±1 mg/dl, respectively with high phosphorous levels of 14.73±1.22 mg/dl (Table 2). Kaneko *et al* (2008) reported normal blood urea nitrogen range from 10-28 mg/dl. Increase in urea in renal failure is caused by impaired ability to excrete proteinaceous catabolites because of marked reduction in GFR. Urea is also directly related to the protein content of the diet. Urea can also be increased by gastrointestinal hemorrhage, enhanced protein catabolism, decreasing urine volume and glucocorticoids (Robertson and Seguin 2013). Lee *et al* (2011) observed that BUN levels were higher in dogs having gastrointestinal signs of vomiting or diarrhea along with renal failure. Creatinine is produced from the metabolism of creatinine in muscles. It is released into the blood at a relatively constant rate and is excreted largely by glomerular filteration. Creatinine is not significantly secreted or reabsorbed by the tubules. For this reason, serum creatinine concentration can be used to evaluate renal function (Squires 2007). Ross (2011) stated that serum phosphorous levels were often elevated; however, the degree of elevation may reflect the degree of reduced GFR rather than duration of disease.

In dogs suffering from renal failure the mean SAP, DAP and MAP were 137±21, 78±14 and 97±16 mm Hg, respectively. On the basis of risk of target organ damage, 21 (75%) out of 28 were having SAP <150 mm Hg i.e in minimum risk category, 2 (7.14%) were having SAP 150-159 mm Hg i.e in mild risk and 5 (17.8%) dogs were having SAP 160-179 mm Hg i.e in moderate risk category.

As per IRIS guidelines, dogs with renal failure were staged on the basis of serum creatinine levels. In present study the overall mean SAP, DAP and MAP in stage 3 dogs (Creatinine 2.1- 5.0 mg/dl) were 148±22.7, 83.5±18.5 and 104±18.6 mm Hg, respectively and in stage 4 dogs (Creatinine >5 mg/dl) mean SAP, DAP and MAP were 135±20.6, 76±13.3 and 96±15 mm Hg, respectively. The blood pressure increased from stage 2 to stage 3 but increase was insignificant. But the blood pressure decreased insignificantly from stage 3 to stage 4. Mann (2013) reported that mean SAP gradually increased with increasing stage although it was in normal range. Mean DAP of dogs in stage 2 and 3 was within normal range and above reference range in stage 4 and also found that 30.7% of stage 3 and 36% of stage 4 renal failures was having blood pressure more than 150/ 95.

Finco (2004) found an association between increased systemic arterial pressure and renal injury. Coulter *et al* (1984) cited that in dogs with chronic renal failure, the mean systolic blood pressure was in the normal range although it increased with increasing stages. Porciello *et al* (2004) observed that in dogs suffering from chronic renal failure and dirofilariasis, both systolic and diastolic blood pressure were statistically higher than the control group while for diabetic dogs only the diastolic values were higher. Jacob *et al* (2003) observed that there was increased risk of developing a uremic crisis and of dying in dogs having high systolic blood pressure with chronic renal failure. Spayed and neutered dogs with Acute Kidney injury had significantly lower systolic blood pressure as compared to the intact dogs (Geigy *et al* 2011).

Geigy *et al* (2011) in 52 dogs with acute kidney injury observed systolic systemic hypertension (>160 mm Hg) and severe systolic systemic hypertension (>180 mm of Hg) in 37% and 15% cases, respectively. Ramakant *et al* (2013) recorded 50% prevalence of systemic hypertension in chronic renal failure dogs. Due to low blood supply to the kidneys, renin secretion increases, resulting into conversion of Angiotensinogen into Angiotensin I, which further converted into Angiotensin II with the help of ACE, and ultimately leads to vasoconstriction and high blood pressure.

Kidney disease and hypertension are intimately linked. Dogs with CRF are less susceptible to hypertension than humans. Kidney disease can lead to sodium and water retention and resultant extracellular fluid volume expansion. This increases cardiac output, producing systemic hypertension. Further activation of the Renin Angiotensin Aldosterone System elevates systemic arterial blood pressure. On the other hand, high blood pressure causes glomerular hypertension and hyperfilteration, proteinuria and arteriosclerosis.

Brown and Brown (1996) stated that the

Table 1: Hematological findings (Mean ± SEM) in dogs suffering from renal failure with risk classification

CBC (n-8)	Reference value	Renal failure (n-28)	Minimal risk (SAP <150mm Hg) (n=21)	Mild risk (SAP 150-159 mm Hg) (n=2)	Moderate risk (SAP 160-179 mm Hg) (n=5)
Hb (g/dl)	12-19	10.03±0.80 (2.3-18.1)	10.04±1.044 (2.3-18.1)	8.2±0.6 (7.5-8.8)	10±1.21 (7.2-14)
TLC (/¼L)	5000-14100	20975±3457 (5500-78900)	22373±4392 (5300-78900)	12050±1049 (11000-13100)	15208±5453 (6600-38240)
N (/¼L)	2900-12000	19009±3434 (4717-77322)	20668±4341 (4717-77322)	7927±1767 (6160-9694)	13610±5775 (6072-35946)
L (/¼L)	400-2900	1795±272 (245-5976)	1574±319 (245-5976)	3683±276 (3406-3960)	1343±455 (417-2944)
M (/¼L)	100-1400	91±44 (0-880)	0	0	0
E (/¼L)	0-1300	0	20±14.7 (0-269)	440±440 (0-880)	255±142 (0-765)
B (/¼L)	0-100	0	0	0	0

Table 2: Biochemical parameters (Mean ± SEM) in dogs suffering from renal failure with risk classification

Parameter	Reference value	Renal failure (n-28)	Minimal risk (SAP <150 mm Hg) (n=21)	Mild risk (SAP 150-159 mm Hg) (n=2)	Moderate risk (SAP 160-179 mm Hg) (n=5)
TB (mg/dL)	0-0.5	0.97±0.45 (0.1-11.8)	1.1±0.60 (0.1-12)	0.2	0.6±0.37 (0.2-2.1)
ALT (U/L)	21-102	48±8.4 (12-186)	55±11 (12-186)	23±9.5 (13-32)	31±5.29 (18-44)
ALKP (U/L)	1-114	215.3±60 (23-1423)	252±81 (23-1423)	58±24 (34-81)	133±31 (55-238)
TP (g/dL)	5.4-7.5	6.31±.19 (4.4-7.8)	6.3±0.2 (4.8-7.8)	7.7	5.95±0.5 (4.4-7.1)
Albumin (g/dL)	2.3-3.1	2.65±.10 (1.5-3.9)	2.7±0.1 (1.5-3.9)	2.9	2.5±0.23 (2-3)
Globulin (g/dL)	2.4-4.4	3.77±.15 (2.4-5.3)	3.8±0.2 (2.9-5.3)	4.8	3.23±0.4 (2.4-4.2)
BUN (mg/dL)	Aug-28	139±14 (41-270)	148±17 (41-270)	133±65 (68-198)	105±22 (58-159)
Creatinine (mg/dL)	0.5-1.7	9.5±1 (1.9-22.7)	11±1.4 (1.9-23)	6.6±2.1 (4.5-8.7)	5.76±1.21 (3.7-11)
Na (mEq/L)	142-152	142±2 (118-159)	142±2.9 (118-159)	150	141±2.29 (135-146)
K (mEq/L)	3.9-5.1	4.5±0.2 (2.6-6)	4.7±0.2 (3.5-6)	3.7	4.22±0.56 (2.6-5.4)
Ca (mg/dL)	9.0-11.3	9.94±.48 (2.8-13)	10±0.60 (21-13)	9.5	6.05±0.0.56 (8.8-11)
P (mg/dL)	2.9-5.3	14.73±1.22 (3.5-30)	15±1.4 (3.5-30)	26	10.67±2.19 (4.6-16)
Glucose (mg/dL)	65-118	109.68±9 (54-249)	110±13 (54-249)	108	108±13.6 (72-156)
Cholesterol (mg/dL)	135-278	162.81±15.2 (44-266)	150±18 (75-266)	249	164±9.8 (44-239)

elevated blood pressure is transmitted directly to the glomerular capillary bed and this causes increase in glomerular capillary pressure referred as glomerular hypertension which may produce glomerular damage and progressive decrease in renal function. Reduction in glomerular filteration rate result in a decrease in amount of sodium that is filtered at glomerulus with lesser degrees at kidney dysfunction, any reduction in the filtered sodium load should be offset by a reduction in tubular resorption in any remaining functional

nephrons. This suggests that the hypertension in patients with milder forms of chronic kidney disease is linked to impairment of sodium handling in the tubules and/or collecting ducts.

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Evaluation of diagnostic potential of *Echinococcus granulosus* recombinant *Eg*Ag5-38 sub-unit and P-29 antigens for cystic echinococcosis in goats

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Abstract

Echinococcus granulosus is an important zoonotic parasite causing cystic echinococcosis in human and domestic animals and its diagnosis in domestic animals is still a challenge. Two recombinant proteins *Eg*Ag5-38 sub-unit and P-29 of *E. granulosus* were expressed in prokaryotic expression vectors. The diagnostic potential of these two recombinant proteins was evaluated in the detection of cystic echinococcosis in goats in IgG-ELISA. The *Eg*Ag5-38 sub-unit and P-29 recombinant protein reacted fairly with the hydatid infected goats with *Eg*Ag5-38 sub-unit showing sensitivity and specificity of 73.3% and 69.30%, respectively. However, the recombinant P-29 protein showed a higher sensitivity of 80.0% and specificity of 74.3% in the detection of hydatid infection in goats. Cross-reactivity of these recombinant antigens in goats naturally infected with gastro-intestinal strongyle nematodes and *Taenia hydatigena* was studied. The results showed that both these antigens cross-reacted with most of these parasites of goat. Present study is the first report on the evaluation of these two diagnostically potential antigens in goats.

Keywords: Antigen 5-38 sub-unit, Cross-reactivity, Diagnosis, *Echinococcus granulosus*, P-29, Goat, IgG-ELISA;

Introduction

Cystic echinococcosis (CE) caused by the larval stage of *Echinococcus granulosus* is prevalent worldwide and animals and human are infected by incidental ingestion of the parasite eggs in association with dog rearing environments. Serodiagnosis of cystic echinococcosis relies mostly on crude hydatid cyst fluid as the antigen but lacks standardization of the target antigen as reflected by its poor sensitivity and specificity. The literature available on the immunological diagnosis of hydatid infection in humans is extensive with a number of recent reviews (Zhang and McManus, 2006; Craig *et al.,* 2007; Brunetti *et al*., 2010; McManus *et al*., 2012). Research on the development of immunodiagnostic tests for *E. granulosus* infection in domestic ruminants is scanty and the results have been generally disappointing and often contradictory (McManus, 2014). Diagnosis of *E. granulosus* infection in animals is a prerequisite for epidemiological studies and surveillance of echinococcosis in endemic and emergent transmission zones. However, advances in diagnostic approaches for definitive hosts and livestock have not progressed equally over last two decades (Craig *et al*., 2015).

The early detection of hydatidosis in animals has been a challenge. The diagnosis of CE largely depends on imaging scans and serological tests. The imaging techniques have been utilized for confirming the hydatid cysts in human patients but diagnosis of CE with imaging techniques in animals is not feasible in the developing countries due to cost factors. Therefore, in the absence of accurate serological tests infected animals go undetected; adding to the losses to the livestock economy. The only reliable method of diagnosing hydatidosis in animals is by detection of cysts at necropsy. In India fewer reports are available on the development of sero-diagnostic tests for CE in livestock, though all species of domestic livestock are equally susceptible to this infection (Samanta *et al*., 2009; Pan *et al*., 2011). Ag5 and antigen B are the two major lipoprotein antigens present in hydatid cyst fluid and have been considered crucial to immunodiagnosis of CE. Both Ag5 and antigen B have now been well characterized by molecular and biochemical techniques. Ag5 is a very high molecular weight complex comprised of 60-70 kDa components which under reducing conditions dissociate into two sub-units of 22 and 38-kDa, the larger of which contains a phosphorylcholine epitope (Shepherd and McManus, 1987; Lorenzo *et al*., 2003). Likewise, P-29, a protoscolex derived antigen, has shown potential in the serological detection of CE in human (Boubaker *et al*., 2014) but has not been evaluated in animals for detection of this infection. The reports were on diagnosis of CE in animals in view, assessment of the immunodiagnostic potential of two recombinant antigens *Eg*Ag5-38 sub-unit and P-29 of *E. granulosus* in goats.

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Materials and Methods

Two recombinant proteins of *E. granulosus* including *Eg*Ag5-38 sub-unit and P-29 were expressed in the prokaryotic expression vectors. Protoscolices were retrieved from a fertile hydatid cyst from buffalo liver at a local abattoir and total RNA isolated from these protoscolices using Trizol reagent (Invitrogen, USA). Briefly, ~0.3 x10^4 protoscolices were treated with Trizol reagent (1ml) and manually homogenized with a micropestle in a sterile 2.0 ml microcentrifuge tube with several cycles of freeze-thawing at -80°C. Total RNA was isolated from the lysed protoscolices following standard RNA isolation protocol (Invitrogen, USA). The RNA was converted to single stranded cDNA using oligo-dT primer and reverse transcriptase enzyme (MBI Fermentas, USA) following standard protocols of cDNA synthesis. The cDNA coding for each of the above two target proteins was PCR amplified with gene specific primers (Table1). The PCR products were cloned in p^{DRIVE} cloning vector (Qiagen, Germany) and sequence confirmed for each cDNA.

Expression of the recombinant EgAg-5sub-unit and P-29 protein

The cDNAs coding for *EgAg-5 38* sub-unit *and* P-29 proteins were PCR amplified with primers designed with suitable restriction enzyme sites and expressed in prokaryotic expression vector $p^{PROEXHT-b}$ (Table 1). The open reading frame of each cDNA was cloned in the expression vector and insertion of the cDNA in frame with the vector histidine fusion tag was confirmed by sequencing. Expression of the recombinant *Eg*Ag5-38 sub-unit in *Escherichia coli* BL21 (DE3) cells was induced with 1mM IPTG for 5 h at 37 °C. The 2nd target protein P-29 was also expressed in $p^{PROEXHT-b}$ vector at 6 h of 1mM IPTG induction at 37°C. Recombinant *Eg*Ag5-38 sub-unit protein was purified from the *E. coli* BL21 cells following a mild denaturation protocol of urea lysis. Bacterial cells harbouring the recombinant protein were lysed in 8 M urea buffer (pH 8.0), supplemented with 10 mM imidazole and 10 mM β-mercaptoethanol. The protein was purified to complete homogeneity with Ni-NTA affinity chromatography. The recombinant protein was bound to the affinity column (Qiagen, Germany) and was washed with buffer supplemented with 10 mM imidazole at pH 6.5 and elution of the recombinant protein carried out with elution buffer at pH 4.2.

Recombinant P-29 antigen was purified using tris-phosphate buffer containing 6M guanidine hydrochloride as a strong denaturant. IPTG (1mM) induced *E. coli* cells were lysed in the lysis buffer (pH 8.0) containing 6M guanidine hydrochloride and supplemented with 10 mM imidazole and 10 mM β-mercaptoethanol at room temperature. The wash buffer (pH 6.7) was supplemented with 10 mM imidazole and recombinant protein was eluted with elution buffer at pH 4.2. The composition of the lysis, wash and elution buffers used in the purification steps of each recombinant protein was 10 mM tris and 100 mM potassium dihydrogen phosphate containing 6M guanidine hydrochloride or 8M urea as protein denaturant.

Collection of goat sera

Goats (n=116) were screened for hydatid infection at necropsy at a local abattoir at Bareilly, U.P. Out of the 116 animals examined at necropsy, 15 were positive for hydatid cysts in the liver or lungs and remaining 101 were negative for hydatid infection. Sera were retrieved from these animals and screened for anti-hydatid antibodies by IgG-ELISA with the above recombinant antigens. Goats were screened for infection with other parasites including strongyles and *Taenia hydatigena* and sera collected from them for their cross-reactivity studies. Sera collected from healthy goats, maintained at Indian Veterinary Research Institute, Izatnagar, were used as negative control in the subsequent immuno-assays. All experiments on goats were conducted as per the guidelines of the Institute Animal Ethics Committee.

Enzyme Linked Immunosorbent Assay

Checker board titrations were done to optimize the concentration of each antigen. The amount of recombinant *Eg*Ag5-38 sub-unit and P-29 antigens coated on each well of the 96-well microtitre plate was optimized to 1.0 µg /ml and 2.0 µg/ml of coating buffer, respectively. The optimal dilutions of serum samples and anti-goat IgG-HRP conjugate (Sigma Chemicals, USA) used in the assay were 1:100-1:200 and 1:6000-1:12000, respectively for these antigens. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the cut-off value for each recombinant antigen. Levels of sensitivity were plotted against the levels of one minus specificity at each cut-off point on a ROC curve. Cut-off values were selected

that gave the highest sum of sensitivity (%) and specificity (%), as described by Amagai *et al*. (1999). The area under the ROC curve (AUC) was the parameter used to define the antigen's discriminatory values between ELISA positive and negative animals.

Results and Discussion

Goats were screened for anti-hydatid antibodies with the two recombinant antigens *viz*. *Eg*Ag5-38 sub-unit and P-29. Necropsy examination of the lungs and liver of 116 goats confirmed 15 (12.9%) animals positive for hydatid cysts. ELISA was standardized for detection of the IgG antibodies in each animal. The *Eg*Ag5-38 sub-unit antigen showed positive reactivity with 11/15 necropsy confirmed positive animals with OD_{492} above the cut-off value 0.14. However, 4/15 necropsy positive animals were negative with *Eg*Ag5-38 sub-unit ELISA leading to the sensitivity of 73.3%. The IgG-ELISA with this antigen showed 40/101 (30.3%) necropsy negative animals for hydatid cyst as sero-positive (Fig.1). With antigen P-29, 12/15 necropsy confirmed positive animals were ELISA positive with OD_{492} values above the cut-off (0.16), thereby depicting the sensitivity of 80.0%. The IgG-ELISA with this antigen showed 34/101 (26.0%) necropsy negative animals as ELISA positive (Fig.2). The comparative sensitivity, specificity, positive and negative predictive values of the two assays showed that P-29 antigen has higher sensitivity (80.0%) and specificity (74.3%) than the antigen *Eg*Ag5-38 sub-unit (Table 2).

Studies on the cross-reactivity of each recombinant antigen were conducted with goat sera positive for gastrointestinal (GI) strongyle nematodes and *Taenia hydatigena*. The *Eg*Ag5-38 sub-unit antigen showed immuno-reactivity with sera of 2/5 GI nematode infected goats and 9/21 *T. hydatigena* positive goats with OD_{492} above cut-off (0.14). A single serum sample of a goat harbouring mixed infection of *T. hydatigena* and GI nematodes showed OD_{492} above positive cut-off. Two goats with a mixed infection of hydatid and gastrointestinal nematodes when probed with this antigen reacted in only one case and no sero-positive reaction was observed in a single specimen of goat infected with hydatid and *T. hydatigena* (Fig.3a).

The P-29 antigen showed reactivity with 2/5 sera positive for GI nematodes and 9/21 *T. hydatigena* positive sera with OD_{492} above cut-off (0.16). The serum of a single goat with mixed infection of *T. hydatigena* and GI nematodes showed OD_{492} above negative cut-off. Goats with a mixed infection of hydatid and *T. hydatigena* also reacted with the antigen P-29 (Fig.3b). These results showed that both *Eg*Ag5-38 sub-unit and P-29 antigens reacted equally with the GI nematodes and *T. hydatigena* infected goat sera.

The recombinant *Eg*Ag5-38 sub-unit and P-29 antigen of *E. granulosus* were evaluated in the present investigation for the detection of CE in goats. The Ag5-38 sub-unit showed a sensitivity of 73.3% and specificity

Table 1: Primer sequences designed for PCR amplification of two target genes coding for *Eg*Ag5-38s and P-29 proteins

	Gene	Primer name	Primer length	Primer Sequence ($5^1 \rightarrow 3^1$)	Amplicon size
A	***Eg*Ag5-38s**	Ag5-38-S-FOR	21 bp	ATT CTT GCT GGA AAA AGC GCA	890 bp
		Ag5-38-REV	22 bp	TAG ACT GCG TAG CGG TTG ATC C	
	P-29	P-29-FOR	22 bp	ATG TCC GGA TTT GAC GTT ACT A	717 bp
		P-29-REV	24 bp	CTA CTC GCC CAG CAT CAT ACT GCA	
B	***Eg*Ag5-38s**	Ag5-38s FOR-EX	31 bp	***CCA TGG ATC C***AT TCT TGC TGG AAA AAG CGC A	910 bp
		Ag5-38s REV-EX	32 bp	***ACC TGA AGC TT***A GAC TGC GTA GCG GTT GAT CC	
	P-29	P29-FOR-EX	33 bp	***CCA TGG GAT CC***A TGT CCG GAT TTG ACG TTA CTA	738 bp
		P29-REV-EX	34 bp	***GCT TTC TAG A***CT ACT CGC CCA GCA TCA TAC TGC A	

*Bold sequences indicate restriction enzyme sites incorporated in the primer sequences for cloning in respective expression vectors

Table 2: Comparative sensitivity and specificity of two recombinant antigens in IgG-ELISA with goat sera

Antigen	AUC	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Ag5-38s	0.77	0.14	73.3	69.3	26.2	94.6	69.8
P-29	0.76	0.16	80	74.3	31.6	96.2	75

of 69.3%, respectively. However, P-29 antigen showed a higher sensitivity of 80.0% and specificity of 74.3%. The recombinant Ag5-38 sub-unit was found to cross-react equally with the sera of goats infected with gastrointestinal strongyle nematodes and *Taenia hydatigena*. Similarly, P-29 antigen cross-reacted with the above parasites in the goat host. The results indicate that these two proteins share cross-reacting epitopes between the taeniid cestodes and strongyle nematodes there by compromising the specificity of these two recombinant antigens. This study confined to the probing of total IgG immunoglobulins in the hydatid infected host serum and the immunoglobulin IgG sub-classes have not been evaluated in the diagnosis of CE in goats using different recombinant antigens. Therefore, further studies detecting serum IgG sub-classes may enable for better sensitivity and specificity of these antigens in goats. Peptide based ELISA for improving the sensitivity and specificity of these antigens in the sero-detection of CE in goats may also be evaluated.

The identification of the arc 5 (Ag5) band formed on immunoelectrophoresis (IEP) has been regarded as the most highly specific immunodiagnostic test for cystic echinococcosis in human (Capron *et al*., 1970; Guisantes *et al*., 1975; Varela-Diaz *et al*., 1976; Kagan, 1976). However, the results have shown that antibodies precipitating arc 5 in *E. granulosus* cyst fluid were also present in the sera of some sheep infected with *T. ovis* and *T. hydatigena* and the arc 5-IEP test was further limited by poor sensitivity (Yong and Heath, 1979). Hydatid cyst fluid has been used most frequently as a source of *E. granulosus* antigen and its components have been comprehensively investigated for their applicability in serological tests in human hydatid patients. But, much less research work has been directed towards the development of immunodiagnostic tests for *E. granulosus* infection in domestic ruminants and the results have been generally disappointing and often contradictory (McManus, 2014). An accurate serological diagnostic test development for the detection of CE in livestock would represent a major advance in the control of the disease in endemic regions. Diagnosis of hydatidosis in ruminants is based mainly on necropsy findings with no specific ante-mortem serological test being developed. Accurate serological diagnosis of hydatid infection in livestock is hindered due to the serological cross-reactivity of different antigens with several taeniid cestodes and other parasites of the host (Yong *et al*., 1984; Lightowlers and Gottstein, 1995).

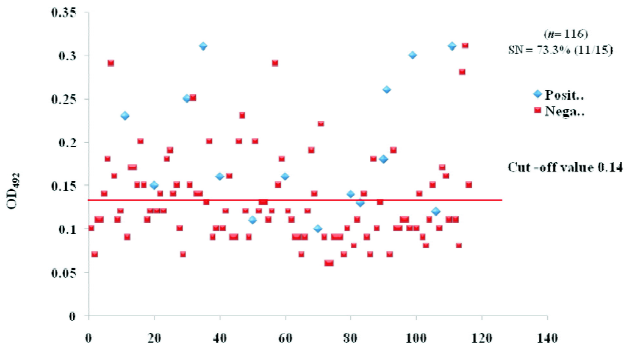

Fig. 1: IgG-ELISA showing immuno-reactivity of goat sera with recombinant Ag5-38 sub-unit

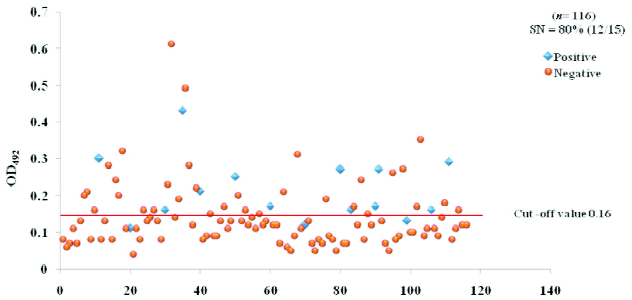

Fig. 2: IgG-ELISA showing immuno-reactivity of goat sera with recombinant P29 antigen

Several serological assays such as

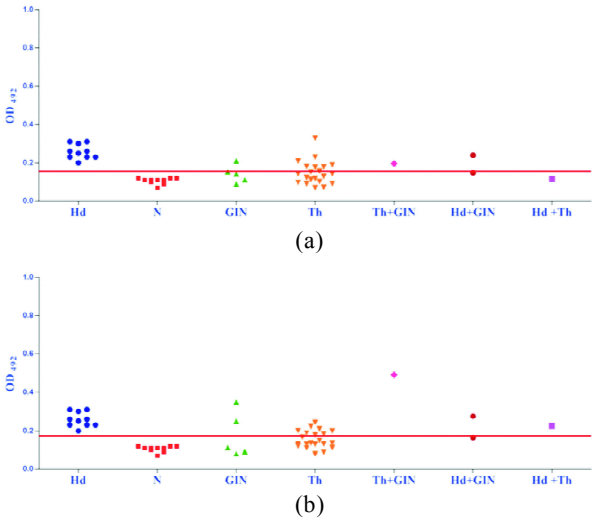

Fig. 3: IgG-ELISA of goat sera showing cross reactions of recombinant antigen Ag5-38 sub-unit (a) and P-29 (b) with various parasites of goat (Hd-Hydatid positive sera, N-Healthy goat sera (Negative control), GIN -Gastro Intestinal Strongyle Nematodes, Th-*Taenia hydatigena* positive sera)

immunoblotting and ELISA have been used with several antigens in efforts to diagnose CE in animals. In general, the problem of insensitivity and serological cross-reactions between *E. granulosus* and other parasites limit the specific diagnosis of hydatid infection in sheep when using crude or fractionated parasite antigens, secretions produced during *in vitro* cultivation of protoscolices or hydatid cyst membranes (Dueger *et al*., 2003; Simsek and Koroglu, 2004; Ghorbanpoor *et al*., 2006; Gatti *et al*., 2007; Golassa *et al*., 2011). Similar to antigen B, cross-reactivity of antigen 5 with antigens of other cestodes has been a recurrent challenge (McManus, 2014). However, Pagnozzi *et al*. 2016 have developed a robust chromatographic technique for enriching the Ag5 antigen from the hydatid cyst and reported this antigen as highly sensitive and specific in the detection of human CE. This study seems to revive interest in the use of Ag5 in CE diagnosis in human. Therefore, more studies on the use of Ag5 sub-units in the diagnosis of hydatid infection in domestic animals are required to fully exploit the potential of this antigen.

In the present study, the recombinant Ag5-38 sub-unit and P-29 antigens showed a fair degree of sensitivity but cross-reacted with the parasites investigated in goats. The issues related to this cross-reactivity of the recombinant antigens with other helminths in goats compromise the specificity of the test. However, these antigens can be used in the sero-surveillance of the goat flocks and not for individual screening of animals for cystic echinococcosis in the endemic regions of the country. The present results provide first information on the cross-reactivity of the two potent human CE diagnostic molecules *Eg*Ag5-38 sub-unit and P-29 of *E. granulosus* with some common helminth parasites of goat. Present investigation was carried out on a smaller number of goats. However, screening of larger sample size and determining the cross-reactivity with more species of helminths parasitizing the goat is needed for validating the utility of these two antigens in the sero-diagnosis of cystic echinococcosis in goats.

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Short Communication

Clinico-epidemiological and electrocardiographic study of canine dilated cardiomyopathy

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Abstract

The aim of this study was to investigate the incidence of dialated cardio myopathy (DCM) in dogs presented to Refferal Veterinary Poly Clinic of this institute. Ecocardiography, electrocardiography and serum biomarkers of cardiac disease where used in diagonising DCM cases in dogs. Dilated Cardiomyopathy was recorded in 16/374 (4.27%) dogs screened during study period suggesting the higher incidence of disease in canine population. The medium to large breed dogs were found more commonly affected however the study also showed the substantial incidence of DCM in small breeds such as Pomeranian. The mid age group dogs were found more susceptible for DCM while males have were found more frequently affected than the females The physiological parameters may vary from dog to dog and usually not conclusive of DCM. The electrocardiographic finding in conjunction with echocardiography could be proved useful in detection of DCM.

Keywords: Canine, Dilated Cardiomyopathy, Ecocardiogrpahy, ECG and Epidemiology.

Introduction

The cardiovascular diseases are being increasingly reported in dogs in past few decades. The overall prevalence of cardiac diseases in the dog is around 4.4% (Manczur *et al.,* 2003). Dilated Cardiomyopathy (DCM), or heart muscle disease, describes a group of heterogeneous conditions that affects the heart muscle functionally and/or structurally (Tidholm and Jonsson, 2005). DCM is one of the most common acquired heart diseases in dog. Canine Dilated cardiomyopathy has been recorded in several breeds, notably Boxer dogs, Dobermanns, English cocker spaniels, and in giant breed dogs (Tidholm, 1996). The most commonly observed symptoms of cardiomyopathy are exercise intolerance, dyspnoea, tachypnoea, and coughing (due to left sided heart failure); abdominal enlargements, jugular distension and pulsation (due to right sided heart failure), cold extremities, loss of weight, syncope, low-intensity systolic murmur, weak femoral arterial pulses, and cyanosis (Tidholm *et al.,* 1997). The study of incidence DCM in Indian canine population are scanty, hence the present study was designed to investigate the incidence, clinical picture and electrocardiographic features of DCM in dogs.

Materials and Methods

The present study was conducted on clinical cases of dogs reported during year 2010-12 at Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, India. Eighty two dogs were selected for screening, out of which 16 were found positive for DCM. The identified dogs were examined on the basis of physical examination, clinical examination, blood pressure (B.P), electrocardiography (ECG). Six healthy dogs were kept as control. The dogs found positive for cardiomyopathy were categorized as per breed, age (0-1yr, 1-3 yr, 3-5yr, 5-7 yr, 7-10yr and >10 yr) and sex.

ECG was recorded in standard body position (Tilley, 1985) with animals restrained in right lateral recumbancy using standard ECG recorder (Cardiart-408-BPL) at paper speed of 50 mm seconds with sensitivity of (1cm=1 mV) without use of filter. A minimum of 10 complex in bipolar limb leads (I,II,III) and augumented unipolar limb leads (aVR, aVL and aVF) were recorded. Blood pressure was recorded by Non-invasive blood pressure (NIBP) instrument (SurgiVet,) using pediatric cuff from radial artery.

Echocardiographic examinations were performed using two-dimensional and motion mode (M-mode) echocardiography machine (Pie Medicals, Netherlands) with 5MHz annular array transducer. All the measurements were made using leading edge method as per the recommendations of the American society of Echocardiography from frozen images on the screen (Thomas *et al.,* 1993).

Result and Discussion

The present study recorded 16/374 (4.27%)

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Table 1 : Physiological parameters (Mean±SE) in healthy and dogs with DCM

Parameters	Healthy control (n=06)	Dogs with DCM (n=16)
HR (/min)	118.33±4.69	102.85±8.94
SBP (mmHg)	133.33±2.72	139.06±9.73
DBP (mmHg)	76.00±2.18	89.75±8.46
MAP (mmHg)	90.66±4.15	108.38±7.13*
RR (/min)	35.50±1.73	37.94±3.88

*Denotes significance (P<0.05) between the groups.

Table 2 : Electrocardiographic changes in dogs with DCM

Types of abnormality	(N=16)	%
Second degree AV block (Mobitz type II)	1	6.25
Low voltage QRS, , P mitrale and Atrial Fibrillation	7	43.75
Biventricular enlargement	2	12.50
Atrial stand still	1	6.25
Atrial fibrillation	1	6.25
Electrical alternanace	1	6.25
Ventricular tachycardia (VPCs)	1	6.25
Right ventricular enlargement (Deep Q-waves)	1	6.25
Left ventricular enlargement	1	6.25

dogs were affected with DCM which was confirmed by echocardiography. The clinical signs exhibited in dogs with cardiomyopathy were exercise intolerance- 16/16 (100%), coughing-05/16 (31.25%), dyspnoea 04/16 (25%), syncope -03/16 (18.75%), weaknes/lethargy/ drowsiness-12/16(75%), pedal edema- 02/16 (12.50%), cyanosis of tongue- 03/16 (18.75%), emaciation- 04 / 16(25%), overweight -03/16 (18.75%), ascites 04 (25%). Almost all the dogs 16/16 (100%) were having cardiac murmurs of auscultation.

Cases of DCM were most commonly found in Labrador (07/16) followed by German shepherd (03/ 16), Pomeranian (03/16), Pug (01/16), Great Dane (01/ 16) and Cross-bred (01/16). The DCM was most commonly found in age group between 1 and 3 years (4/16), 3 and 5 years (3/16), 5 and 7 years (4/16), 7 and 10 years (3/16) and > 10 years (01/16). The DCM was more frequently encountered in males (12/16) than females (4/16).

The physiological parameters of dogs having cardiomyopathy were compared with healthy dogs (Table-1). The heart rate, systolic blood pressure, diastolic blood pressure, and respiration rate did not differ significantly with healthy control dogs respectively. MAP of cardiomyopathic dogs were found significantly (P<0.05) higher than the healthy dogs. The most frequent ECG findings in the DCM cases low voltage QRS followed by biventricular enlargement (Table-2).

The clinical signs exhibited by cardiomyopathic dogs in the present study were exercise intolerance, coughing, dyspnoea, syncope, weakness/lethargy/ drowsiness, pedal edema, cyanosis of tongue, emaciation, overweight, ascites and cardiac murmurs on auscultation. Exercise intolerance and dyspnoea were the result of forward failure due to reduced cardiac output (Meurs *et al.,* 2002). Ascites was recorded concurrently with DCM due to right side heart failure. Ascitic fluid could result in reduced electrical conductivity on the body surface (Pandian, 2005).

The cardiomyopathy was highest in age group between 1and 3 years -04/16 (25%) and 5 and 7 years - 4/16 (25%). Broschk and Distl (2005) reported that the age of onset of DCM varies between 3 and 7 years is in agreement with the findings of present study. Wess *et al.* (2010) reported DCM prevalence in various age groups was as follows: age group between 1 and 2 years (3.3%), 2 and 4 years (9.9%), 4 and 6 years (12.5%), 6 and 8 years (43.6%), and >8 years (44.1%). The DCM was found more in male than female. The similar findings of higher incidence of cardiomyopathy in male has also been reported by earlier workers (Kumar *et al.,* 2010).

The highest incidence of cardiomyopathy in was recorded in Labrador (43.75%) followed by 18.75% in German shephered and Pomeranian. In present study, the large breed of dogs was found to be most commonly affected with dilated cardiomyopathy. The similar finding was also recorded earlier by various other workers (Kumar *et al.,* 2010). Further it has been reported that prevalence rate are much higher in prospective studies (screening for the presence of DCM, without clinical signs) viz. 63.2% in Doberman (O'Grady, and Horne, 1998); 24.2% in Irish Wolfhound (Vollmar, 2000) ; 17.6% in Newfoundland (Dukes-McEwan, 1999).

The physiological parameters of cardiomyopathic dogs were compared with healthy dogs. The HR, SBP, DBP and RR in present study were within the physiological ranges (Weiser *et al.,* 2008). The most common ECG abnormality found in present study was Low voltage QRS followed by biventricular enlargement. The higher number of low voltage

complexes recorded in present study could be due to ascites (Pandian, 2005).

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'J' wave syndrome in dogs- An electrocardiographic study

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Abstract

'J' wave is a deflection at R-ST junction and has been reported in humans under physiological and pathophysiological condition. The present study revealed the occurrence of 'J' wave in 3.0% dogs of different age and breeds among the population of 2000 dogs. Forty five dogs showing 'J' wave were clinically healthy and 15 dogs were hypothermic (temp. < 92 ° F).In healthy dogs 'J' wave was buried or partially burried in QRS complex. While in hypothermic dogs 'J' Wave was distinct having mean amplitude of 0.112±0.0003 mV and duration of 22.8±2.44 ms. A distinct and well formed 'J' wave seems to be pathognomonic in hypothermic dogs.

Keywords*:* Dogs, hypothermia, 'J' wave

'J'wave is a deflection at R-ST junction in electrocardiogram (J point) and has been observed in humans under physiological and pathophysiological conditions (Detweiler, 2010). It has also been detected in dogs while studying the effect of hypothermia on respiratory and cardiac functions and termed as 'Osborn' wave. In humans a typical well formed 'J' wave has been found associated with severe ischaemia (Yan and Antzelevitch 1996), hypothermia, hypercalcaemia, brain injury, subarachnoid haemorrhage, Chagas disease, sepsis, vasospastic angina, Brugada syndrome, acute myocardial ischaemia, Prinzmetal angina, hypertrophy of the left ventricle, cocaine or haloperidol overdosing and idiopathic ventricular fibrillation in inferior leads. Recently Agudelo and Schanilac (2015) have reported 'J' wave in dogs in Czech Republic. However, there is lack of information about the incidence and characteristic of 'J' wave in canine population in India. Therefore, an attempt was made to review the position of 'J' wave in electrocardiogram of dogs referred at the Hospital.

Materials and Methods

Two thousand electrocardiograms of the dogs, taken at the hospital during January, 2008 to April, 2016 for evaluating heart in health and diseases, were reviewed for the occurrence of 'Osborn' or 'J' wave. Electrocardiograms were recorded on the dogs in right lateral recumbency employing hex axial lead system in a room with calm and quite surroundings using Magic RX (Maestros Mediline Systems Limited) electrocardiographic machine at a paper speed of 25 mm /second calibrated to display voltage signals at 1 cm/mV. Owners were allowed to be with the dogs to make them comfortable and minimize stress. No electronic filter was used. Amplitude and duration of 'J'wave was calculated manually with the help of hand lens.

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Results and Discussion

The presence of 'J' or Osborn wave (Fig. 1,2,3) was detected in 60 dogs of varying age (4 months to six years) and breeds (German shepherds, Labrador, Mastiff, Rottweiler, Great Dane, Pomeranian, Doberman, and Non descript) making its prevalence as 3.0 % in the population of 2000 dogs. In the present study the prevalence of 'J' wave was very low as compared to ear report (Rudling *et al*., 2016) in a study consisting of 206 healthy dogs of 11 breeds. They further reported that the prevalence of 'J' was very high in dogs of Petit Basset Griffon Vandeen breed (21/23, 91%). No such breed predisposition was clear in the present study. The 'J' wave was mainly detected in lead II and/ or avF. Forty five dogs showing 'J' wave were clinically healthy and were not associated with any pathological condition. Remaining 15 dogs showing 'J' waves were hypothermic (core body temperature < 92^0 F). In 77.77% (35/45) of the healthy dogs 'J' wave was buried in QRS complex (Fig.1). In rest 25 dogs (10 healthy and 15 hypothermic) 'J' was partially buried or markedly distinct. Overall 'J' wave amplitude and duration was 0.112±0.0003 mV (median 0.10 mV); and 22.8±2.44 ms (median 20.0 ms) respectively. 'J' wave in hypothermic dogs has been attributed to delayed ventricular depolarization and early repolarization, tissue anoxia and acidosis (Bruson *et al*. 2005). The presence of a prominent action potential notch in

Electrocardiograms showing 'J' wave

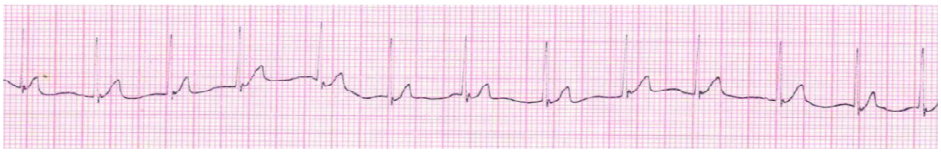

Fig.1. Electrocardiogram of an adult healthy male German Shepherd dog showing 'J' wave buried in QRS complex.Lead II.

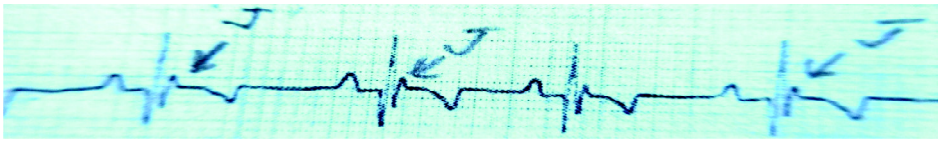

Fig.2. Electrocardiogram of a male hypothermic non-descript dog showing distinct 'J' wave. Lead II.

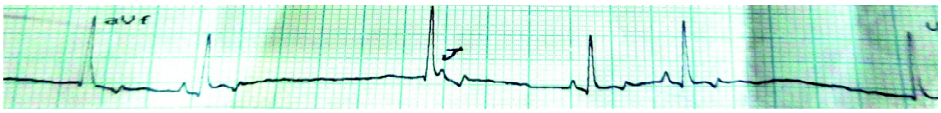

Fig.3. Electrocardiogram of an adult female hypothermic nondescript dog showing bradycardia and distinct 'J' wave in Lead II.

epicardium but not endocardium is shown to provide a voltage gradient that manifests as a J (Osborn) wave or elevated J-point in the ECG (Yan Gan-Xin and Antzelevitch, 1996). Agudelo and Schanilec (2015) detected 'J' wave in 40% control and in29.1% DMVD (degenerative mitral valve disease) affected dogs and found no effect of submaximal exercise test on size and shape of 'J' wave. In both humans and animals a prominent 'J' wave in electrocardiogram has been considered pathognomonic of hypothermia (Eagle, 1994),hypercalaemia (Sridharan and Horon, 1984) and a marker for substrate capable of generating life threatening ventricular arrhythmias (Antzelevitch and Yan,2010).

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Methicillin-resistant *Staphylococcus aureus* isolated from domestic and wild animals of Kerala and Karnataka

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) organisms are a major public health problem because of their multiple antibiotic resistance properties. A total of 73 swab samples were taken from nasal and wound lesions of domestic and wild animals (53dogs, 6 cattle, 8 goats, 5 deer and 1 monkey) from non-governmental organisations (KARUNA and CUPA) of Bangalore, Karnataka and teaching veterinary clinical complex and center for wild life studies, Veterinary College, Pookode, Kerala. Out of 73 samples, 56 were found positive for *S. aureus* by biochemical tests and by PCR. Molecular confirmation of MRSA was carried out by PCR. MRSA was detected in 9 dogs (16.07%) and 1 goat (1.78%) yielding a total of 10 (17.85%) MRSA isolates. Out of these 10 MRSA isolates, 2 were isolated from wound lesions (20%) and 8 (80%) from nasal mucosa. All MRSA isolates were subjected to antimicrobial susceptibility test and were found sensitive to tetracycline (100%) and chloramphenicol (90%) and resistant to gentamicin, co-trimoxazole, ciprofloxacin, methicillin, ampicillin, amoxyclav, cefuroxime, vancomycin and erythromycin indicating their multidrug resistance. The results clearly show increasing resistance of *S. aureus* organisms to β-lactam antibiotics paving way for the emergence of MRSA.

Keywords: Domestic and wild animals, MRSA, Karnataka, Kerala,

The Staphylococcus aureus is one of the most virulent pathogen for humans. It is the causative agent of a variety of deep-seated invasive and toxin-mediated diseases, as well as superficial infections (Lowy, 1998). Methicillin-resistant *Staphylococcus aureus* (MRSA) is emerging as a zoonotic bacterial pathogen of public health importance. It causes nosocomial and community onset infections. MRSA was first identified in UK and was recognized as a hospital-associated pathogen worldwide (hospital-associated MRSA (HA-MRSA) (Deurenberg *et al.,* 2008). MRSA has the capacity to acquire resistance to β-lactam antibiotics by β-lactamase production and acquisition of mec determinants (Lowy, 1998). Indiscriminate use of antibiotics and drug prescription without proper susceptibility tests for quick medication are some key factors for emergence of resistant organisms against antimicrobial agents especially in developing nations (Okeke *et al.,* 2005). The emergence of antibiotic resistance among the pathogens is a rising issue of global worry (Zhang *et al.,* 2006). MRSA express resistance to multiple antibiotics, such as tetracyclines, fusidic acid, fluoroquinolones, macrolides, lincosamides, aminoglycosides, and glycopeptides (Rybak and LaPlante, 2005).

In recent years, MRSA has been increasingly reported as emerging problem in veterinary medicine, particularly in small animal and equine practices. Strains isolated from pet animal cases usually indistinguishable from those isolated from human. Pets become infected through contact with infected people and those pets in turn pass MRSA back to humans. MRSA are not only carried by pet animals but can also cause clinical disease in animals. Since then, MRSA has been found in a variety of other domestic species including dogs (Van Duijkeren *et al.,* 2004; Walther *et al.,* 2008), cats (Bender *et al.,* 2005), cows (Moon *et al.*, 2007), horses (Vanderhaeghen *et al.,* 2010), sheep (Goni *et al.,* 2004), pigs (Meemken *et al.,* 2008), guinea pig. rabbit, turtle, bat, parrot (Walther *et al.,* 2008) and chickens (Kwon *et al.,* 2006).

In absence of appropriate surveillance of antimicrobial resistance, growing increase in MRSA bacteria appended worries in the incidence of infections among variety of wounds and demands constant bacteriological monitoring of pathogen even from minor injuries to know their current antibiotic susceptibility pattern (Khullar *et al.,* 2016). MRSA strains differ in their human/animal affiliation and pathogenic potential. As most reported animal isolates of MRSA are from clinical infections following failure of empirical therapy, there are few epidemiological studies of the prevalence of MRSA in animals (Rich *et al.,* 2005).

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The main objective of the present study was to ascertain the prevalence of MRSA in various animals and to know the antimicrobial susceptibility pattern of obtained MRSA isolates.

Materials and Methods

Collection of samples

A total of 73 sterile swab samples (53 dogs, 6 cattle, 8 goats, 5 deer and 1 monkey) were collected from both nostril and wound lesions of dogs, cattle, goats, deer and monkey from non-governmental organisations (KARUNA and CUPA) of Bangalore and from teaching veterinary clinical complex and centre for wildlife studies, Veterinary College, Pookode, Kerala. The samples were then carried to the laboratory in insulated containers for further processing.

Isolation of *Staphylococcus aureus*

A standard protocol described by Lancette and Bennett (2001) was used for the isolation of *S. aureus*. Swab tips were suspended in 10 ml of staphylococcal enrichment broth and incubated at 37°C for 24h. A loopful of inoculum from the enrichment broth was streaked onto duplicate plates of Baird-Parker (BP) agar medium and incubated at 37°C for 24h. After incubation, the colonies having characteristic appearance (circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, grey black to jet black frequently with light coloured margin surrounded by opaque zone and often with outer clear zone) on BP agar medium (fig.1) were selected and transferred onto nutrient agar slants and incubated at 37°C overnight. The isolates were stored at refrigeration temperature for characterisation. The isolates were further subjected to staining, biochemical tests (Table 1) and molecular reactions (PCR).

Table 1: Confirmation of *S. aureus* isolates by biochemical test

Tests	*Staphylococcus aureus*
(a) Primary test reactions	
Grams staining	+
Motility	-
Catalase	+
Oxidase	-
VP	+
Urease	+
(b) Sugar fermentation reactions	
Lactose	+
Glucose	+
Maltose	+
Sucrose	+

Identification of MRSA

Identification of MRSA isolates to the species level was verified by PCR using primers specific for *S aureus* thermo-stable nuclease, the *nuc* gene and the genotypic identification of MRSA using *mec*A gene (Brakstad *et al.,* 1992). The presence of *nuc* and *mec*A gene was verified in *S. aureus* isolates using the following primers. For *nuc* gene, *nuc*1 5'-GCGATTGATGGTGATACGGTT-3' and *nuc*2 5'-AGCCAAGCCTTGACGAACTAAAG-3'and for *mec*A gene, *mec*A1 5'-AAAATCGATGG TAAAGGTTGGC-3' and *mec*A2 5'-AGTTCTGCAGTA CCGGATTTGC-3' are expected to yield a PCR product of 279 bp and 533 bp for *nuc* and *mec*A gene (fig. 2) respectively. PCR was performed in a 25µl reaction mixture with a PCR buffer containing 200 µm concentration of each deoxynucleoside triphosphate (dNTP), 10 mM Tris-HCL (pH 8.3), 1.5 mM MgCl2, 1 unit of Taq polymerase (Promega), 0.25 µM concentration of each primer and 2.5 µl of DNA template. DNA amplification was carried out for 34 cycles in 25 µl of reaction mixture as follows: denaturation at 94°C for 50 s, annealing at 57°C for 50 s and 58°C for 50 s for *nuc* and *mec*A gene respectively, extension at 72°C for 50 s with a final extension at 72°C for 5 min. The gel is visualized through the agarose gel electrophoresis.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was carried out for the MRSA isolates using disc diffusion method according to Clinical and Laboratory Standards Institute methods (CLSI, 2007). Briefly, each of the isolate was inoculated into nutrient broth (HiMedia) and incubated at 37°C for 24 h. Turbidity of the growing culture was adjusted to correspond with that of a barium sulphate (0.5 MacFarland) standard. About 0.1 ml of the nutrient broth culture was subsequently inoculated onto Mueller Hinton agar plates and spread over the surface with sterile L rods (Spreaders). Antimicrobial discs were then placed on the surface of each plate by means of antibiotic disc dispenser and incubated at 37°C for 24 h. Diameters of inhibition zone were measured using a transparent ruler and the interpretative breakpoints for resistance were determined by comparing zone diameters as recommended by Clinical and Laboratory Standards Institute (CLSI, 2007) and readings were recorded. The methicillin-resistant *S. aureus* isolates were tested

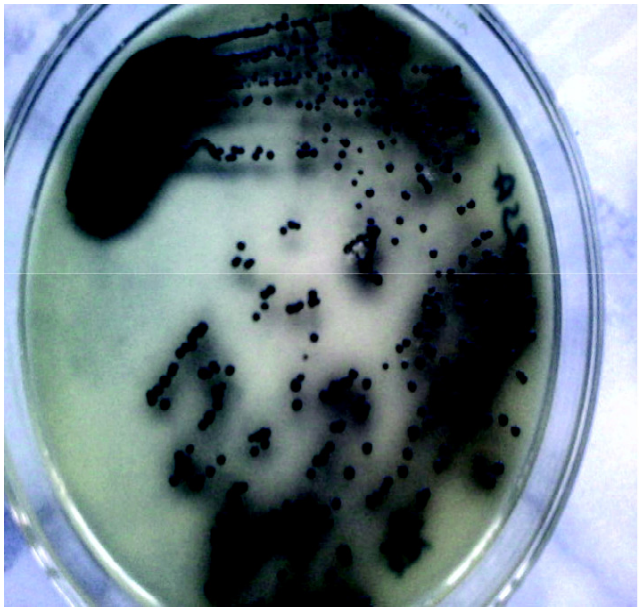

Fig. 1. Colonies of *S. aureus* on BP agar

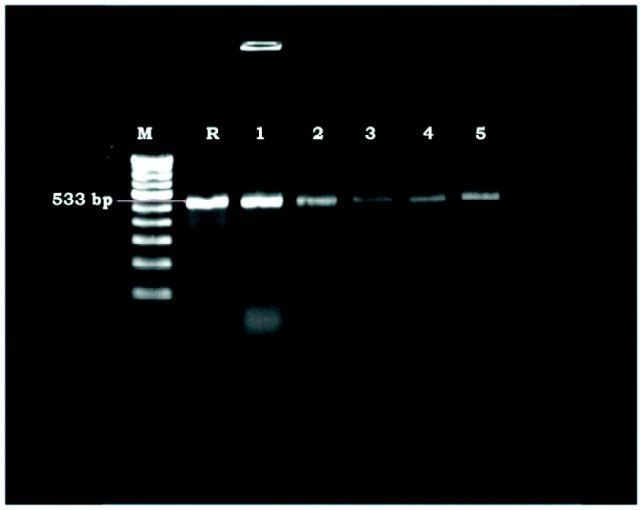

Fig. 2. Gel electrophoresis of *mecA* gene M-Mareker, R-Reference, 1-5: positive samples

against a panel of 11 antimicrobials namely; ampicillin (10 µg), amoxyclav (amoxicillin and clavulanate) (30 µg), cefuroxime (30 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), methicillin (5 µg), tetracycline (30 µg) and vancomycin (10 µg).

Results and Discussion

Prevalence of *Staphylococcus aureus* and MRSA

Out of 73 swab samples, 47 (88.67%) dog, 3 (50%) cattle, 4 (50%) goat and 2 (40 %) deer samples were positive for *S. aureus* (fig. 3). In total, 56 samples

Table 2: Prevalence of MRSA in different species

Species of Animals	Total No of Samples	No of *S. aureus* isolates	No of MRSA isolates	Prevalence of MRSA
Dog	53	47	9	16.07%
Cattle	6	3	0	0%
Goat	8	4	1	1.78%
Deer	5	2	0	0%
Monkey	1	0	0	0%
Total	73	56	10	17.85%

were found positive for *S. aureus*. Thus, the overall prevalence of *S. aureus* was 76.71%. Out of these 56 *S. aureus* isolates, MRSA was isolated from 9 dogs (16.07%) and 1 goat (1.78%) yielding a total of 10 (17.85%) MRSA isolates (Table 2).

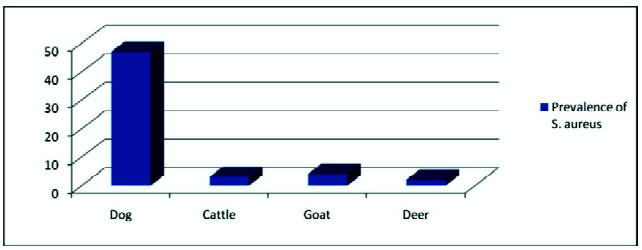

Fig. 3. Prevalence of *S. aureus* in different species

Prevalence of MRSA in different lesions

Out of these 10 MRSA isolates, 2 were isolated from wound lesions (20%) and 8 (80 %) from nasal mucosa (fig. 4).

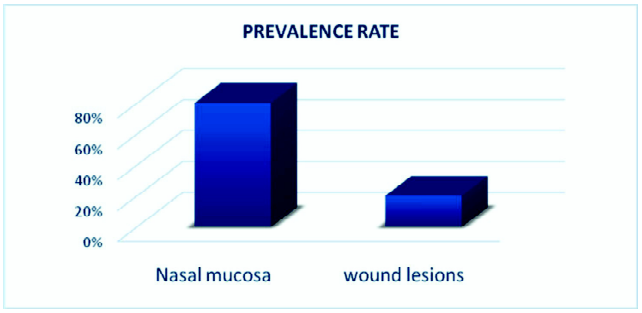

Fig 4. Prevalence of MRSA in wound and nasal lesions

Antimicrobial susceptibility test

All the MRSA isolates were subjected to antimicrobial susceptibility test. The isolates were found highly sensitive to tetracycline (100%) and chloramphenicol (90%) and found resistant to gentamicin, co-trimoxazole, ciprofloxacin, methicillin,

ampicillin, amoxyclav, cefuroxime, vancomycin and erythromycin (fig. 5).

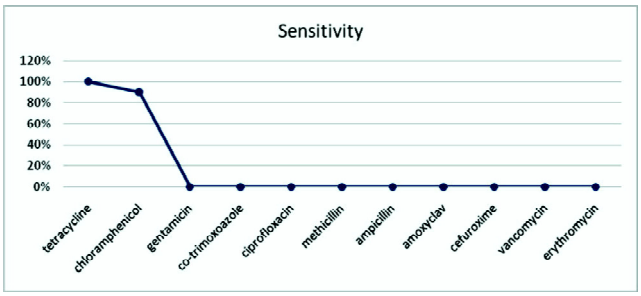

Fig. 5. Antimicrobial susceptibility of MRSA isolates

MRSA is now increasingly recognised in animals worldwide. Pets have been implicated as a source of infection (Sing and Hormansdorfer, 2008). MRSA pose a potential threat to human health through occupational exposure and ease of spread during the increased international movement of livestock and agricultural personnel. Historically, MRSA infections in companion animals involved Human Associated MRSA strains and the direction of spread was linked from humans to animals as the strain causing infections in these animals were closely related to human strains prevalent in the community and the hospitals of same geographical regions (Loeffler *et al.,* 2005). As MRSA becomes established in different animal and human populations, elimination of all risk will be impossible. Asymptomatic colonisation and shedding of MRSA by veterinary and agricultural personnel, together with selection pressures because of antimicrobial feed additives and injudicious usage of antibiotics may contribute to the establishment of MRSA in the food chain and domestic animals (Morgan, 2008). It has become apparent that MRSA can be readily transmitted between humans and animals in the community (Van Duijkeren *et al.,* 2005).

In the present study, prevalence of *S. aureus* and MRSA was found to be 76.71% and 17.85% respectively. MRSA was isolated at a percent of 8.9 from kenneled dogs (Loeffler *et al.,* 2005). In an Irish veterinary hospital, more than half of their 25 MRSA animal isolates were of canine origin, with 8 horses, 1 cat, 1 rabbit and 1 seal also infected (O'Mahony *et al.,* 2005). Twenty nine percent of dog samples were found positive for MRSA (Walther *et al.,* 2008). A study has been conducted on alarming proportions of MRSA in wound samples from companion animals in Germany and reported that MRSA was isolated from 62.7 % (121) of canine wound lesions (Vincze *et al.,* 2014). Two dogs with wounds were found positive for MRSA (Van Duijkeren *et al.,* 2004). A study was conducted on MRSA carriage in different free-living wild animal species in Spain and MRSA was isolated from 0.37% of deer samples (Porrero *et al.,* 2013).

Observations made in the study revealed that all the MRSA isolates were found cent percent sensitive to tetracycline followed by chloramphenicol (90%) whereas completely resistant to gentamicin, co-trimoxazole, ciprofloxacin, methicillin, ampicillin, amoxyclav, cefuroxime, vancomycin and erythromycin. All the MRSA isolates were multidrug resistant. These were in accordance with antibiotic sensitivity pattern observed by Tiwari *et al.* (2012). The results were supported by findings of other study which evidenced that MRSA isolates recovered from wounds showed resistance of 100% and 96% to ampicillin, respectively (Yaseen *et al.,* 2013; Hemamalini *et al.,* 2015). Similar findings were documented earlier by Bozdogan *et al.* (2003) and Falagas and Karveli (2001). Work done in developed countries highlighted the emergence and spread of resistance in *S. aureus* against penicillin, Methicillin, tetracycline and erythromycin groups of drugs with pace (Kaur and Chate, 2015). O'Mahany *et al.* (2005) also supported the present findings in which MRSA was isolated from 25 animals with recovery of 56% from canines and MRSA isolates were resistant to macrolides and flouroquinolones group of antimicrobials which are in conformity with our findings.

Comparatively higher prevalence of MRSA from dogs could be due to close contact of humans with pet animals. Earlier records also suggest that colonization of hospital strain of MRSA is increasingly documented from pets including dogs and cats, which is an indicative of close contact of humans and dogs (Kelly *et al.,* 1999). In this way pets are acting as a reservoir of resistant MRSA strains for re-infecting in-contact human population. This is supported by the literature (Walther *et al.,* 2012) which mentioned the homology of MRSA isolated from animals with human MRSA through PFGE. It is documented that farm animal MRSA and community acquired MRSA are genetically more identical as compared to hospital acquired MRSA (Weiler *et al.,* 2011).

Conclusion

The present study reported the higher prevalence of MRSA in pet animals and all the MRSA isolates were found multidrug resistant which is an important finding. MRSA can transfer among the closely kept animals and also from animals to humans. Hence the pet owners, farm workers, veterinarians, persons working at abattoirs are more prone and are at more risk.

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Bovine mastitis in Kashmir: epidemiology and therapeutic study

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Abstract

The aim of this study was to find epidemiological factors associated with incidence of mastitis in cows and its management. Higher prevalence of mastitis was recorded in crossbred cattle aged between 4 and 5 years during summer season. Animals mostly suffered within few days of post-partum. Left quarters affected more than right ones with characteristic changes in colour and consistency of milk. The disease appeared in acute, chronic and sub clinical forms. Organisms isolated from the affected quarters were *Staphylococcus sp., Streptococcus sp., Escherechia sp. Corynaebacterium sp., Klebsiella sp., Pseudomonas sp.* and *Nocardia* species. Gentamycin was found highly effective both in *in-vivo* and *in-vitro* studies.

Keywords*:* Cattle, Mastitis, Prevalence.

Mastitis is oldest diseases of milch animal ranks second to FMD as most challenging disease in high yielding dairy cows in India. The disease is most important in terms of its effect on quality and quantity of milk besides public health hazards due to antibiotic residues and therapeutic agents in milk. The disease causes heavy economic losses and a costliest production disease in dairy herds worldwide (Miller *et al*., 1993). In India, losses due to mastitis amounting to the tune of rupees 2009.32 crores per annum have been reported (Sirohi and Sirohi, 2001). The disease is complex in nature with a diverse nature of etiological agent. The epidemiology of mastitis differs with environment. The environment and bacterial fauna of Kashmir is different from other parts of India and accordingly the pathogen density may differ. With this hypothesis the present study was undertaken to record the prevalence and management of mastitis under the study area or other similar temperate areas.

Materials and Methods

A total of 207 cases of bovine mastitis presented both at the farmer's house and also at the Clinical Complex of the Faculty were served as animals for this study. All the cases were confirmed for mastitis on the basis of California mastitis test. In these animals, prevalence with respect to breed, age, season, stage of lactation, previous disease history, disease associated with calving, affection of quarters, milk characteristics, effect of disease on milk production and type of mastitis/ state of udder were recorded. Milk samples from the affected cows were collected aseptically and subjected to cultural sensitivity test with isolation of the organisms. Antibiotic sensitivity was seen against ofloxacin, gentamycin, amikacin, enrofloxacin, ciprofloxacin, chloramphenicol, oxytetracycline, ampicillin/cloxacillin, amoxicillin and penicillin. Based on cultural sensitivity, treatment was administered with the specific antibiotic through parenteral route to see the in vivo efficacy of each drug.

Results and Discussion

The epidemiological data is presented in Table 1. Maximum prevalence was observed in crossbred (91.79%) cattle followed by nondescript (8.21%) cattle. This might be due to more occurrence of former breed than later one as local cattle are being replaced by crossbred one in most part of this region. Maximum prevalence was observed in summer season (42.03%; 87/207) followed by autumn (31.40%; 65/207) and spring (24.64%; 51/207) and minimum in winter (1.93%; 4/207). The higher prevalence in summer could be due to humid climate, which makes the environment suitable for growth of pathogenic organisms (Radostitis *et al.*, 2000). Similar results were obtained by Singh and Pachouri (2004), who recorded maximum incidence in the month of August when environmental temperature and humidity were 31^{0}C and 75% respectively. Singh *et. al*. (1996) recorded higher incidence of mastitis in monsoon season. During these months, the floors of the cow shed also remain wet (Deore, 2001). The teats also

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become moist, which also favours microbial growth and colonization.

Highest numbers of animals affected with mastitis was during 1st calving .85(41.06%) followed by 3rd calving 45 (21.74%) then 2nd calving 27 (13.04%), 4th calving 25 (12.08%), 5th calving 12 (5.80%), 6th calving 10 (4.83%) and lastly lowest prevalence in case of 7th calving 3 (1.45%). The prevalence decreased after 3rd calving. From this observation it was found that the disease was mostly observed in younger animals. Very few animals were affected beyond 6th parity. Generally in Kashmir animals are not kept beyond 6th parity and are sold, which probably resulted in occurrence of few mastitis cases in this group.

The prevalence in early, mid and late lactation was recorded as 55.56, 20.29 and 24.15 per cent respectively. Highest percentage (42.03%, 87/ 207) of animals suffered from the disease within 1st month of calving and that too within few days (preferably 3-4 days) after parturition. Oliver and Calvinho (1995) indicated that bovine udder appeared to be markedly susceptible to new inflammatory infections during physiologic transition of the mammary gland from lactation to involution. Since milk production remains high in early lactation, chance of transmitting infection to udder is also high as milk is a favourable media for bacterial growth and multiplication.

Out of total animals affected, 16.91% (35/207) animals had the history of some illness in their previous lactation which comprised of abortion (1.45%), post parturient udder edema (7.25%) and mastitis (9.18%). It was seen that 8.70% animals suffered from repeated infection of mastitis even after successful treatment on the previous occasion. This might be due to the fact that successive infections were not caused by strain of the same species, or even if caused by strain of the same species, the virulence of the later strain might have been much higher than the virulence of the former strain. A total of 11.11% animals suffered from other diseases at the time of calving, which included milk fever (3.86%), dystocia (1.93%), post parturient paresis (4.35%) and udder edema (0.97%).

Table 1: Involvement of quarters in mastitic cow

Quarters affected	Numbers of animals affected (%)
Left Fore (LF)	29 (14.01)
Left Rear (LR)	28 (13.53)
Right Fore (RF)	27 (13.04)
Right Rear (RR)	10 (4.83)
LF+RF	19 (9.18)
LF+LR	17 (8.21)
LF+RR	2 (0.97)
LR+RR	33 (15.94)
LF+RF+RR	4 (1.93)
LR+RF+R	4 (1.93)
All quarters	34 (16.43)

Quarters affected: Involvement of the disease in different quarters has been shown in Table-1. Single quarters were involved in 45.41% cases; however, two and three quarters were involved in 34.30 and 3.86% cases. Left sided quarters affected more as compared to right sided one. Under village condition milking is usually practiced from left side and if milker's hands are not cleaned properly organism may transmit from hand to the udder.

Milk characteristics: Changes in milk characterized by presence of pus (28.02%,58/207) or blood (2.2.71%, 47/207) or combination of both (7.25%, 15/207), or watery (5.80%, 12/207) or curdling (15.94%, 33/207) in appearance.

Status of milk yield: On average milk production ranged from 8.33 kg/ day/ animal (5-15 kg) before disease to 4.21 kg/ day/ animal (0.25-8 kg) after disease. This shows that mastitis results in drastic reduction of milk yield and thereby leads to heavy economic losses.

Type of mastitis/ state of udder: The disease was acute, chronic and sub clinical in 46.86 (97/207), 26.57 (55/207) and 17.39% (36/207) cases respectively. In addition to this, 7.25% cases of obstructive mastitis (1.45% partial and 5.80% complete) and 1.93% cases of spider teats were also observed. In acute condition, most of the animals (43%) showed hot, painful and swollen udder, where as in chronic condition udder was usually hard which was observed in 19.81% cases. In 6.76% cases udder was fibrosed and these cases did not respond to conventional antibiotic treatment. In subclinical form udder was found normal with only changes in milk characteristics. Cows with subclinical mastitis are characterized by no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% (Holdway, 1992). The subclinical form is considered 15-40 times more prevalent than clinical form and accounts for greater losses in terms of milk production (Harmon, 1994). This

Table 2: Antibiotic sensitivity pattern of mastitic milk

Antibiotic (no. of samples studied)	In-vitro study			In-vivo study
	Highly sensitive	Moderately sensitive	Resistant	
Ofloxacin (32)	11 (34.37%)	15 (46.88%)	6 (18.75%)	
Gentamycin (32)	10 (31.25)	16 (50.00%)	6 (18.75%)	91.04% (61/67)
Amikacin (32)	7 (21.88%)	17 (53.13%)	8 (25.00%)	
Enrofloxacin (31)	5 (16.13%)	18 (58.06%)	8 (25.81%)	88.57% (62/70)
Ciprofloxacin (30)	4 (13.33%)	20 (66.67%)	6 (20.00%)	56.76% (21/37)
Chloramphenicol (34)	4 (11.76%)	22 (64.71%)	8 (23.53%)	57.75% (41/71)
Oxytetracycline (31)	4 (12.90%)	18 (58.06%)	9 (29.03%)	
Ampicillin/ cloxacillin (31)	3 (9.68%)	17 (54.84%)	10 (32.26%)	
Amoxicillin (34)	3 (8.82%)	16 (47.06%)	15 (44.12%)	
Penicillin (34)	3 (8.82%)	13 (38.24%)	18 (52.94%)	

form represents a reservoir of infectious organisms, and it is related to approximately 70% of economic losses of mastitis (Varshney and Naresh, 2004).

Etiological factors: In the present study most of the cases were of bacterial (93%) origin. However, 6.25% cases were of fungal in nature. Organisms isolated were S*taphylococcus, Streptococcus, E. coli, Corynaebacterium, Klebsiella, Pseudomonas* and *Nocardia* species.

Antibiotic sensitivity: The antimicrobial sensitivity results are presented in Table 2. Out of the total samples subjected to cultural sensitivity testing 34.37, 31.25, 21.88, 16.13, 13.33 and 11.76% were highly sensitive against ofloxacin, gentamycin, amikacin, enrofloxacin, ciprofloxacin and chloramphenicol respectively (Table-2). Oxytetracycline, ampicillin/cloxacillin, amoxicillin and penicillin were found resistant against the organisms. Chanda *et. al.* (1989) recorded highest sensitivity of 40% against gentamycin followed by ampicillin, tetracycline, chloramphenicol, kanamycin and nitrofurantoin. The variation of results may be due to development of resistant organisms to different antimicrobial agents owing to indiscriminate use of antimicrobial agents in a particular locality. The selection of antibiotic for treatment of mastitis should therefore be made on the basis of sensitivity as evidenced by cultural sensitivity testing and pharmacokinetic characteristics of the drug (Srivastava, 2001).

The in vivo testing of these drugs showed best results against gentamycin followed by enrofloxacin. Chloramphenicol and ciprofloxacin also found satisfactory but not to that good cure rate.

Conclusion

On the basis of the study, it may be concluded that Higher prevalence of mastitis was recorded in crossbred cattle aged between 4 and 5 years during summer season. Animals mostly suffered within few days of post-partum. Left quarters affected more than right ones Organisms such as *Staphylococcus sp., Streptococcus sp., Escherechia sp. Corynaebacterium sp., Klebsiella sp., Pseudomonas sp.* and *Nocardia* sp were main cause of mastitis in Kashmir. Gentamycin was found highly effective for treatment of mastitis in this region

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Spirocerca lupi infection in Labrador dog and it's management

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Abstract

A male Labrador dog of two years age was presented with a history of continuous vomition particularly just after feeding for more than two months. The dog had altered behavior and eats on foreign bodies like pieces of carpet, ground grass, stones etc. just after feeding but before vomiting. There was drastic loss of body weight around 7 kg during last two months. The dog was completely avoiding solid foods. The fecal sample examination was found positive for *spirocerca lupi.* The dog was administered with levamisol @ 5mg/kg bwt subcutaneously fortnightly for four injections and diethyl carbamizine @ 10mg/kg bwt daily for 15 days. The clinical recovery was noticed after four days of treatment and the fecal sample examination remained negative for the said nematode ova till three months after end of the treatment.

Keywords: Diethyle Carbamazine, Levamisole, ***S****pirocerca lupi,*

The *Spirocerca lupi* is a nematode parasite of dogs with worldwide distribution, particularly in the regions with warm climate like India (Kumar *et al*, 1981:, Ramchandran *et al*, 1984). The clinical severity in infested dogs depends on location of larva and adult worms. The migration of infected larvae through walls of gastric arteries and gastroepiploic artery produces continuous vomition (Bailey, 1972). The adult worms produce esophageal granuloma, aortic aneurysm and interfere with swallowing leading to persistent regurgitation and vomition to sudden death due to rupture of arteries resulting in hemothorax (Aroch, *et al,* 2011, Fox, *et al*, 1988, Ivoghli,, 1977).

The disease can be diagnosed by detecting embryonated eggs in faeces which is very rare (Traversa,, *et al,* 2008) and metastatic lesion to nodular granuloma on the wall of esophagus which can be detected by barium meal (Fox *et al.,* 1988). The report on *S. lupi* in Indian is scanty. The present paper reports *S. lupi* infection in Labrador dogs, it's diagnosis by fecal sample examination and successful treatment.

A male Labrador dog of two years age was presented to the outdoor of Teaching Veterinary Clinical Complex with the history of vomition for more than two months. There was continuous vomition just after eating. The dog was madly searching for some foreign objects like grass, soil; carpet pieces etc., just after eating or drinking and was vomiting immediately thereafter. The feed intake was reducing gradually. At the time of presentation, the dog was completely avoiding solid foods. The health condition deteriorated drastically with loss of 7kg body weight within last two months. The dog had high affinity to eat cow dunk. The biochemical parameters of the blood sample for liver and kidney function showed the values like AST – 35 I.U/L, ALT-30 I.U/L, Alkaline phosphatase 48 I.U/L, Serum cholesterol 233 mg/dl, fasting plasma glucose 83mg/dl, BUN and Creatinine values were also within the normal range. The complete blood count report showed eosinophillia with normal hemoglobin and TLC.

The dog was previously treated for gastritis with pantoprazol, ondasetron, ceftriaxone with tazobactum, I.V, lyphilised liver extract I.M, and digestive enzymes orally. The clinical signs did not subside after continuous treatment for 15 days.

The dog was referred for digital X-ray and C-arm view of esophagus, pharynx, lungs and mediastinal lymphnodes but there was absence of any nodular or metastatic lesion. The barium meal test could not be done as the owner did not agree for force full swallowing of barium meal. The fecal sample was sent to the parasitology laboratory and was found positive for *Spirocerca lupi* ova both in direct smear and floatation method with saturated zinc sulphate and sugar floatation technique.

The dog was treated with Levamisole @ 5mg/kg bwt subcutaneously at an interval of 15 days for four injections and diethyl carbamizine @ 10mg/kg bwt for 15 days. The vomiting tendency after eating solid food was reduced by more than 50% and there was no

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vomition after drinking milk as reported by the owner 2 days after treatment. The dog recovered uneventfully and the clinical signs completely disappeared after 4 days of treatment. Multivitamins, aminoacids and digestive enzymes were prescribed for 15 days for oral route. The diethyl carbamazine was effective in ameliorating the typical clinical signs of vomition and regurgitation in animals with esophageal nodule, and improving the overall health status of the animal (Lisel *et al*, 2008).

The fecal sample was again examined microscopically through floatation technique which was negative after 15, 30 and 90 days after the 4th dose of Levamisol injection. The body weight of the dog slowly improved after 15 days of treatment.

The present case was diagnosed at its uncomplicated stage and the dog recovered uneventfully. This study documented infection in successfully management of *S. lupi* using levamisol @ 5mg/kg bwt subcutaneously fortnightly for four injections and diethyl carbamizine @ 10mg/kg bwt daily for 15 days.

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Therapeutic management of snake bite in buffalo- A Case Report

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Abstract

This report describes successful management of snake bite in a seven year old Murrah buffalo and lesions in other animal which died on the way to TVCC, RAJUVAS, Rajasthan . The buffalo was exhibiting the excessive frothy salivation, respiratory distress, mild rise of body temperature, restlessness and bluish discoloration of skin with fangs marks. The haematological analysis showed reduced levels of Hb, TEC and PCV and elevated levels of TLC and neutrophills. The biochemical analysis revealed increased AST, ALT, triglyceride, total bilirubin, total protein, albumin and globulin and no changes found in serum creatinine, BUN and ALP. Necropsy findings of heifer revealed punctured wound; petechial to ecchymotic hemorrhages on buccal mucosa peritoneum, epicardial and the subendocardium surface, congested lung, spleen and liver parenchyma and froths in trachea. The affected buffalo was treated with an anti-snake venom serum (ASVS) with NSS, tetanus toxoid, glucocorticoid, antibiotic, fluid therapy and B-complex. Buffalo recovered after 3 days of commencement of treatment.

Keywords*:* Anti-snake venom serum, Buffalo, Haemato-biochemical, Snake bite

Case Presentation

A seven year old high yielding Murrah buffalo and another two year old buffalo heifer were presented at Teaching Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner with complaint of snake bite from a nearby village. The owner of buffalo reported that heifer was died during transportation to clinics. The attendant of animals had seen cobra snake moving in the vicinity of buffalo yard. The buffalo and heifer were healthy with no previous history of any clinical illness before this incident.

The buffalo was exhibiting the excessive frothy salivation, respiratory distress, mild rise of body temperature (102.8°F) and restlessness. The skin of the animal was cynotic and it appeared to be bluish in color, pupil was dilated and mild swelling at the site of bite. Fang marks were observed on back perhaps due to bite in sitting posture. Fangs marks were noticeable after retraction of skin with accumulation of sero-sanguineous fluid and some blood clots at the site matching to the fang marks. On close examination of skin revealed presence of pair of 1.5 mm elliptical and red puncture wounds. The respiration rate and pulse rate were increased to 42 per minute and 76 per minute, respectively. The haematological analysis showed reduction in haemoglobin (8.4 g/dl), total RBC count ($5.1x10^6$/µl) and PCV (23%) values; elevated levels of Total Leucocyte Count (TLC) $16.8x10^3$/µl and neutrophillia (N 73%) was seen on the differential leucocyte count. The biochemical analysis revealed increased in values of serum AST (249 U/L), ALT (122 U/L), triglyceride (38 g/dl), total bilirubin (0.9 mg/dl), total protein (9.1 g/dl), albumin (4.3g/dl) and globulin (4.8 g/dl). The values of serum creatinine (1.6mg/dl), BUN (32 mg/dl) and ALP (93 IU/L) were within the normal range. Similar findings were also reported by Pal *et al.* (2012) and Farooq *et al.* (2014).

Necropsy of buffalo heifer was conducted on request of animal owner to find out exact cause of death. There was punctured wound with oedema and haemorrhage on the right forelimb. The buccal mucosa showed the petechial hemorrhages. There were petechial to ecchymotic hemorrhages on the peritoneum, epicardial and the subendocardial surface of heart, linear streaks were present on the right ventricular myocardium. Congested lung, spleen and liver parenchyma were observed; intestines were hemorrhagic. The lumen of trachea revealed frothy contents. These findings were accordance with Banga *et al.* (2009) and Farooq *et al.* (2014).

Treatment and Discussion

The affected buffalo was treated with anti-snake venom serum (ASVS) 4 vial (40 ml) with two liter NSS, tetanus toxoid (5 ml i.m.), glucocorticoid therapy (Dexamethasone @ 2 mg/kg b. wt. i.v.), antimicrobial (Amoxicillin @ 10 mg/kg i.v.), fluid therapy (5% DNS i.v. @ 30 ml/kg b.wt) and B-complex (10 ml i.v.). The animal was kept under close observation for providing

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careful monitoring. The buffalo recovered fully and became clinically active after 3 days of commencement of treatment.

The composition of snake venom is highly complex containing many proteins, enzymes and strongly basic polypeptides (Jiminez–Porras, 1968). Cobra snake are highly poisonous and can deliver about 200–500 mg of venom on average (Shea, 2005) and their bites are very common in the morning and evening than during night (Punde, 2008). Venom secretion in all venomous snakes appears to vary in seasons; more in warmer months with high morbidity and fatality (Bawaskar and Bawaskar, 2015). The venom of snakes of family Elapidae is predominantly neurotoxic which causes paralysis and death due to respiratory failure within 20 minutes to 6 hours (Prasad and Koley, 2006; Radostits *et al.,* 2007). Neurotoxins present in the snake venom attack the central nervous system and results in failure of cardiovascular system. Krait and cobra venom contains acetylcholine (Ach) esterase, phospholipase B and glycerophosphatase. Phospholipase A2 is found in the majority of venom. It destroys mitochondria, RBCs, leukocytes, platelets, peripheral nerve endings, skeletal muscles, vascular endothelium, presynaptic neurotoxicity, has opiate-like sedative effects, and cause auto pharmacological release of histamine. Hyaluronidase promotes the spread of venom through the tissue. Proteolytic enzymes are responsible for local changes in permeability leading to edema, blistering, bruising and local necrosis (Williams *et al*., 2010; Warrell *et al.,* 2013).

Polyvalent antivenom available in India acts against krait, cobra, Russell's viper and Echis. It accelerates the dissociation of the toxin-receptors complexes and reverses the paralysis. Anti-snake venom neutralizes circulating venom and it has no action once the venom is attached to the receptor site. Antivenom should be administered as soon as signs of systemic or severe local swelling are noted. The mean times between envenoming and death are 8 h (12 min to 120 h) in cobra (Williams *et al.,* 2010; Warrell, 2010). It is suggested that some precautionary measures should be adopted to control the snake population in nearby areas of animal sheds. Moreover, snake population could be controlled by searching their hides and destroy them.

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Clinical Article

Ultrasonographic diagnosis of adrenal gland tumor in a dog

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Abstract

An eight years old intact male dog weighing 17 kg was brought to the Madras Veterinary College Teaching Hospital with the history of lethargy, polyphagia and weight loss. Upon clinical examination the dog's mentation was dull with a BCS of 4/9 and was lying down throughout the examination procedure. The blood pressure was recorded Doppler apparatus and the dog was found hypertensive (Systolic blood pressure :180 mmHg). Complete blood count, serum biochemical analysis and urinalysis performed on the dog were unrewarding. The total T4 levels were within reference limits. The dog was then subjected to abdominal ultrasonography and echocardiographic procedures. Although echocardiography revealed normal study, abdominal ultrasound revealed a mass above the kidneys bilaterally. Upon visualising from multiple windows, it was confirmed as an adrenal gland mass of dimensions,

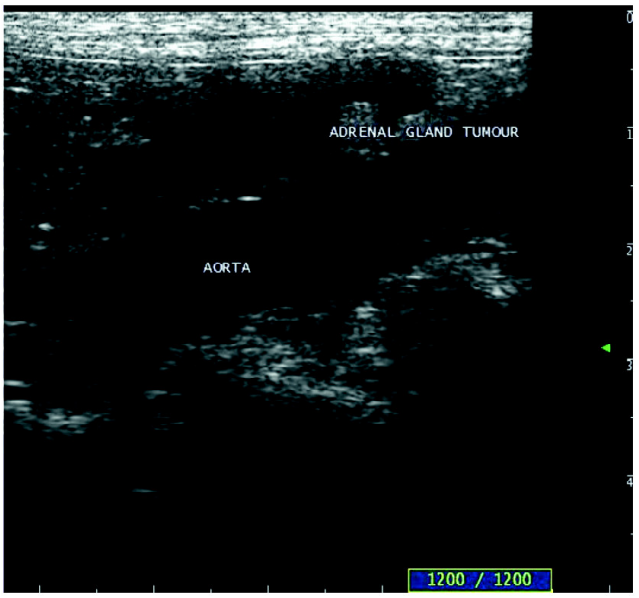
Keywords: Adrenal gland, Blood Pressure, Depression Ecography ultrasonography.

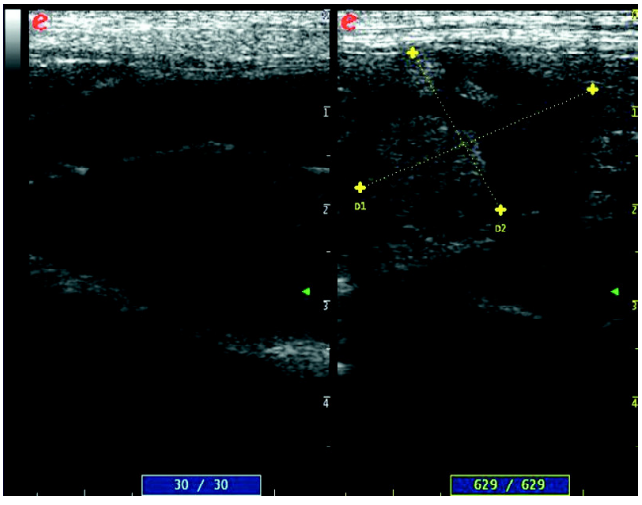
Case Presentation

An 8 years old intact male dog weighing 17 kg was brought to the Madras Veterinary College Teaching Hospital with the history of lethargy, polyphagia and weight loss. Upon clinical examination the dog's mentation was dull with a BCS of 4/9 and was lying down throughout the examination procedure. The dog was hypertensive (180 mmHg) which was diagonsed using a Doppler apparatus. Rest of the routine explorations were normal. A complete blood count, serum biochemical analysis and urinalysis performed on the dog were unrewarding. The total T4 levels were within reference limits. The dog was then subjected to abdominal ultrasonography and echocardiographic procedures. Although echocardiography revealed normal study, abdominal ultrasound revealed a mass above the kidneys bilaterally. Upon visualising from multiple windows, it was confirmed as anadrenal glandmass of dimensions, 2.6 cm by 1.8cm. Liver showed multiple areas of hyperechogenecity and mixed echogenicity of the parenchyma suggestive of metastases. However, thoracic radiographs showed no signs of metastasis.To confirm that the mass was a fully functional growth causing the signs in the dog, a standard ACTH test and a Low Dose Dexamethasone Suppression Test was planned, however the owner denied approval.

Treatment and Discussion

Radical adrenal gland removal being the gold standard of therapy was suggested to the owner who was not willing to consent to it. The pet did not make it to the end of the day besides palliation and a necropsy was performed. Bilateral adrenomegaly was appreciated





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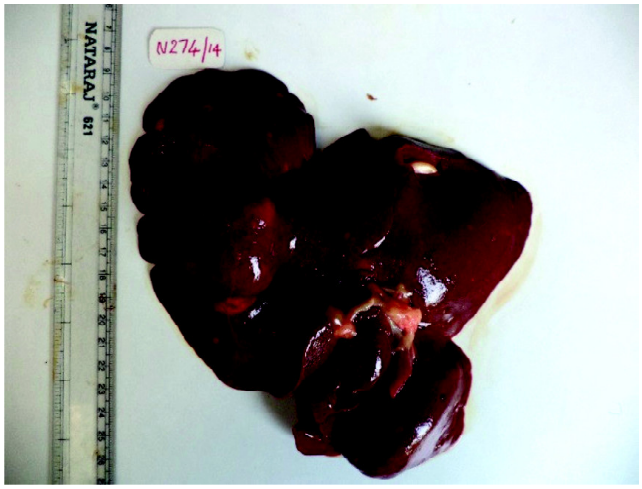

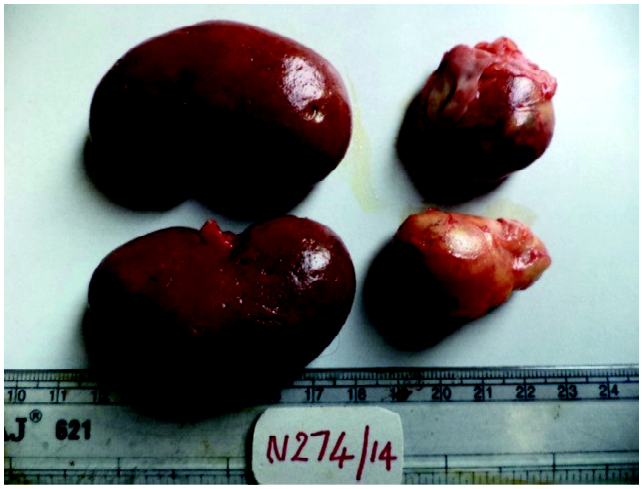

with gross parenchymal changes. Liver showed multiple discoloured nodules which were later confirmed as metastasesof the adrenal adenocarcinoma by histopathological examination.

Primary adrenal gland tumors are a rare diagnosis in canine and are often an accidental finding. Their occurrence is limited to 1-2% of all canine neoplasia (Bailey and Page 2007). The most common types of tumors of the adrenal gland are the adenoma and the adenocarcinoma. Due to the wide range of clinical signs and varying presentations, making a confirmatory diagnosis clinically has been challenging prior to the incorporation of ultrasonographic imaging in veterinary science. The presented case describes the diagnosis of a bilateral arenal gland tumorin a dog.

Abdominal ultrasonography has become the test of choice for determining the origin of hyperadrenocorticism in dogs (Loste *et al*.). Furthermore ultrasonography has also been playing a vital role in the diagnosis of metastasis till liver via the invasion into caudal vena cava. Even with such advanced diagnostic support most adrenal gland tumors slip from being diagnosed due to their clinically silent nature. Such masses discovered accidentally during ultrasonographic procedures for other suspected issues are tagged as incidentalomas (Singh and Buch2008; Terzolo et al. 2009; Galac *et al*. 2010).

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Chronic renal failure due to ehrlichiosis in a dog

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Abstract

A 3 years old, male Labrador was presented to TVCC, CVAS, Bikaner with history of dullness, marked weight loss, off feed, vomiting, diarrhoea and decrease urine output, subnormal temperature, elevated pulse and respiratory rate and dehydration. History revealed that dog was diagnosed and treated for ehrlichiosis with doxycycline. Haematology, urinanalysis, serum biochemistry, ultrasonographic and radiographic findings revealed that dog was suffering from IRIS graded stage 4th of chronic renal failure .Radiographic examination revealed kidney length to L2 vertebrae ratio 1.8. Ultrasonography showed enlarged spleen and small sized kidney with hyperechoic periphery, loss of architectural detail of renal parenchyma, indistinct contours of renal cortex and lack of demarcation of corticomedullary junction. During three week of therapy, there was no improvement and ultimately animal was euthanized on request of owner. Post mortem showed small sized kidney with loss of corticomedullary junction , urinary bladder with normal mucosa, enlarged spleen , normal heart and liver, congested lungs, trachea containing frothy exudate, empty intestine and stomach with haemorrhagic mucosa.

Keywords*:* Chronic renal failure, Doxycycline, Dog, Ehrlichiosis

Case Presentation

A 3 years old, 29 kg, male Labrador was presented to TVCC, CVAS, Bikaner with history of dullness, marked weight loss, off feed, vomiting, diarrhoea and decrease urine output since 2 weeks. On detail examination dog was found to have subnormal temperature, elevated pulse and respiratory rate, dehydration, increase capillary refill time (5 sec), increase skin tenting time (8 sec), sunken eyes, pale mucous membrane, melena and uremic breath. Further, previous history revealed that dog was diagnosed and treated for ehrlichiosis three months before and recovered successfully. Haematology, urinanalysis, serum biochemistry, ultrasonographic and radiographic findings revealed that dog was suffering from IRIS graded stage 4th of chronic renal failure (Table 1 and 2). Radiographic examination revealed kidney length to L2 vertebrae ratio 1.8. Ultrasonography showed enlarged spleen and small sized kidney with hyperechoic periphery, loss of architectural detail of renal parenchyma, indistinct contours of renal cortex and lack of demarcation of corticomedullary junction. Chronic ehrlichiosis was suspected to be the cause of the chronic renal failure in the present case.

Treatment and discussion

Dog was managed by intravenous fluid therapy with 5% DNS @ 40ml/Kg b.wt. and Ringers lactate @ 40ml/Kg b.wt., antibiotic cefotaxime @ 25 mg/kg i/v bid, inj. nandrolone decanoate @50 mg/week i/m, and tab. Fosbait (lanthanium carbonate) 500 mg, daily po to reduce blood urea nitrogen and phosphorus levels, respectively. Injection Botropase was given to check internal haemorrhages and recombinant human erythropoietin @50 IU/kg was given for erythropisis. Injection calcium gluconate 10ml slow poision i/v was given to increase blood calcium level and injection Lasix @ 0.2 mg/kg was given to increase urine output. Nausea and vomiting was suppressed using antiemetic ondansetron hydrochloride @0.5 mg/kg bid and antacid pantoprazole @1mg/kg bid. Supportive therapy was ascorbic acid 500mg/day i/v, Neuroxin M (containing methylcobalamin 500 mcg, pyridoxine 50 mg and nicotinamide 50 mg/ml) @ 3 ml, i/v for seven days. During three week of therapy, there was no improvement (Table 1) and ultimately animal was euthanized on request of owner. Post mortem showed small sized kidney with loss of corticomedullary junction (Fig. 1), urinary bladder with normal mucosa, enlarged spleen (Fig. 2), normal heart and liver, congested lungs, trachea containing frothy exudate, empty intestine and stomach with haemorrhagic mucosa.

In this case, the haematological analysis did not reveal *Ehrlichia* organism when presented again in clinic, probably due to previous successful treatment but the present clinical findings are due to renal failure caused by chornic ehrlichiosis. Ehrlichiosis as a cause

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of renal failure is also documented previously by Luckschander *et al.* (2003).

E. canis infection is a multisystemic disease which may cause a large variety of different clinical manifestations. Many of the clinical and pathological abnormalities that develop during the chronic phase are due to immune reactions against the intracellular organism (Waner and Harrus, 2013). In chronic cases, the bone marrow becomes hypoplastic, and lymphocytes and plasmacytes infiltrate various organs. Variable clinical findings based on the predominant organs affected. These include marked splenomegaly, glomerulonephritis, renal failure, interstitial pneumonitis, meningitis and severe weight loss (Jennifer, 2016). Etiology of anaemia in renal failure (King *et al.,* 1992) is multifactorial and major significance is deficiency of erythropoietin due to inadequate erythropoietin production by the diseased kidneys (Chew and Dibartola, 1998b). Options for treating anemia of CRF include hormone replacement therapy, anabolic steroids, and correcting factors promoting red blood cell loss or impairing red blood cell production (Sharma *et al.,* 2015a). Leucopenia observed in this case suports the findings of Kumar *et al*. (2011), who reported decreased total leucocyte count in end-stage renal failure dogs, which reflected the immunocompromised status of animal. Neutrophilic leukocytosis (Sharma *et al*., 2015b) and decrease platelet counts (Srivastava *et al.,* 2011) due to insufficient thrombopoietic activity have been reported in dogs suffering from CRF (Gafter *et al*., 1987). Increase in blood urea in renal failure is caused by impaired ability to excrete proteinaceous catabolites because of marked reduction in glomerular filtration rate (GFR) (Osborne *et al*., 1972), also, increase in creatinine may result from

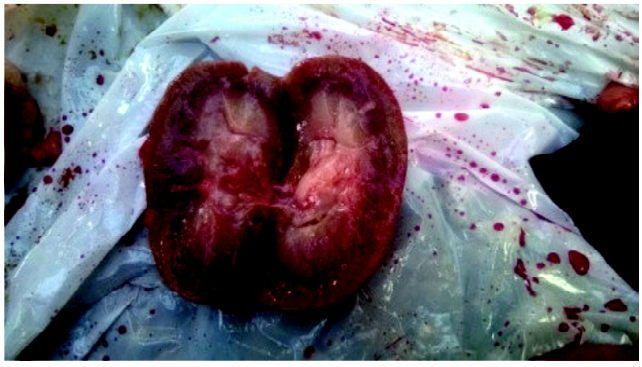

Fig 1. Small size kidney with loss of architectural detail of renal parenchyma

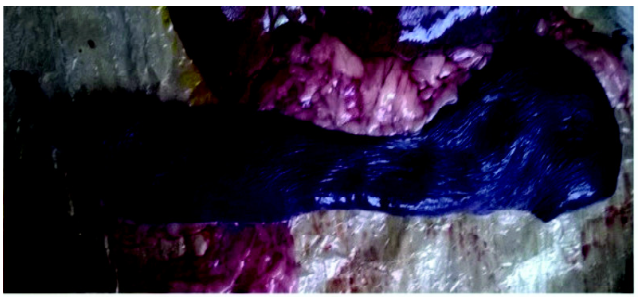

Fig 2. Enlarged spleen

decreased renal excretion (Allen *et al*., 1987). Finding of hyperphosphatemia was due to the progressive decrease in GFR and filtered load of phosphate decreased as GFR decreased which results in hyperphosphatemia (Sharma *et al*., 2015b). Serum phosphorus concentration has been reported to increase with the loss of e"85% of renal function (Chew and Gieg, 2006). Suggestive reason of hypocalcemia may be the less absorption of calcium in the intestine due to less production of calcitriol from the damaged kidney (Nagode *et al*., 1996). Suggestive reason of elevated ALKP might be secondary renal hyperparathyroidism (Center, 1996). Above findings were also observed by Ross *et al.* (2007) and Meyers *et al*. (2008) in CRF.

Increased urine turbidity or changes in urine sediment may due to accumulation of WBC, RBC, renal

Table. 1 Haematology and serum biochemistry of dog suffering from CRF

Parameters	On first day	On 25th day
Hb (gm %)	3	3.5
PCV (%)	18	20
TEC ($\times 10^6/\mu L$)	2.5	3.0
TLC ($\times 10^3/\mu L$)	8	6
Platelets ($\times 10^6/\mu L$)	1.2	1.13
N (%)	78	82
L (%)	21	18
E (%)	0	0
M (%)	1	0
B (%)	0	0
BUN (mg/dL)	130	154
Creatinine (mg/ dL)	12.5	12.8
BUN/serum Creatinine	10.4	11.66
ALKP (IU/L)	179	182
SGPT (IU/L)	87	100
SGOT (IU/L)	10	11
Sodium (mmol/L)	142	137
Potassium (mmol/L)	5.1	5.6
Phosphorus (mg/dL)	16	13
Calcium (mg/dL)	7.4	5.5
Total Protein (g/dL)	5.8	5.1
Albumin (g/dL)	3	2.3

Table. 2 Urinanalysis of dog suffering from CRF

Test	Result
Colour	Yellow
Transparency	Slightly turbid
Specific gravity	1.020
pH	6.9
Protein	Trace
Glucose	Nil
Ketone	Nil
Bilirubin	Nil
Urobilinogen	Normal
RBC	Nil
Pus cells	15-20/ HPF
Epithelial cells	5-6/ HPF
Cast	Occasional Granular
Crystal	Calcium Oxalate
Urine protein creatinine ratio	0.5

epithelial cells or cellular or granular casts (Srivastava *et al*., 2012). Increased pH of urine was due to development of metabolic alkalosis in end stage renal failure in context loss of Na^+, Cl^-, H^+ and volume by vomiting and also due to treatment with base like bicarbonate, antacids and preparations containing calcium (Kumar *et al*., 2011). Unchanged specific gravity of urine may be due to oliguric phase of renal failure (Kumar *et al*., 2011). Finding of proteinuria may be due to the severe glomerular injury with nephrotic syndrome (Cowgill and Francey, 2005), tubule-interstitial inflammation or could be due to endothelial dysfunction and the presence of pyuria (Vaden *et al*., 2005). Significant increase in epithelial cell count is indicative of the disease process in kidney and presence of pus cells indicate inflammation anywhere in urinary tract (Osborne *et al*., 1972). Similar findings were also observed by (Kumar *et al*., 2011) in renal failure in dogs. Dog was found borderline proteinuric (UP/C 0.2 to 0.5) according to International Renal Interest Society (IRIS) recommendations (Polzin *et al*., 2005). The UP/C provides an indication of the magnitude of proteinuria. Higher UP/C value indicates more severe or extensive glomerular lesion (Nabity, 2010).

Radiographic findings of decrease kidney size in dogs could be related to end stage renal failure due to scarring and fibrosis (Polzin, 2011). Normal kidney length to L2 vertebrae ratio on lateral radiographs in dogs should be 2.79 ± 0.46 (Lobacz *et al*., 2012). Ultrasonographic findings of the present study were in agreement with Margeschel *et al*. (2007) loss of architectural detail is a significant feature of renal diseases due to gradual loss of functional nephrons (Verma, 2005). Splenomegaly is a prominent pathological and clinical finding in both the acute and chronic stages of the disease (Reardon and Pierce, 1981).

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Successful treatment of snake envenomation in a Murrah buffalo

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Abstract

Snake envenomation in a Murrah buffalo was diagnosed based on history of owner sighting the snake bite and physical examination which revealed fang-marks just above the coffin joint/ hoof. The haemato-biochemical analysis showed elevated levels of aminotransferase and muscle enzymes while a reduction in haemoglobin concentration and haematocrit value. The treatment of snake-bitten buffalo with a polyvalent antivenom serum at a dose of 50 ml i.v. in dextrose-normal saline along with ancillary treatment was successful. No antivenom reactions were seen and buffalo recovered fully in 3 days.

***Keywords*:** Antivenom; Envenomation; Fang-marks; Hematocrit

Poisoning from snake venom in animals is a veterinary emergency and requires immediate attention (Ananda *et al.*, 2009). Most snake-bites happen when the snake is trodden on in the dark by feet of large animals or snake may be picked up unintentionally. Some bites occur when the snake (usually a krait) comes in to the animal shed at night in search of its prey. Although there is often insufficient toxin injection to cause death in large animals, a serious secondary bacterial infection may be set up in the local swelling and cause the subsequent death of the animal. The toxins in venom include: neurotoxins, causing flaccid paralysis, pupillary dilatation and respiratory failure; cytolisins, causing tissue necrosis; hemolysins leading to a hemorrhagic tendency and myotoxins, causing muscle necrosis and myoglobinuria. Systemic treatment should include antivenom, antibiotics, and antitoxin (Radostitis *et al*., 2007). This study reports a rare clinical condition of snake envenomation in an adult Murrah buffalo.

Case Presentation

A five years old female Murrah buffalo (body wt.~300 kg) was presented to the Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izatnagar at night time with the history of depression, excessive frothy salivation, ataxia, excitement, recumbency, rapid breathing and oliguria. The owner reported progress of snake in animal barn and mentioned the frequent snake problem in the area. The snake was black in colour with few white dorsal bands and white ventral side and without hood. Snake. The vital parameters *viz*. rectal temperature, heart rate and respiratory rate were recorded as 101.2^0 F, 47 per min. and 32 per min., respectively. The close physical examination of the right hindlimb revealed painful swelling and cyanosis of tissues along with presence of fang marks (fig. 1.) of venomous snake just above the coffin joint/ hoof. Before the transport of buffalo to veterinary polyclinic, a tight tourniquet, as a part of first-aid treatment, was applied around the upper part of the bitten limb.

To make the diagnosis, jugular blood was submitted for complete haematology and serum sample was also sent for biochemical analysis. The 20-minute whole blood clotting test (20WBCT) conducted using 2 ml jugular blood was found to be negative and thus, revealed the absence of consumption coagulopathy. On hematological examination, marked neutrophil leucocytosis along with a mild decrease in haemoglobin concentration and hematocrit value. Serum biochemistry indicated severe muscle damage and a mild hyperbilirubinemia.

Table 1. Haemato-biochemical alterations in snake-bitten buffalo

Parameter	Snake-bitten buffalo	Reference values
TLC ($x10^3/\mu L$)	17.2	6-12
TEC ($x10^6/\mu L$)	4.83	5-10
Neutrophils (%)	54	30-40
Hematocrit (%)	25	30-40
Hemoglobin (g%)	9.20	10-14
AST (U/L)	169	78-132
ALT (U/L)	63	11-40
Creatine kinase (U/L)	366	35-280
Total bilirubin (mg/dL)	0.82	0.01-0.5
Total protein (g/dL)	5.38	5.7-8.1
BUN (mg/dL)	17.97	6-27
Serum creatinine (mg/dL)	1.41	1-2

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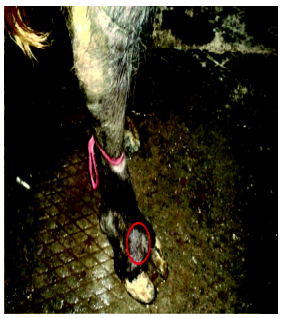
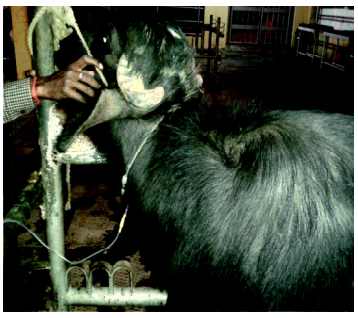

Fig. 1. Fang-marks (encircled) Fig. 2.Ongoing antivenom therapy

Based on history, clinical signs and laboratory examination (Table 1) the case was diagnosed as snake envenomation by an Indian krait.

Treatment and Discussion

The treatment was initiated immediately with lyophilized polyvalent antivenom serum (Snake Venom Antiserum I.P., Bharat Serums and Vaccines Limited) (fig. 3.) where 50 ml reconstituted freeze-dried antivenom was diluted in 1000 ml of dextrose-normal saline and infused slow intravenously (fig. 2.) over a period of 30 minutes provided careful monitoring for early antivenom reactions was done during the first hour following treatment.

Furthermore, tetanus toxoid at a dose of 5 ml i.m. (Tetanus toxoid, Serum Institute of India), dexamethasone 30 mg i.v. (Dexona, Zydus AHL), pheniramine maleate 10 ml i.m. (Avil vet, Intervet), atropine sulfate at the rate of 0.04 mg/kg i.m. (Atrotas, Intas) and long acting enrofloxacin 30 ml i.m. (Flobac SA, Intas) were simultaneously admininstered.

The buffalo showed signs of improvement to treatment after 4 hours as it became active and did not show late anaphylactic reactions as well, as reported by the owner. The animal was kept under regular review and it recovered completely in 3 days.

Clinical signs like depression, frothy salivation, muscular weakness and ataxia observed in the present case may be attributed to the enzymatic and non enzymatic compounds in the snake venom. The cyanotic edema observed at the site of bite may be attributed to enzyme hyaluronidase which acts as a spreading factor (Wolff, 2006). The alterations in the haematological parameters might be due to damage to the blood cells by snake venom. The increased biochemical values like aspartate aminotransferase and creatine kinase may be due to muscle damage caused by snake venom (O'Shea, 2005). Hyperbilirubinemia can be due to extravasation of blood (Warrell, 2010). Sometimes lyophilized polyvalent anti-snake venom may cause anaphylactic reactions (Sai *et al*, 2008), to overcome the untoward effect to antivenom and edema of bitten part, dexamethasone was given. Prophylactically, tetanus toxoid and broad spectrum antibiotic were administered, as the fangs of the snake are supposed to be contaminated with various types of bacteria.

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Transmissible venereal tumour in dog : A case report

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Abstract

A four year old mongrel dog was brought to Teaching Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar LUVAS (HISAR) with history of nodular growths dispersed on the whole body involving all the regional lymph nodes and eyes, bloody urination, normal appetite and defecation. Clinical examination revealed nodular growths on penis, prepuce, scrotum, neck, lateral thoracic, dorsal and ventral abdominal regions. Haematology revealed slight anaemia, normal total leukocyte count and blood smear was positive for *Hepatozoon canis*. Histopathological examination revealed large, round or oval cells with indistinct outlines, frequent mitotic figures and it was diagnosed as metastatic TVT. Dog was treated with vincristine sulphate @ 0.025mg/kg b.wt. weekly along with antiemetic, antibiotic (Tab. Cephalexin) , Vitamin C, antihistaminics and topical antibiotic sprays applied on lesion twice a day four weeks. The dog recovered completely after four weeks of chemotherapy without any further complications and reoccurrence.

Keywords: Canine, Histopathology ,Transmissible Venereal tumour ,

Case presentation

A four year old sexually mature mongrel male dog, weighing 15 kg was brought to TVCC, LUVAS (HISAR) with a history of tumorous nodular growth of variable sizes dispersed on whole body involving all the regional lymph nodes (lymphadenopathy) as well as eye along with blood in urin. Dog had normal behavior, appetite, body temperature, pulse and respiration rate. The consumption of water and diuresis was also normal. Initial physical examination revealed hard non-fluctuating neoplastic nodules affecting genitalia i.e. penis, prepuce and scrotum as well as extragenital sites including neck, dorsal and ventral abdominal and eye (ocular CTVT). Exudative nodules discharging creamy pus were also present on skin and scrotum.

Blood (3 ml) was collected into sterilized vial containing ethylene diamine tetra acetic acid (EDTA) for haematological study. The various haematological parameters viz. total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated. Blood smears were prepared immediately, stained with Giemsa stain and screened for presence of haemoprotozoan parasites.

The detailed gross observations with specific locations of growths were recorded. Tissue biopsies were collected from different sites after administration of local anaesthesia around the growth. Then samples were fixed in 10% buffered formalin for histopathological studies. After proper fixation of tissues, these were cut into small pieces of 2-3mm thickness and then embedded in the paraffin by standard procedures. The paraffin embedded tissues were cut into 4 micron thick sections., and stained with hematoxylin and eosin (H.&E.) as per conventional procedures (Culling, 1995). Toluidene blue staining was done on duplicate sections to differentiate it from mastocytoma (Luna, 1968).

Treatment and Discussion

Dog was treated with vincristine sulphate @ 0.025mg/kg b.wt. IV weekly for four consecutive doses along with antiemetic (Injection Emset® 1 ml IV). To control bacterial infection antibiotic (Tab. Cephalexin® @ 10 mg/kg b.wt BID) therapy along with supportive

Table 1: Hematological observations in the mongrel dog

Parameters	Observed value
Hb (g %)	10
TEC ($\times 10^6/\mu L$)	5.33
PCV (%)	32
TEC($\times 10^3/\mu L$)	11.2
MCV(fL)	60.03
MCH(pg)	18.76
MCHC(%)	31.25
Differential Leucocytic Count	
Neutrophils (%)	52
Lymphocytes (%)	40
Eosinophils (%)	08
Haemoprotozoan parasite	*Hepatozoon canis*

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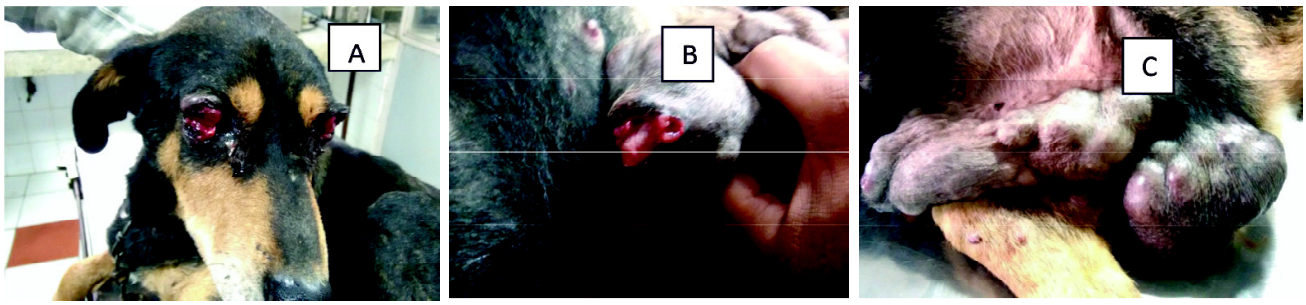

A. Ocular CTVT involving both whole eyes, B. Multilobulated, lesions on the tip of the penis, C. Nodular growth on the coudal aspect of penis as well as scrotum

Fig.1 Canine transmissible venereal tumour (Before treatment)

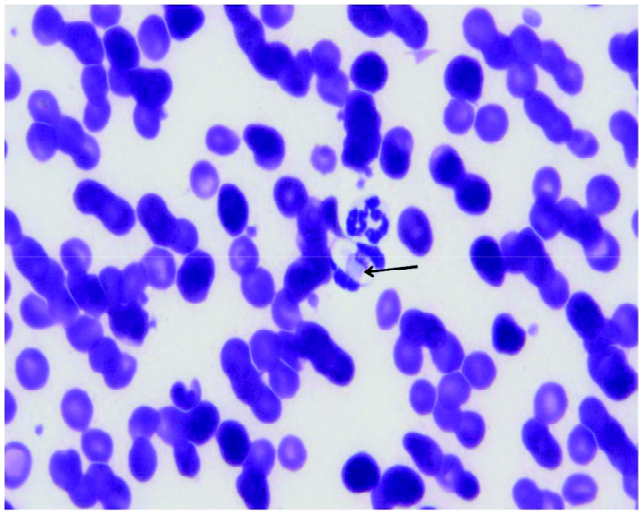

Fig. 2 Blood smear showing ellipsoid shaped *Hepatozoon canis* gamonts in neutrophil (Arrow). Field stain×1000.

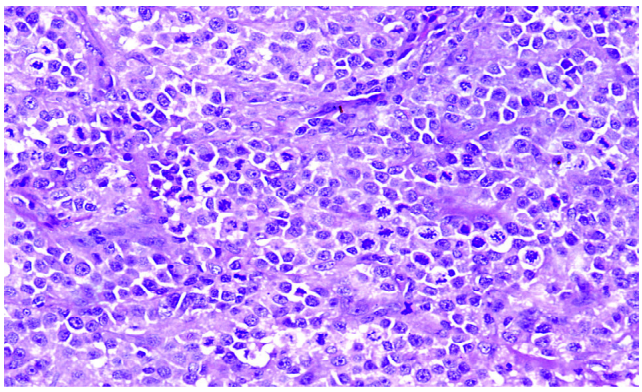

Fig. 3 Section showing diffuse sheets of round cells and scanty connective tissue stroma and numerous mitotic figures. H&E ×400

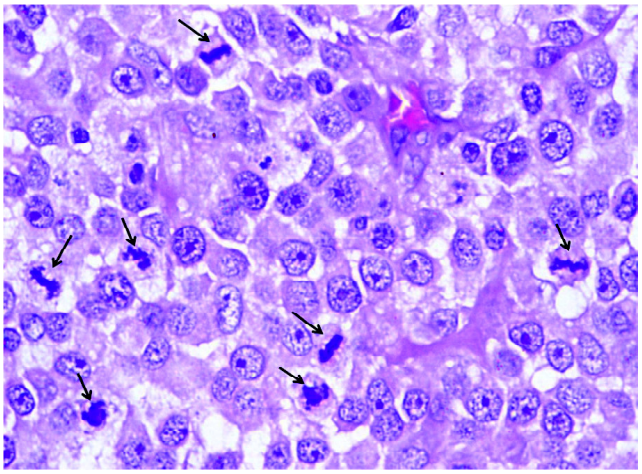

Fig.4 Section showing neoplastic cells with oval to round nucleus, distinct nucleolus, stippled chromatin, granular cytoplasm and frequent mitotic figures (Arrow). H&E ×1000

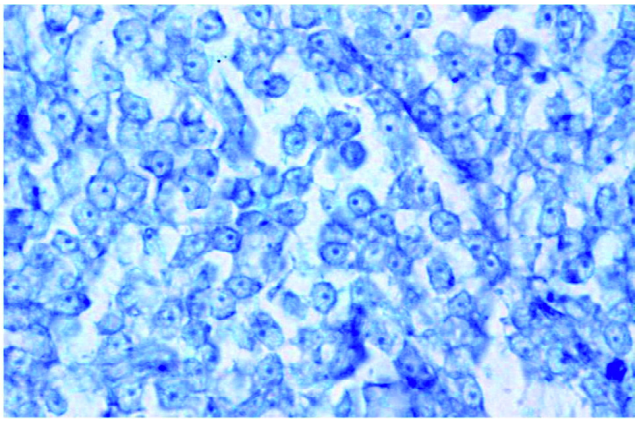

Fig. 5 Neoplastic cells devoid of metachromatic granules. Toluidene Blue ×1000

treatment was also initiated which included Tab. Vitamin C (100 mg BID) , antihistaminics (Injection Avil® 1ml IM) for 7 days and topical antibiotic spray on lesions twice a day for four weeks.

On presentation hard non-fluctuating neoplastic nodules dispersed on the whole body were present. Anorexia, loss of body condition, depression respiratory distress along with pus discharge from neoplastic nodules present on eye, penis etc. were observed (Figure 1a,b,c).

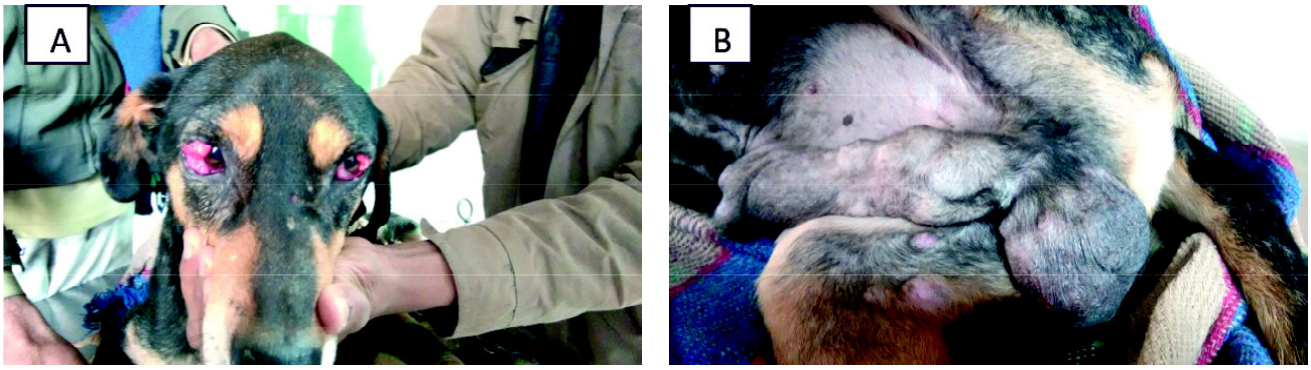

Fig. 6 Canine transmissible venereal tumour (after 2 weeks of treatment) Partial resolution of Ocular as well as genital lesions

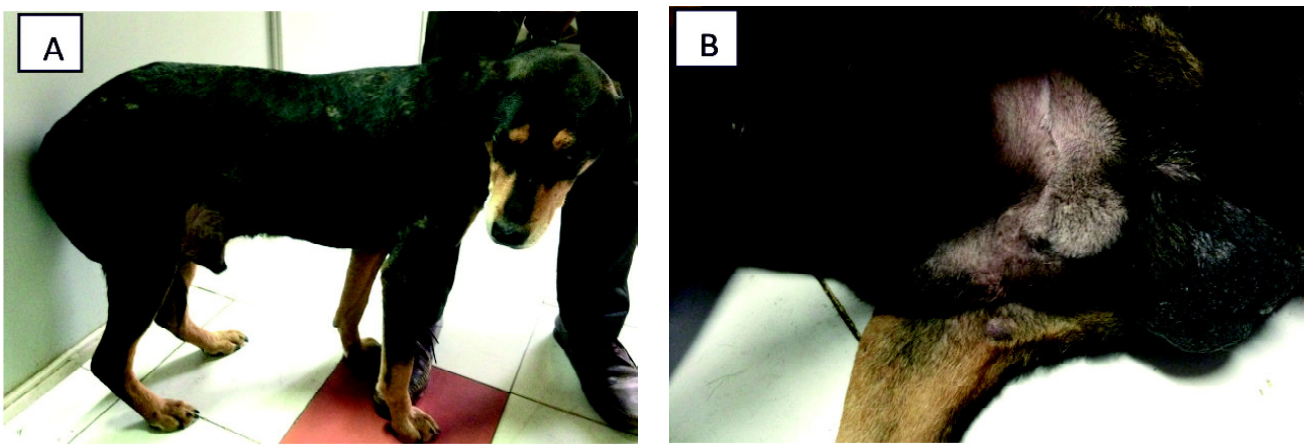

Fig.7 Canine transmissible venereal tumour (after 4 weeks of treatment) Complete resolution of lesions from the body

Haematology revealed slightly lowered values of Hb, TEC and PCV indicating mild anaemia. The overall haematological values are presented in table 1. Blood smear examination showed presence of gamonts of *Hepatozoon canis* in neutrophils (Figure.2).

Grossly, the nodular growths were observed on various parts of body involving regional lymph nodes, neck, lateral thoracic, dorsal and ventral abdominal regions, subcutaneous tissues, prepuce, scrotum and penis. The growths were variable sized and oval to round in shape. Microscopically, the tissue sections from different growths revealed the similar type of histopathological changes. Histopathological examination revealed large oval to round cells arranged in rows or sheets separated by thin fibrous connective tissue stroma (Figure.3). The nuclei were large, round or oval with single prominent nuclei. Mild to moderate anisokaryosis was observed. Neoplastic cells revealed slightly granular or vacuolated cytoplasm with high nuclear-cytoplasmic ratio. Mitotic figures were frequent (Figure.4). Toluidene blue staining on duplicate sections did not reveal metachromatic granules in the neoplastic cells (Figure.5). Based on detailed clinical examination, location and patho-morphological findings it was diagnosed as a case of metastatic transmissible venereal tumour.

A presumptive diagnosis of metastatic canine TVT was made based on the combination of clinical findings epidemiological factors (middle-aged sexually active dog living in an endemic region) and the primary location of the tumors (genitalia involvement). Definitive diagnosis of TVT, with differentiation from other round cell tumors was made by histopathological investigation of the biopsied nodules.

The dog recovered completely after four weeks of chemotherapy without any complications and reoccurrence (Figure.6 a, b and Figure. 7 a, b).

TVTs can occur in any breed, age and sex of dog but in young dogs (2-5 years) with maximum sexual activity or dogs with a compromised immune system, tumors have a greater tendency to metastasiz. Metastases

were more frequent in males than in females. In the present case, patient had a thin to emaciated body condition with concurrent infection of *Hepatozoon canis* with metastatic cutaneous lesions may be due to dissemination of tumor cells. Concurrent infections likely to compromised the immunity of patients leading to the occurrence of generalised venereal tumors. Prognosis of systemic and advanced state of the disease due to metastases to the visceral organs reported to be unfavourable (Chikweto *et al*., 2013).

Chemotherapy with Vincristine Sulfate, as single agent given IV once weekly is the most effective and practical treatment protocol known to be the available for CTVT (Calvet *et al*., 1982; Nak *et al*., 2005). Complete regression of lesions was observed in the present case after four treatments with vincristine sulfate, with no recurrence. Common side effects with vincristine sulfate are gastrointestinal upset and leukopenia (Milo and Snead, 2014). Vincristine reduces the rate of development of tumorous cells which causes dam-age to germ cell DNA

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Management of Osteoarthritis using in homeopathic Combination in dogs

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Abstract

Twenty client owned dogs with hind limb lameness, inability to get up unassisted, reluctance to move, and/or altered behavior for last one to five month were diagnosed with osteo-arthritis of Hip and/or Stiffle joint (s) on radiological evaluation and were treated with a homeopathic combination remedy consisting of Arnica M.30C, Ruta G.,30C, Rhus Tox 30C, Hypericum P. 30C, Kali Phos 30C and Mag Phos 30C in equal proportions at the dose rate of 4 pills four times daily orally for 4 weeks. Clinical response was monitored weekly and radiological evaluation was done at the end of 4th week .There was progressive decline in clinical score from 1st to 4th week and radiographic recovery was marked at the end of 4th week with an improvement in quality of life. It seems homeopathic combination remedy can form an alternate protocol for the management of osteoarthritis in dogs.

Keywords: dog, homeopathic combination remedy, lameness, osteoarthritis, pain, radiography

Canine osteoarthritis is a chronic progressive disease of dogs associated with pain, discomfort, stiffness of joints, and lameness disabling them to lead normal life. Although oral non-steroidal anti-inflammatory drugs including COX-2 selective inhibitors are being used for the treatment of osteoarthritis but these drugs do not significantly alter the disease progression and sometimes caused gastric disturbances. Chondro-modulating agents are beneficial at early stage of osteo-arthritis and in some cases can also reverse cartilage damage but individual variations in response are very high and long term follow up is a costly affair. In human homeopathy many drugs have been claimed for the treatment of arthritis (Boericke, 2001). During recent years a homeopathic treatment has shown promising results in the management of canine osteoarthritis in Finland (Hielm-Bjorkman *et al*., 2009). Despite prevalence and seriousness of canine osteo-arthritis no such studies seem to have been conducted in India. Therefore, the present study was undertaken to evaluate a homeopathic combination remedy in the management of canine osteoarthritis.

Case Presentation

Twenty client owned dogs with the history of lameness, and altered behavior formed the material for the present study. The dogs were subjected to detailed clinical and radiological examinations as per standard procedures. Their body condition score was assessed on a 5 point body condition score system (Lund et al., 1999). To facilitate recognition of subtle changes owner questionnaire as recommended by Hielm-Bjorkman *et al.* (2009) were used. Clinical score of the dogs was assessed using an Ordinal Scoring System pre and post therapy (0, 1st, 2nd, 3rd and 4th week post therapy) on 20 point scale (McCarthy *et al*., 2007). Radiographic scoring was done using Takahashi Scoring System (Takahashi *et al*., 2004). At the time of referral blood smears were screened for blood protozoa and ehrlichial infections and blood samples were analyzed for serum proteins, serum creatinine, blood urea, serum alkaline phosphatase and SGPT to rule out blood infections and /or renal and hepatic diseases as per standard procedures using fully automatic Biochemistry Robochem analyser.

A homeopathic combination remedy consisting of Arnica M.30C, Ruta G.,30C, RhusTox 30C, Hypericum P. 30C, Kali Phos 30C and Mag Phos 30C in equal proportions was prepared in pill form . The remedy was administered @ 4 pills four times daily orally for four weeks with the informed consent of the owners. The duration of the treatment for evaluating radiographs was fixed as four week if clinical response was evident within a week. No other supportive therapy was given. After 2 weeks of initial rest dogs were given controlled leash walk.

At the end of four week therapy subjective evaluation of clinical outcome of treatment was performed using a subjective grading system from 0 to 3 (0= worse / no change, 1= improvement, 2= great improvement, 3= returned to normal /near normal) as described by Hayashi *et al* . (2011).

Results and Discussion

Lameness, altered behavior, and inability to getup unassisted was persisting for one to five months (mean duration 2.9 months ± 0.29, median 3.0 months). Their initial total index score for pain varied from 7 to 10 on the basis of owners' questionnaire as recommended

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by Hielm-Bjorkman *et al*. (2009). Detailed clinical examination revealed normal rectal temperature (101.0 to 102.6 ^{0}F); lameness; limitation in mobility of hip and/ or stifle joints; pain on palpation of hip/ stifle joints during extension and flexion of the limbs; and limited weight bearing on affected hind limb(s). Osteoarthritis was observed in both small (9) and large (11) breeds but most of the dogs were either overweight or obese on a 5 point body condition score (Lund *et al.,* 1999). It appears that the males were more (85%) affected as compared to females (15%).The clinical signs observed in present cases were in agreement with those described for canine osteo-arthritis (Nelson and Couto, 1998). Clinical score varied from 11.0 to 18.0 (Mean 14.4 ± 0.53 , Median 13.5) on a 20 point scale for lameness, joint movement restriction, pain and weight bearing at the time of referral. Haematological investigations ruled out blood protozoan and ehrlichial infections and involvement of liver and kidneys. Gastro-intestinal parasitism was excluded by the negative coprological findings. Initial radiographic changes at the time of referral showed a non-uniform joint space, narrowing of hip and/or stifle joints giving an impression of asymmetric joint spaces confirming moderate to severe ,radiological gradation 2 to 3, osteoarthritis (Fig. 1 a). Bone density was almost normal. Involvement of hip alone, stifle alone and hip and stifle both was observed in 13, 3 and 4 dogs respectively. Out of 13 dogs with hip involvement, both hips were affected in 10 dogs and one hip in other three. In three dogs both stifles were affected. Four dogs had involvement of either one hip or one stifle, both hips and one stifle or one hip and both stifles.

Chronic pain due to deterioration of articular cartilage, periarticular changes and localized inflammation in the joint is the hall mark of osteo-arthritis in dogs. Therefore primary aim of treatment is to relieve pain, reduce inflammation, increase joint mobility, prevent further damage of cartilage, and improve quality of life of the affected dogs. Modern medicine relies heavily on non- steroidal anti-inflammatory drugs for the management of osteoarthritis in human and veterinary medicine (Nelson and Couto,1998). Their side effects restrict their prolonged use. In homeopathic medicine, Arnica, Ruta, Hypericum, Cal.phos, Rhux Tox, Bryonia, Pulsatila, Belladonna have been indicated for such symptoms in humans. Based of pathology of the disease and properties of homeopathic drugs a homeopathic formulation was developed and evaluated in dogs with confirmed

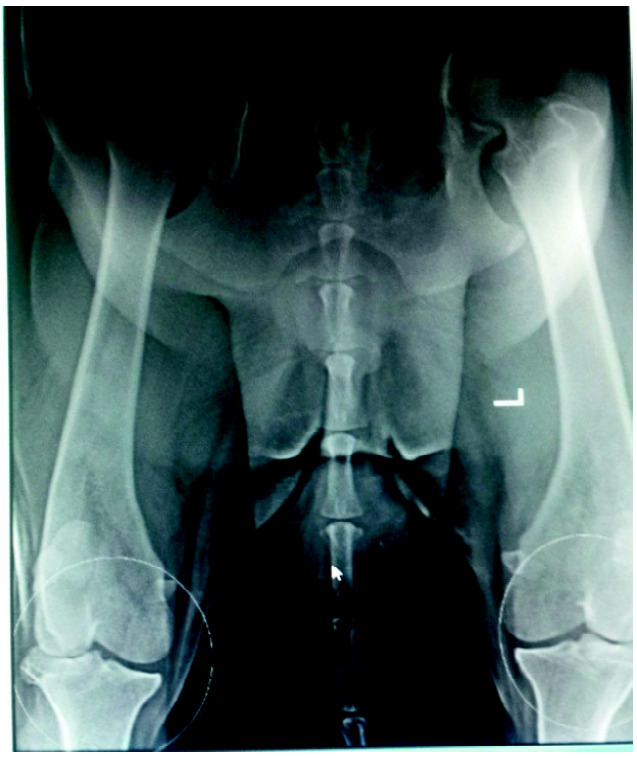

Fig.1a. Radiograph of an adult German shepherd 4th week post therapy with Joint space due to osteophytes.

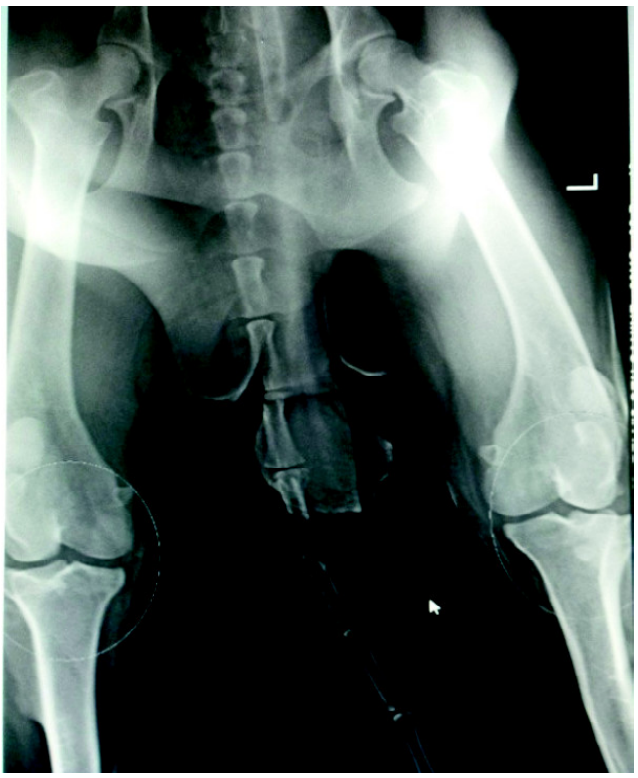

Fig.1b. Radiograph of the same German Shepherd showing osteoarthritis of both Stiffle joints.There is marked narrowing of Homeopathic combination remedy. Note marked improvement in joint space of both stiffles.

Table 1: Effect of Homeopathic therapy on lameness

Sl. No.	Duration of lameness on day '0'	Number of Observations	Clinical Score
1.	0-1 month	03	3.0± 0
2.	1-2 month	04	2.75±0.25
3	2-3 month	05	2.25±0.20
4	3-4 month	03	2.0± 0.0
5	4-5 month	03	1.66±0.33

0= worse / no change, 1= improvement, 2= great improvement, 3= returned to normal /near to normal

osteoarthritis with the informed consent of the owners. Two of the 20 dogs were dropped from the study as they collapsed on day 4th and 5th post therapy due to hyperthermia (heat stroke).Mean clinical score declined progressively from 14.4 ± 0.53 (range 11.0 to 18.0, median 13.5) on day 0 to 12.55 ±0.41(range 10.0 to 17.0, median 12.0) at the end of 1st week to 10.05 ±0.44 (range 7.0 to 14.0, median 10.0) at the end of 2nd week to 6.5 ±0.23 (range 5.0 to 8.0, median7.0) at the end of 3rd week to 4.72 ± 0.135 (range 4.0 to 6.0, median 5.0) at the end of 4th week) and reached almost at normal level. Improvement in clinical score was associated with radiological evidence of reduction in degenerative joint fill up and increase in joint space at the end of four week therapy (Fig.1b). Scoring of clinical outcome at the end of 4th week therapy (table1) indicated that dogs having clinical signs for shorter period at the time of referral had better clinical outcome as compared to those having clinical signs for longer period. The effectiveness of the homeopathic complex in the management of osteoarthritis of hips/stifles/both in dogs could be ascribed to Arnica for its effect on traumatic injuries; Ruta for its action on periosteum and cartilage where there is tendency of deposit formation in joints; Hypericum for excessive pain in hind limbs; Rhus tox for its action on joints (Boericke, 2001); Kali phos for lameness in extremities which aggravates on exertion- a common accompaniment in osteo-arthritis in dogs; and Mag Phos for its ability to increase bone density or arrests bone loss (Sojka and Weaver,1995) .In Finland a homeopathic combination preparation consisting of different homeopathic drugs has also shown promising results in alleviating chronic orthopedic pain in dogs suffering from osteoarthritis (Hielm-Bjorkman *et al.*, 2009).

It can be concluded that the homeopathic combination remedy holds a great promise in the management of osteo-arthritis in dogs. However, controlled blind studies are indicated further.

Acknowledgements

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Ultrasonography detection of stum pyometra in a labrador bitch

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Abstract

A seven year old female Labrador retriever weighing 27 Kg was brought with the history of foul smelling purulent discharge from the vulva even after spaying. The owner also reported that the animal was anorectic and vomition since two days. Abdominal ultra sonography revealed anechoeic sound below the bladder that was conformed as stump pyometra**.** Surgically it was corrected and the animal had an uneventful recovery.

Keywords: Bitch, ovariohysterectomy , stump pyometra.

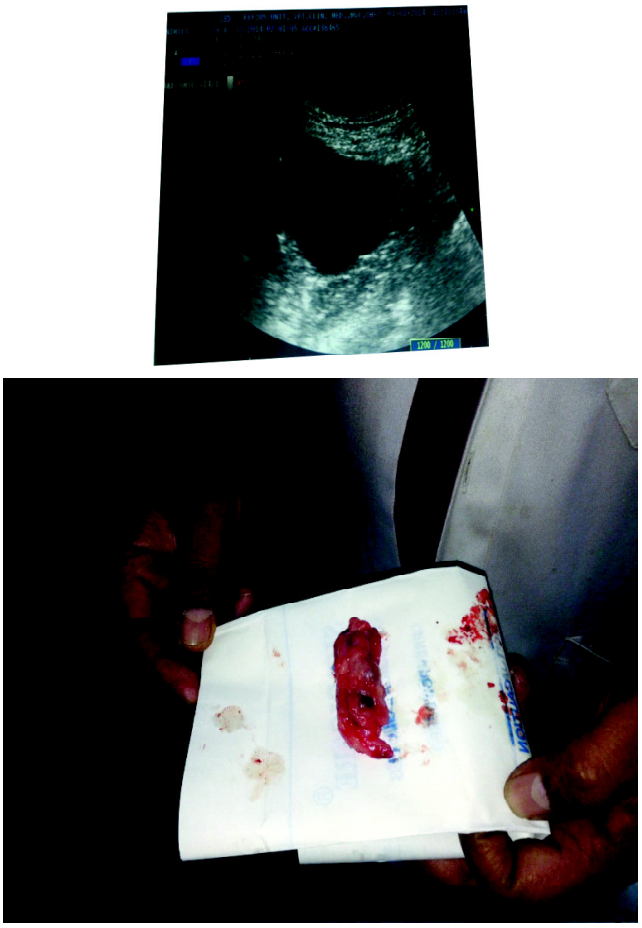
Case Presentation

A seven year old female Labrador retriever weighing 27 Kg was brought with the history of foul smelling purulent discharge from the vulva even after spaying. Sterilization was performed on sixth year. The owner also reported that the animal was anorectic, Pyrexia, lethargy, weight loss, vomition, diarrhea, excessive thirst and urination since two days. Physical examination revealed elevated temperature (40.3p C) and heart rate (130 bpm) and congested mucous membrane. Abdominal palpation revealed tensed and vaginal examination showed purulent foul smelling discharge and mucous membrane was congested and edematous. Haematobiochemical values observed neutrophilia and elevated blood urine nitrogen (32mg/dl). Survey radiography of abdomen revealed no abnormalities. Abdominal ultra sonography showed anechoeic sound below the bladder (Fig.1) that was conformed as stump pyometra and surgical correction was resorted too.

Treatment and Discussions

Cefotaxime and meloxicam were administered intravenously @20 mg/kg and 0.2 mg/kg body weight respectively. General anaesthesia was induced with administration of propofol @ 4mg/kg BW and maintained by quarter to half the dose, as and when it required. Caudal coelotomy was performed and ovarian remnants were ligated and resected.

Ovariohysterectomy has been described as a surgical method of contraception in carnivores (Dillon and Henderson 1992). Stump pyometra is the infection and luminal purulent distention of the uterine tissue remaining from the incomplete removal of ovaries and / or uterine tissues in ovariohysterectomy operation (Johnston *et al.,* 2001. The hormonal influence especially progesterone secretion which is essential in the formation of stump pyometra (Nelson and Feldman,1986). Major clinical symptoms are similar to that of pyometra or



Stump pyometra

Fig.1 Unechoic structure seen below the urinary bladder suggestive of stump pyometra

cystic endometrial hyperplasia, which are like haemorrhagic or purulent vaginal discharge, depression and anorexia. Contrast media in retrograde vaginography reveals arborisation while pyometra like image is generally observed parallel to the bladder in abdominal ultra sonography. One of the severe complications of ovariohysterectomy is incomplete removal of one or two ovary, which is also known as ovarian remnants. This condition is usually followed by stump pyometra which can be described as the infections of the uterine body tissue remaining after the operation however both conditions may also be encountered separately (Johnson *et al.,* 2001). Okkens *et al*., 1981, have reported various complications of ovariohysterectomy in 909 cases as incomplete removal of the ovaries, reaction of surgical suture material, flank fistules and intra abdominal adhesion affecting the functions of other organs, while stump pyometra was found in 20% of the cases, either alone or along with the other complications.

Surgical therapy consist of the removal of infected tissue and the remaining ovarian tissues(Johnston *et al.,* 2001).. In the present case ultrasonography aided for the diagnosis of stump pyometra and to prevent this proper removal of both ovaries is necessary.

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Author Index

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Subject Index

ISVM Awards and Rules

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

General rules applicable to all the awards:-

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

1. SHRI RAM LAL AGRAWAL GOLD MEDAL

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

2. INTAS YOUNG SCIENTIST AWARD

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

3. DR. D.C. BLOOD GOLD MEDAL

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

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

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

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

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

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

6. DR. G.N. DUTTA MEMORIAL AWARD

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

7. P. K. DAS GOLD MEDAL

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

8. AWARD OF FELLOWSHIP OF ISVM (FISVM)

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

9. FIELD VETERINARIAN AWARD

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

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

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

this purpose.

10. ISVM MERIT AWARD FOR POST GRADUATE RESEARCH:

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

11. BEST CLINCAL ARTICLE AWARD

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

12. BEST RESEARCH ARTICLE AWARD

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

13. ISVM APPRECIATION AWARD

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

Award Application procedure

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

Remark Note:

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

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

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

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

GENERAL GUIDELINES FOR CONTRIBUTORS

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

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

In case the research work includes experimentation on animals, authors has to submit a certificate that the work carried out is with the approval of the **Institutional Ethics Committee or as per the laws in force in the country in which it has been conducted**. A certificate to this effect should be signed by corresponding author on behalf of all the authors**.** Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

For a article to be published in IJVM it is mandatory that atleast one of the author should be a life member of the Indian Society for Veterinary Medicine. In case none of the author (s) is permanent member of the Indian Society for Veterinary Medicine he/she may apply for the permanent membership to "The General Secretary, Indian Society for Veterinary Medicine" in a prescribed format given at end of the journal.

The official language of journal is English. The articles should be sent to **"The Associate Editor, Indian Journal of Veterinary Medicine, Division of Medicine, Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, U.P. (India)"** along with a self addressed envelope of A4 size having postage tickets of Rs 30 for further correspondence**.** The manuscript should be typewritten in A4 size paper in Times New Roman, font size 12 on one side of the paper with wide margins (2.5 cm all around the page) and double spacing throughout the article except in abstracts, footnotes and references which should be in single spacing. **It should be sent in duplicate.** Each page of the manuscript should be numbered on the top corner including title page, references, tables, etc. All the pages should contain running title of the paper at the top.

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

Authors are requested also to return the original version along with original comments of the reviewer to the editorial office for reference and records. The modified articles should be submitted to editorial office within 30 days of receipt, failing which the publication of article may be delayed. A demand letter will be sent to the corresponding author for payments of processing and publication fee of the article. Only on receipt of full payments, the article will be taken up for publication and the author will be informed accordingly.

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

1. **Title Page:** Should be typed on separate page contain full title of the article, name of the author(s) along with their affiliation, name of the place (Department, College, University etc.) where work was done. Name of the corresponding author, complete postal address including Pin-code along with phone number and the email address at the bottom of the page.
2. **Manuscript:** In general should be arranged as fallows: (Contributers should take care that name of the author(s), their affiliation and the institution name should not be included in this section and only be mentioned in the title page only.)
   - **A. Title:** Title of the article should be clear, self descriptive in nature and should not contain abbreviation or symbols
   - **B. Abstract:** Abstract should not exceed 300 words and should outline briefly the purpose of the study, important findings and conclusions. Repetition and generally known information should be avoided.

C. Key words: 4 to 5 Key word.

D. Introduction: No subtitle should be given and briefly state the nature and purpose of the work together with the important findings of previous workers.

E. Materials and Methods: The author(s) should describe materials, methods, apparatus, experimental procedure and statistical methods in detail to allow other workers to reproduce the results. Sub-heading may be used in this part.

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

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

H. Acknowledgement(s): This should be short. Grants and technical helps provided should be acknowledged.

I. References: All publications cited in the text should be presented in the form of a list of references arranged alphabetically according to authors' surnames. Don't give serial numbers. Use the following system for arranging the references.

a. For periodicals:

Bartley, E.E., Wheatcroft, K.L., Claydon, T.J. Fountaine, F.C. and Fairish, D.V. 1951. Effect of feeding aureomycin to dairy calves. *J. Anim. Sci.* **10**: 1036-1038.

***b.* For books:**

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

c. For chapter in a book:

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

d. For thesis:

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

e. For proceedings of symposia/conference:

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

I. **Tables:** These should be as few as possible and typed on separate sheets and numbered in roman numeri-cal. Each table should have a brief and self-explanatory title. Table format should be in accordance with the format of *Indian J. Vet. Med.* that is containing grids and cell.

J. Figures: High-resolution (300-600 dpi or greater) and should be initially saved in a neutral data format such as JPEG. Illustrations should be numbered as cited in the sequential order in the text, with a legend on a separate sheet. The editors and publisher reserve the right to reject illustrations or figures based upon poor quality of submitted materials.

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

Body weight	b wt	Litre	I
Calory	cal	Meter	m
Centimeter	cm	Microlitre	µl
Counts per minute	cpm	Millligram	mg

Cubic centimeter	cm^3	Millilitre	ml
Degree centigrade	°C	Minute(s)	min
Degree Fahrenheit	°F	Once a day	od
Decilitre	dl	Parts per million	ppm
Gram	g	Percent	%
Hour(s)	hr	Picogram	pg
Inch	in	Revolution per min	rpm
Intramuscular	im	Second(s)	sec
Intraperitoneal	ip	Square centimeter	cm^2
Intravenous	iv	Subcutaneous	sc
Kilo calories	kcal	Thrice a day	tid
Kilogram	kg	Year(s)	yr
Twice a day	bid	Volts	v

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

Footnotes: These should be used only when absolutely essential. When used, they should be numbered in text, indicated by superscript numbers and kept as short as possible.

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

Short communication

They should be in the same general format as full length papers, should contain between 2000-2500 words but not more than six type pages including tables and illustrations. The manuscript for this head should be clearly marked 'Short Communication' at the right corner on the top of the first page of manuscript. The abstract (not more than 150-200 words), key words (not more than 3 words) and subheading, except for acknowledgement and references, should not be written in the manuscript. Each short communication should contain a definite conclusion of the findings.

The references should be given as per format for the research articles.

Clinical articles

Clinical case reports of interesting and rare nature are published under this heading. The article sent for publication under this head, should contain between 1000-1500 words but not more than three typed pages including references and illustrations and should be marked 'Clinical Article' at the right upper corner of the first page of manuscript. An abstract (not more than 100-150 words), Key words (not more than 3 words). The manuscript should contain history and impor-tant clinical observations of the case, tentative diagnosis and its confirmation, line of treatment used and fate of the case. At last, it should have a brief discussion on the line of treatment and conclusion. All these can be given in separate paragraphs sequentially and sub-heading is not required. The acknowledgement, if necessary, may be given but it should be as short as possible and should bear subheadings. Each article should have significant clinical findings.

The references should be given as per format for the research articles.

Processing and publication fee

Indian J. Vet. Med. charges article processing and publication fee per accepted article as fallowing:

Research article : Rs. 1000 per accepted article

Short communication : Rs. 800 per accepted article

Clinical article : Rs. 600 per accepted article

Note: The decision of the Editor is final in all matters pertaining to the publication of the articles. No reason shall be given for the non-acceptance of the article. Editor/editorial board has the right to do final editorial revision of the accepted articles, restriction of number of pages, tables and figures.

iii. _____

iv. _____

(d) Scientific publications (Give No. only)

i. Research_____ (Indian J.) _____(Foreign J.) _____

ii. Popular_____ iii) Books/Monographs_____

e) No. of post graduate students guided

i. M.Sc./MVSc. _____

ii. PhD/DSc._____

f) Any other relevant information (s)_____

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I am enclosing a demand Draft of Rs. 1500.00 in favour of Indian Society tor Veterinary Medicine, payable at Pantnagar, Branch of SBI (Code No. 01133) for the above purpose. (Strike out if not required)

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Recommendation by a life member of ISVM

I am recommending the name of Dr._____ for consideration as a life member of the society.

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Address: _____

(For Secretariat records)

Membership of Dr._____ is accepted/could not accepted because _____and his/her name has been enlisted in the state of_____at serial no._____.

General Secretary **Treasurer**

INDIAN JOURNAL OF VETERINARY MEDICINE

DECLARATION

FORM IV (See Rule 8)

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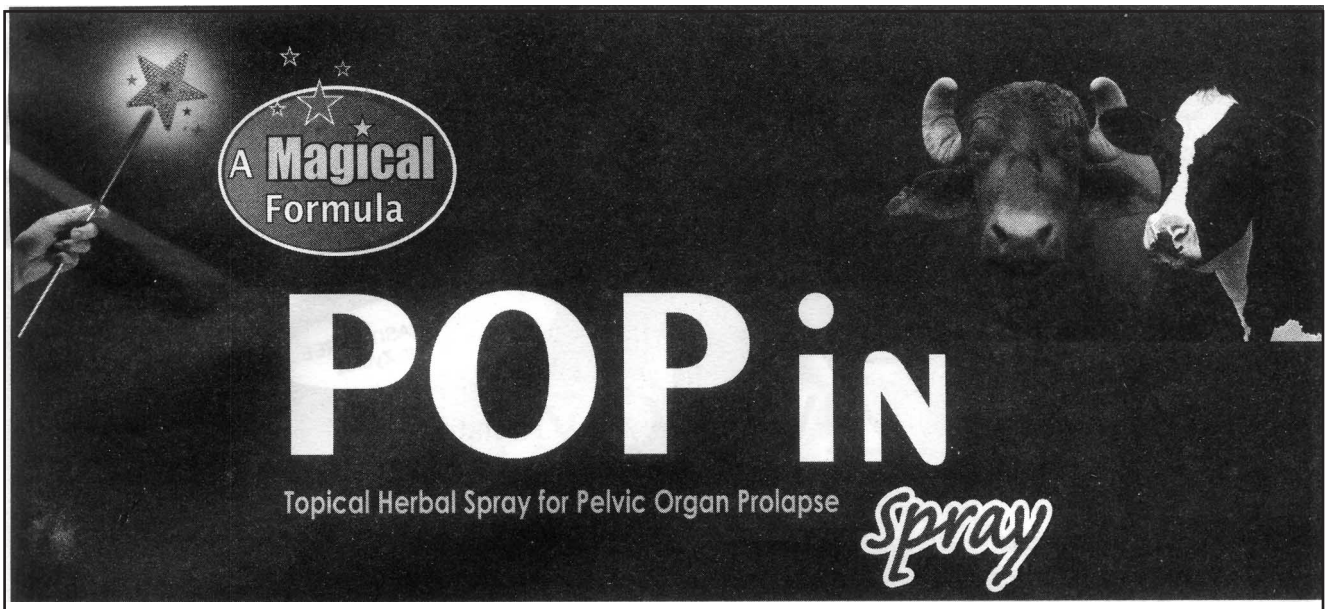
Nationality	:	Indian
Address	:	Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India.
6. Proprietor's Name	:	Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India.

I, S. Dey, Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated: 31st December, 2016 **(S. Dey)**



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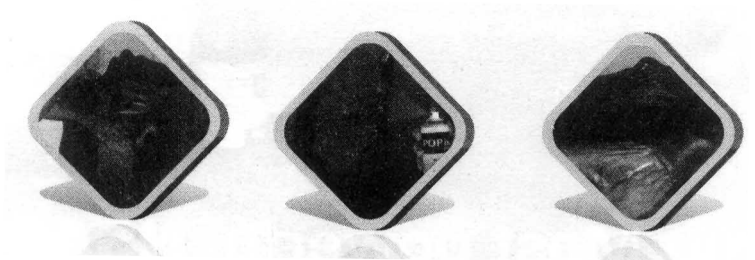

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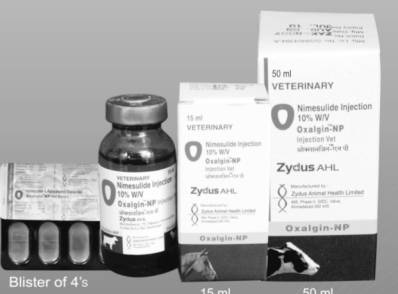

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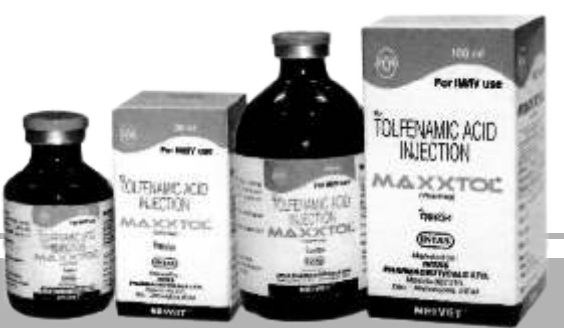

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Research Articles

Short Communication

CLINICAL ARTICLES

Clinical Articles