Gross Morphology of Interior of Rumen in Buffalo (Bubalus bubalis)*

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ABSTRACT

Establishing the morphological features of the rumen becomes highly essential for the study of the pathological deviations. Present study was carried out on six buffaloes to characterize the gross morphology of rumen. Anatomical measurements were taken following standard procedures described. The findings of present study were larger reticulo-rumen (wet contents 15.1 ± 0.74% of body weight), unevenly distributed ruminal papillae across the internal surface of rumen, un-papillated and strong ruminal pillars. The results of present study were analyzed statistically with measurements published for other ruminants. The gross morphology of rumen in buffaloes showed several anatomical characteristics typical for other grazers.

Key words : Gross morphology, Rumen, Buffalo, Grazer

The rumen is the largest compartment of the forestomach which plays an important role in the digestive physiology of ruminants. Each of the compartments of the forestomach has its own morphological features that play an essential role in the rumination mechanism (Scala et al., 2011). Using evidence from 65 ruminant species, Hofmann (1989) divided ruminants according to the differences in the size of their digestive organs, density and size of forestomach papillae and dietary preferences. Three feeding types have been identified:

1. Browsers (concentrate selectors - CS) are focused on the best digestible parts of plants and have the smallest stomach with the largest papillae of the highest density.
2. Intermediate feeders (IM) without specific feeding requirements.
3. Grazers (Grass and roughage eaters -GR) are capable of coping with the least digestible food with high fibre content. Therefore, their stomach has the largest capacity and is distinctively segmented internally. Grazers have the smallest rumen papillae which may be missing on ruminal pillars and in the dorsal sac.

There is scanty information on the gross morphology of interior of rumen in buffaloe Hence, the present study has been undertaken.

MATERIALS AND METHODS

The body weight of buffaloes was registered and rumen samples were collected immediately from slaughter houses and post mortem at Veterinary colleges, Hassan and Shimoga, Karnataka. Cause of death for all animals was ascertained before collection of the specimens due to unrelated affections of gastrointestinal system. Anatomical measurements were taken as per the standard procedures described by Hofmann (1989). In brief, the rumino-reticulum was placed on its left side, and the height and width of rumen were measured with soft measuring tape.

The tissue pieces of rumen collected were studied using stereozoom microscope (Lynx, Lawrence and

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Mayo) and the images were captured by camera (Nikon, COOLPIX P5100, Japan).The thickness of ruminal wall and ruminal pillar were measured using Digimatic caliper (Mitutoyo®). The distribution of ruminal papillae on the wall and on the pillar, their shape, color and surface of papillae were observed.

A square centimeter sample from the bottom of the ventral sac of rumen was used to determine papillae density (No. of papillae/ square centimeter of rumen mucosa). Ten papillae were chosen randomly to measure maximum length and width. Width was measured as distance at midpoint of papillae using Digimatic caliper (Mitutoyo®). The means of various parameters were calculated and used for subsequent statistical analysis. Then, surface enlargement factor (SEF) was calculated according to Hofmann and Nygren (1992).

$$SEF = \frac{(2 \times \text{Papillae Surface Area}) \times [\text{Papillae number} + \text{Base surface}]}{\text{Base surface}}$$

Papillae Surface Area = Papillae length X Papillae width

Base surface = Area of subsample (A square centimeter sample which was used to determine papillae density)

RESULTS AND DISCUSSION

The average wet rumino-reticular (RR) content (% of BW) of buffaloes was 15.1±0.74 in the present study. Clauss et al. (2003) showed that the relative wet RR content on total bodyweight differed significantly between the ruminant feeding types. The regression lines plotted for buffaloes were above grazer line (Fig 1A). Therefore, considered as grazer type as reported by Hofmann (1989) who stated the presence of a heterogeneous papillation pattern typical of grazing species which was also the finding in the present observations (Fig 2A & B). Stratification has long been accepted as a mechanism for increased particle retention (and hence more efficient digestion of dietary fiber that would benefit species feeding on grass) in the rumen of domestic taxa. Several anatomical traits associated with stratification, including the papillation gradient showed significant correlation with the per cent grass composition of species' natural diets. Thus, the papillation gradient in grazer-type species was interpreted as the consequence of adaptations to a grazing niche, adaptations that would have lead to elevated fluid through the rumen, with the resulting benefit of an increased microbial protein harvest and formation of a fiber raft that aids in particle retention (Clauss et al., 2003). This could be due to the fact that gastrointestinal contents increase isometrically with body weight (Van Soest 1994).

The average rumen height was recorded as 49.5±3.62cm in the present study. These results are nearly similar to those of Clauss et al. (2010), where they showed that the relative rumen height on total bodyweight differed significantly between the ruminant feeding types. The measurements taken from buffaloes closely matched the regression lines for grazer type (Fig 1B). Hofmann (1989) described that the browsers tend to have larger, more muscular, mostly divided rumen / reticulum, than do browsers. This adaptation may serve to retard the passage of digesta to lower tract, giving more time for fermentation of plant fiber (cellulose). Further, Clauss et al. (2009) explained that the differences in papillation between ruminant species were due to sheer effect of body size and rumen size. Larger the rumen, more distinct are the characteristics of its contents in different layers.

The rumen pillar thickness was 9.5 ± 1.1mm in buffaloe. The data from present study matched with the regression lines drawn according to Clauss et al. (2003), in which buffaloe was fitted with grazer line (Fig. 1C). They also proposed that major anatomical adaptations in grazers was due to strong rumen pillars and observed that they prefer grass forage under natural conditions.

In the present study, papillae were larger and denser in the ventral sac of the rumen (Fig 2A & B). The results are correlated with findings of Scott and Gardner (1973) who found that the papillae were largest and most dense in the ventral sac of the ruminal areas,

where the papillae were exposed to the highest concentrations of soluble nutrients.

The color of papillae was brown in buffaloe. The variations in color of papillae could be due to type of forage in ruminants. Nockels et al. (1966) predicted that the dark brown colour of papillae appeared to be a combination of keratinized tissue, resulted from rapid growth and limited abrasion, high supply of iron, and an acid pH. Rasha (2007) revealed that the diet changes the colour of papillae i.e. papillae from hay-fed sheep or sheep fed concentrate for 4 weeks had light brown colour. However, dark brown coloured papillae were observed in 6 and 12 weeks concentrate-fed groups.

In the present study, the rumen papillae were distributed unevenly on the mucosal surface as similar to that of Oryx dammah which is a grazing ruminant of the bovidae family (Perez and Jerbi, 2012). Ghosh (2009) stated that the mucous membrane of bovine rumen is heavily studded with large variety of papillae except at the edges of the pillars and the colour of mucous membrane was brown. Konig and Liebich (2009) observed that the ruminal papillae are not developed over the centre of the roof or the free margins of the pillars. Individual papilla showed a great variation in form and size. They varied from low round elevations through conical and tongue like forms to flattened leaves. Prominence, form and density largely depended on the diet of the animal. Increasing the amount of roughages resulted in shortening of the papillae, whereas increasing the energy content of the diet caused the papillae to become longer as seen in cows during lactation.

The papillae were absent on the ruminal pillars of buffalo. The length, width and surface area of papillae were recorded as 3.57±0.09 mm, 0.81±0.01 mm, and 2.88±0.07 mm^2 respectively in buffaloes. Although, papillae served as absorptive structures, the total ruminal volume and surface area had a significant influence on nutrient transport (James et al., 1983), thus changes in papillary size indicate a marked increase of relative rumen epithelial absorptive surface. Lentle et al. (1996) reported that the quality of ingested food affects the size of rumen papillae in red deer. The results of this study clearly demonstrate the effect of the type of forage they select, on the development of the ruminal papillae.

The density of ruminal papilla was 69.3±1.79 in buffaloes. According to Hofmann (1989) these data corresponded with the classification of food specialization of the studied species. The variations in density of rumen papillae observed between species corresponded with the presumption that the development of rumen papillation depended on quality of the ingested forage-on production of volatile fatty acids (VFA) which stimulate papillary growth (Tamate et al., 1962 and Hofmann, 1989). Rasha (2007) demonstrated that the decrease in the number of papillae per cm^2 mucosa was due to increasing the duration of concentrate feeding in sheep. But there was no indication of fusion of several papillae into one papilla. In the present study, the highest density of papillae was in the animals of grazing type.

The densely packed papillae might be due to decrease in thickness of individual papillae, thus, accommodating more papillae per unit area. Tiwari and Jamdar (1970) demonstrated that areas of the rumen wall with large number of papillae absorb more volatile fatty acids than do areas with few papillae. However, Lentle et al. (1996) reported that the quality of ingested food affects the density of rumen papillae in red deer.

The surface enlargement factor (SEF) was 9.75±0.29 in buffaloe. Josefsen et al. (1996) recorded the surface enlargement factor (SEF) in reindeer calves as 5.8-18.6 and papillar length and number of papillae per cm^2 had largest influence on SEF, and these two parameters together accounted for 76-84% of the variation in SEF.

Hofmann (1988) investigated the morphology of rumen and reticulum in roe deer (Capreolus capreolus), and did not find any significant difference between forest and field ecotypes. However, there were differences in rumen papillary development, related to seasonal differences in forage quality and availability.

Forest roe deer showed a wider range of the papillary surface enlargement factor than field roe deer in summer and had their optimal papillary development in autumn. Unfortunately, it was difficult to compare some findings obtained by different researchers because of the lack of uniform nomenclature and illustrations.

CONCLUSION

In the present study, the interior of buffalo rumen showed several anatomical characteristics such as large reticulo-rumen, unpapillated and strong rumen pillar, unevenly papillated mucosa and lesser surface enlargement factor. Further, the regression lines plotted for wet rumino-reticular content, rumen height, pillar thickness were fitted above grazer line, when compared with other ruminants published. All these observations were suggestive of buffaloe as a typical grazer which will be useful for nutritionists and commercial dairy farmers to understand the feeding habit of buffaloes.

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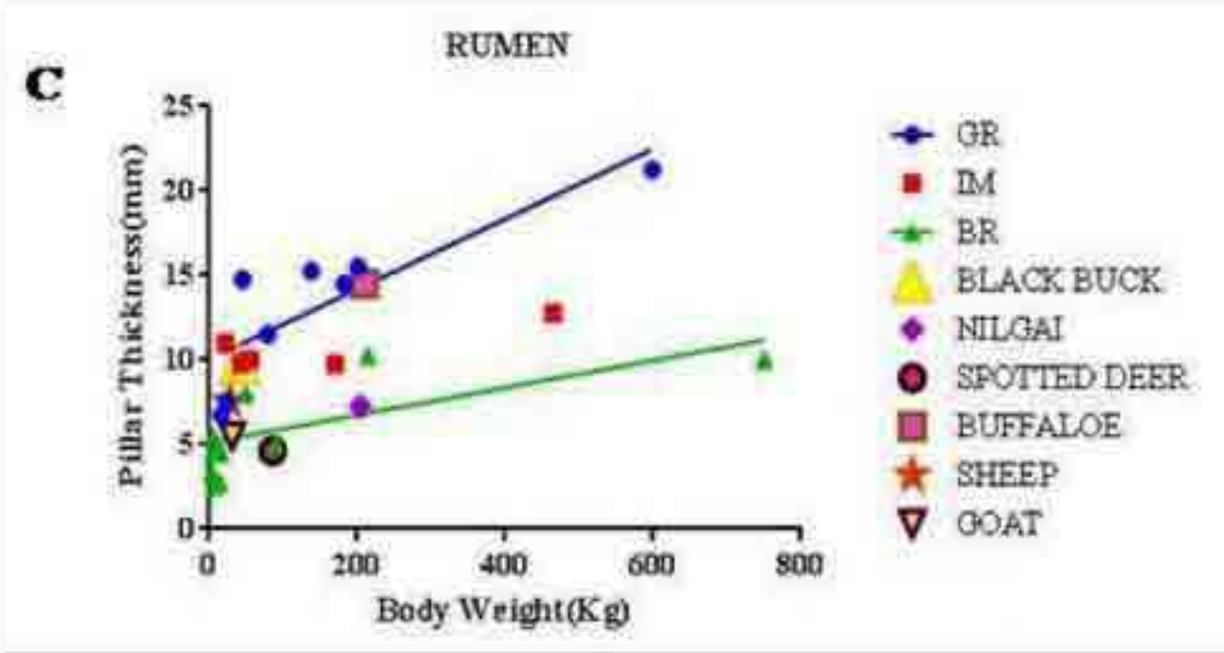


Figure 1: Linear regression data on A) the relative weight of ruminoreticular (RR) wet contents (%BW), B) height C) pillar thickness of rumen with published standard data (Clauss et al., 2003 & 2010). Browser (BR), Grazer (GR) and Intermediate (IM)

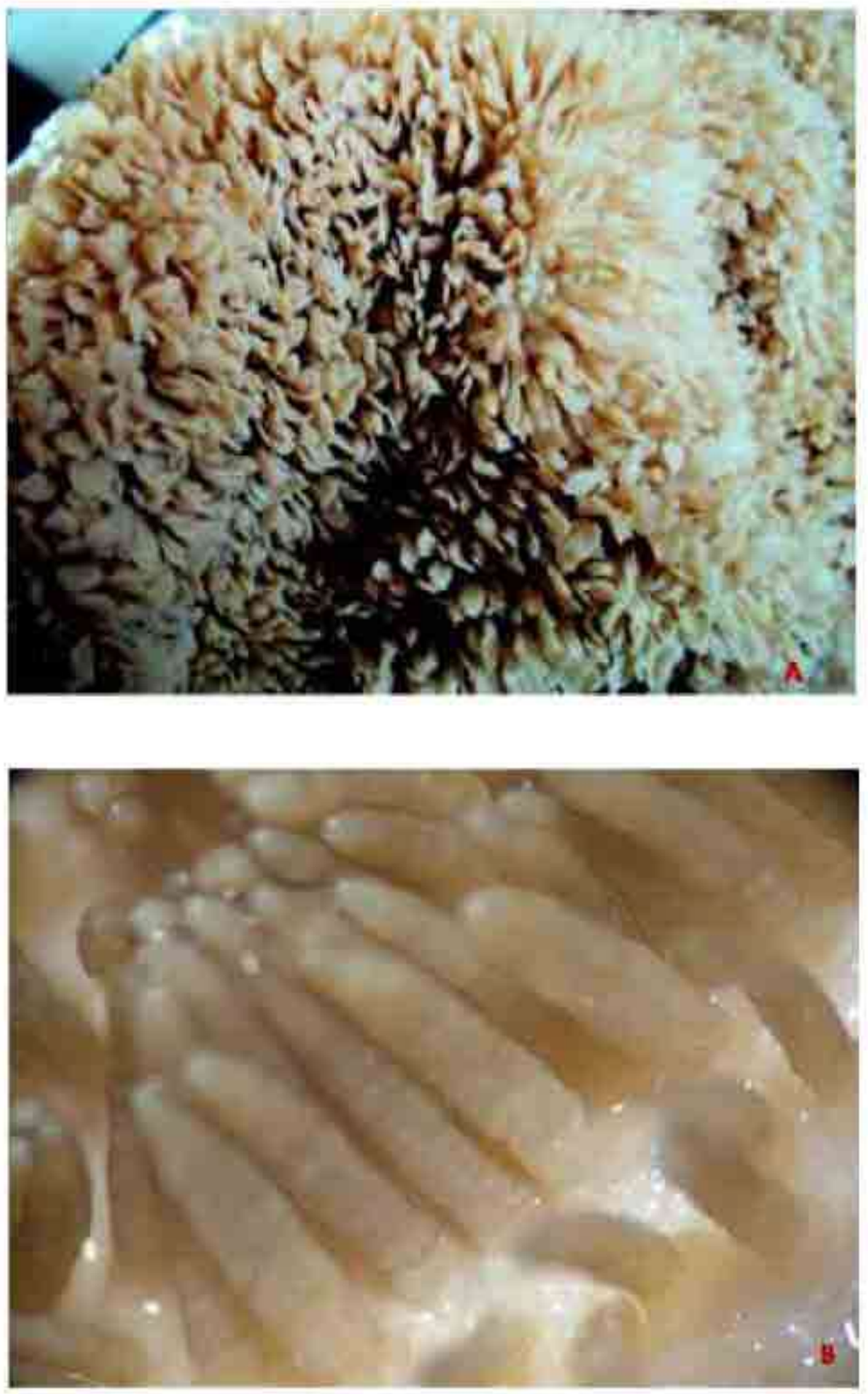
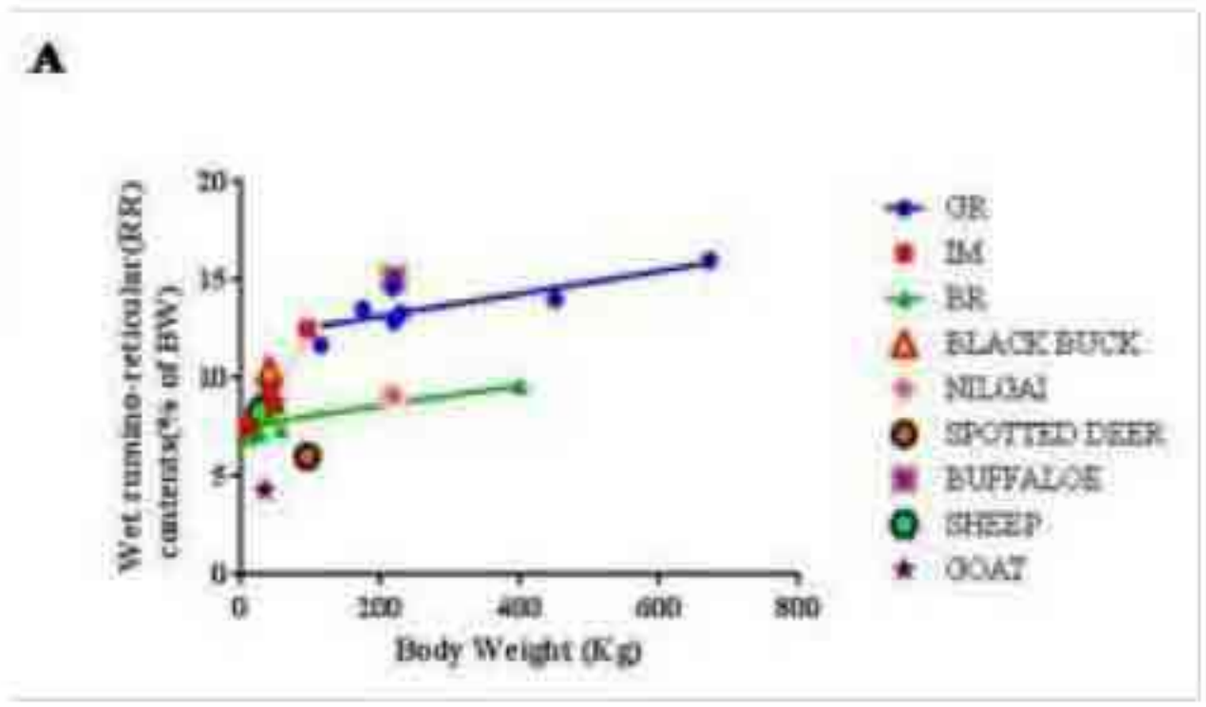
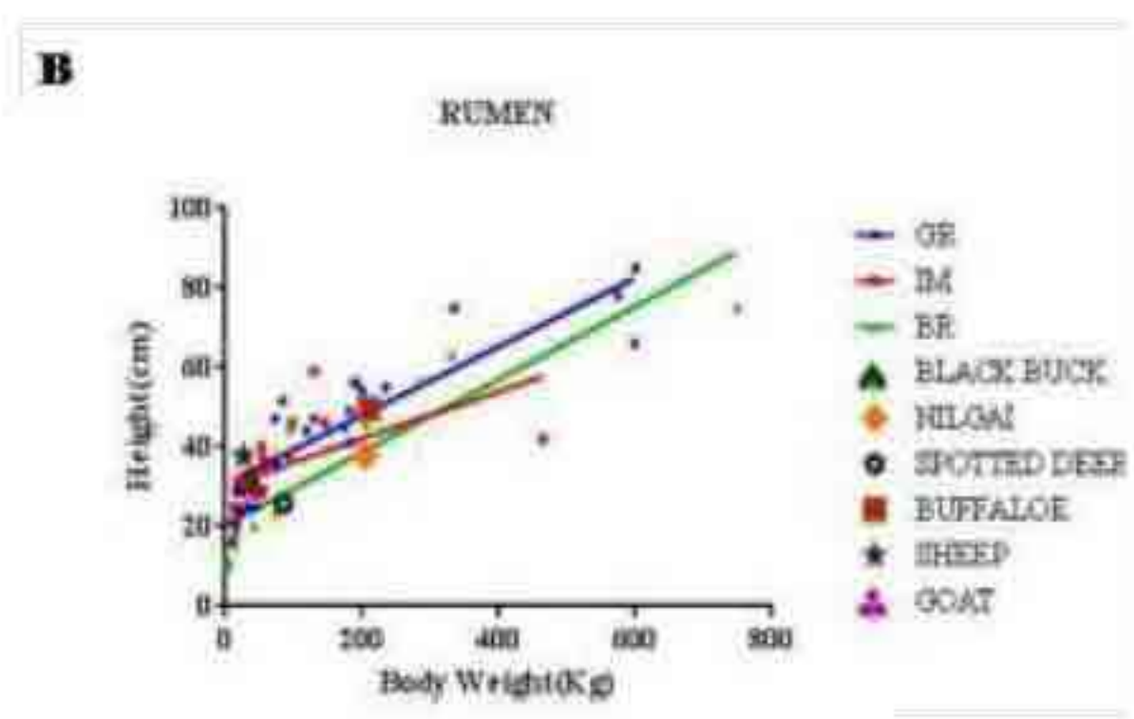


Figure 2: Photograph (A) and stereophotograph (B) showing ruminal papillae on internal surface of ventral sac of rumen in buffalo





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Milk Residue Depletion Kinetics of Cefquinome Sulfate following Therapeutic Administration (Intramammary) in Mastitic Crossbred HF Dairy Cows*

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ABSTRACT

Milk residue depletion kinetics of cefquinome following intramammary administration of cefquinome sulfate (75 mg x 3 infusions at 12 h intervals) was studied in Indian crossbred Holstein Friesian (HF) dairy cows clinically ailing from mastitis. Cefquinome concentration in milk was determined by reverse phase high-performance liquid chromatography (rHPLC). A non-compartmental analysis was employed to derive the kinetic parameters. A peak ($C_{max.}$) concentration of cefquinome in milk of mastitic quarter (664.06±131.24 $\mu g.mL^{-1}$) was observed ($t_{max.}$) at 6 h after last infusion. The elimination half-life ($t_{1/2z}$), the area under curve ($AUC_{0-\infty}$) and mean residence time (MRT) was 5.24±0.51 h, 7915.38±1946.57 $g.mL^{-1}.h$ and 20.97±3.08 h, respectively. The milk concentration of cefquinome persists up to 72 h in mastitic quarter (5.28±0.64 $\mu g.mL^{-1}$) and 36 h in healthy quarter (4.04 $\mu g.mL^{-1}$), respectively after the third infusion in dairy cows. Thus, cefquinome absorption after intramammary infusion as well as its dispersion between the quarters regardless of the pathological status of the udder was adequate. Further, the milk concentration of cefquinome at 72 h post - infusion in the affected quarter was much greater (LOD:0.052 $\mu g.mL^{-1}$) than the reported minimum inhibitory concentration (MIC) against common pathogens causing mastitis.

Key words : Cefquinome, Residue depletion kinetics, Mastitis, HF X dairy cows

Cefquinome sulfate ($C_{23}H_{24}N_6O_5S_2 \cdot H_2SO_4$) is an aminothiazolyl, semisynthetic broad-spectrum antibiotic belonging to fourth generation cephalosporin. Chemically, the zwitterionic structure of cefquinome (Fig.1) can facilitate rapid penetration across biological membranes, including porins of bacterial cell wall. It has a higher affinity to target penicillin-binding proteins (PPBs) and is resistant to β-lactamase enzyme (Dolhan et al., 2014).

Cephalosporins play a prominent role in treating cow mastitis (Bradley and Green, 2009). A minimum inhibitory concentration (MIC) of 0.24 $\mu g.mL^{-1}$ (Zonca et al., 2011) or 0.5 $\mu g.mL^{-1}$ (Yu et al., 2016) was observed against Staphylococcus aureus, a most common pathogen causing mastitis in dairy cows. Cefquinome has excellent activity against both Gram-positive and Gram-negative bacteria such as Staphylococcus sp., Streptococcus sp., Pseudomonas sp., Salmonella sp., Escherichia coli and Klebsiella

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pneumonia in vitro (Chin et al., 1992; Guerin-Faublee et al., 2003; Thomas et al., 2006). Further, cefquinome was found stable to the majority of chromosomally and plasmid-encoded β-lactamase enzyme.

In the Veterinary clinics, cefquinome sulfate was reported to exert broad spectrum of activity against the majority of pathogenic strains (Murphy et al., 1994). In the west cefquinome sulfate was employed successfully in the treatment of meningitis-mastitis-agalactiae syndrome (CVMP, 1995). Cefquinome exhibited time-dependent killing and process post antibiotic effects (PAEs) in vitro increasing with concentration and time of exposure (Ahmad et al., 2015).

The milk residue depletion kinetics of cefquinome sulfate is not known in Indian cross bred HF dairy cows clinically ailing from mastitis. Hence, the milk-depletion kinetics of cefquinome sulfate was determined following therapeutic administration of cefquinome sulphate via intramammary infusion.

MATERIALS AND METHODS

Standards and chemicals: Standard HPLC grade cefquinome sulfate (VETERNAL™, Switzerland; 98.0% purity) was procured from Sigma-Aldrich®, St.Louis USA. Methanol, acetonitrile, formic acid, HPLC- water and trichloroacetic acid were of analytical quality. Deionised water purified by using Milli-Q- system (Millipore, Merck, Mumbai, India) was employed wherever necessary.

Animals with mastitis: Eight cross bred HF cows clinically ailing from mastitis were chosen for the study. These animals (aged about 4-5 years; ~400±50 kg b.wt) were in either second or third lactation yielding 14.0±2.0 kg milk per day. All the animals were hand-milked twice daily at about 6 a.m. and 6 p.m. They were fed with dry forages and allowed for grazing twice a day and had free access to fresh water. The selected animals were not previously exposed to any of the antibacterial or therapeutic agents at least for a period of six weeks. Prior approval from Institutional Animal Ethics Committee was obtained vide No.VCS/IAEC/16/2017-18 dated 10.06.2017 as per CPCSEA regulations for conduct of the study.

Drug administration and milk sampling: A commercial formulation containing 75 mg of cefquinome sulfate (Cobactan LC®, Manufactured by Ms.Intervet International GmbH, Feldstr, Germany; imported and marketed by Ms.Intervet India Pvt. Ltd., Thane, Maharashtra, India) was infused to mastitis affected quarter. Altogether three consecutive infusions at 12 h intervals were undertaken as per the label directions. Following the last intramammary infusion, milk samples (10 mL) from individual mastitic cows were collected in to sterile vials from the treated quarters at '0' (before third infusion) and 6, 12, 24, 36, 48, 72, 96 and 120 h post-administration. Similarly, milk samples were also collected from untreated quarters at 0 ,6,12,24,36 and 48 h. All the samples were storedat -20°C until subjected to assay.

Sample clean-up: Milk samples (1 mL) were transferred into a 15 mL polypropylene centrifuge tubes and 3mL of 10 % trichloroacetic acid was added. The mixture was briefly vortexed for one minute. Later the mixture was gently shaken for 10 min. and centrifuged at 9000×g for 10min. The supernatant was transferred to a glass tube and the residue was then re-extracted once again. The resulting supernatant was loaded onto a solid phase extraction cartridge (SPE cartridge; SUPLICO®, USA; 60 mg/3mL), which was previously activated with 3 mL of methanol and 3 mL of deionized water. The flow rate of the liquids thus added was maintained at a flow rate of approximately 1.0 $mL.min.^{-1}$ and drained under gravity. The SPE cartridge was then rinsed with 3 mL water and dried under vacuum for about 15 min. The retained drug (cefquinome) was eluted from the cartridge with one mL acetonitrile (30% v/v). Finally, the eluate was vortexed and centrifugation at 15000×g for 10 min. The upper layer was collected in to sample vials after passing through nylon filters (0.22ìm diameter) and subjected to liquid chromatography. The analytical recovery for the method adopted was studied by using external standard technique, and it was found to be 94.14%.

HPLC analysis: The assay of cefquinome in milk samples was carried out by using high performance liquid chromatography (HPLC), which consisted of duel pump fitted with a UV-detector system (Shimadzu, LC 20-AD/SPD-20A, Japan). The liquid chromatography was carried out according to Li et al. (2008) with slight modification. Briefly, the mobile phase comprised 90 parts of solution 'A' (Milli 'Q' water with 0.1% formicacid, v/v)and 10 parts of solution 'B' (Acetonitrile, HiMedia® Laboratories, Pvt. Ltd., Mumbai, India). The mobile phase liquid was filtered through membrane filter (0.22 ìm diameter) and later degassed with the help of ultrasonic cleaner. The flow rate of mobile phase was maintained at a rate of 1.0 $mL.min^{-1}$ and pressure maintained at 440 kg. The column temperature was maintained at 40^0C (Uney et al., 2011). The separation of the analyte was achieved using a C18 column (5ìm thickness; Dimension: 4.6 mm x 250mm; S/N: 16C01261; Shimadzu, Japan). Each time the samples that were filtered through 0.22ìm nylon filters were injected (20 ìL) manually in to Rheodyne injector system. The UV- absorbance was measured at a wave length set at 268 nm. The room temperature was maintained at 23^0C during the assay.

Calibration curve: Stock solution of cefquinome sulfate at a concentration of 1000 $\mu g.mL^{-1}$ was prepared by dissolving it in milli-Q water. The stock solution was stored at -20°C and shaded from light until use. All the working standard solutions were freshly prepared and stored at 4°C in the darkness. The calibration curve was constructed in the range of 0.1 to 1000 $\mu g.mL^{-1}$ of cefquinome in milli-Q water. The standard curve was constructed by plotting the concentration of drug (X-axis) against peak area (Y-axis). The standard curve was linear in the range of 0.1 to 1000 $\mu g.mL^{-1}$ with R^2 value 0.999.

Assay precision: The limit of detection (LOD) was determined as the lowest concentration in a sample (analyte), which can be detected from background noise but not quantified with a signal-to-noise of at least 3.3 (Fu et al., 2013). The limit of quantification (LOQ) was defined as the lowest concentration of analyte in a sample quantified with a signal-to-noise ratio of at least 10. The LOD and LOQ of the assay developed was 0.052 and 0.156 ìg.mL^{-1}, respectively. The analytical inter-day coefficient of variation for high and low concentrations of cefquinome was 7.77 and 5.94 per cent, respectively. In a similar way the intra-day coefficient of variation was 2.7 and 9.97 per cent, respectively for high and low concentrations of cefquinome in milk.

Milk cefquinome depletion kinetics: The milk concentration vs time data obtained after intramammary administration of cefquinome sulfate in each mastitic dairy cows was subjected to determine the milk depletion kinetics of cefquinome. A non-compartment model was applied to derive the pharmacokinetic parameters by using a menu-driven add-in program for Microsoft Excel written in visual basic (PK Solver®; version 2.0) validated previously (Zhang et al., 2010). The programme calculates the area under the concentration-time curve (AUC)/ area under the first moment curve (AUMC) based on linear trapezoidal rule. The mean residence time (MRT) was determined as AUMC/AUC while the half-life ($t_{1/2â}$) was calculated as 0.693/â.

Statistical analysis: All the values were expressed as mean ± S.E. The milk - depletion profile of cefquinome in mastitis affected quarter at various time intervals was compared by using Student's 't' test according to Snedecor and Cochran (1994). The milk concentration at different time intervals in drug infused quarter or healthy (unaffected quarter) were compared by two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism, version 7.04, San Digo, Canada, USA). The difference between two values were declared significant when $p < 0.05$.

RESULTS AND DISCUSSION

The individual animal data pertaining to time vs milk concentration of cefquinome after intramammary administration (75 mg x q 12 h x 3 infusions in to mastitic affected quarter) in HFx dairy cows was shown

in Table 1. The semi-logarithmic plot of the time vs mean (±S.E) milk- concentration profile of cefquinome was depicted in Fig.2.

The concentration of cefquinome showed a peak concentration of 664.05 ± 131.17 $\mu g.mL^{-1}$ in milk at 6 h post-administration of last infusion. Cefquinome sulfate was detectable in milk (LOD= 0.052 $\mu g.mL^{-1}$) up to 72 h in mastitis quarter. The perfusion of the cefquinome in to adjacent healthy quarter was good. A peak concentration of cefquinome (19.94 ± 4.50 $\mu g.mL^{-1}$) was observed at 12 h in milk obtained from healthy quarter. However, the concentration of cefquinome in milk of healthy quarter was significantly ($p<0.05$) lesser at 6 h and 12 h when compared to its concentration in milk of infused quarter. The concentration of cefquinome in milk reached to a non-detectable level (LOD = 0.052 ìg.mL^{-1}) at 96 h and 48 h, respectively after last infusion in mastitic and healthy (untreated) quarter (Fig. 2).

The pharmacokinetics (milk depletion kinetics) of cefquinome following therapeutic administration in HFx dairy cows was best described by non-compartment model. The pharmacokinetic parameters derived after analysis of the data tabulated in Table 2. The last detected concentration of cefquinome in milk was 5.28 ± 0.64 $\mu g.mL^{-1}$ at 72 h after third intramammary infusion to mastitic quarter and the ratio of concentration last observed /concentration maximum ($C.last_{(Obs.)}/C_{max.}$) was 0.0088 ± 0.001. It was observed that the elimination half -life of cefquinome sulfate was short after intramammary infusion in dairy cows ($t_{1/2lz}$ = 5.24 ± 0.51 h). The area under the milk concentration-time curve ($AUC_{0-\infty}$) and the area under the movement curve ($AUMC_{0-\infty}$) was 7915.38 ± 1946.57 $\mu g.mL^{-1}.h$ and 140384.21 ± 21744.57 $\mu g.mL^{-1}.h^2$, respectively. The mean residence time (MRT) of cefquinome (sulfate) was 20.97 ± 3.08 h.

Cefquinome sulphate currently widely employed antibiotic in the therapeutic management of mastitis in the country. The current study is aimed to determine the milk depletion kinetics of cefquinome sulfate in Indian crossbred HF dairy cows ailing from clinical mastitis, since such studies in Indian cross bred HF cows and clinically ailing animal patients is lacking. Further, PK- features of drugs are likely to vary according to agro climatic conditions or between the cross breeds of country of origin due to variation in body size and milk production (Zhai et al., 2007).

The time vs milk residue concentration of cefquinome sulfate data was best fit in to non-compartment model. The concentration of cefquinome was detected in the treated and untreated quarter up to 72 h and 36 h after last infusion of cefquinome sulfate in to mastitic quarter, respectively. In the current study the peak ($C_{max.}$) concentration of cefquinome (sulfate) was relatively more than the reported values in healthy HF dairy cows (Li et al., 2014). This may be due to differences in milk production capacity, disease status (mastitis) or body size (Zhaiet al., 2007; Wen et al., 2010).

In the present study the milk concentration of cefquinome in mastitic and the healthy quarters persisted up to 72h (5.28±0.64$\mu g.mL^{-1}$) and 36h (4.04±0.29 $\mu g.mL^{-1}$), respectively. The passage of cefquinome across milk: blood barrier is limited (Yu et.al., 2016). However, the absorption and distribution of cefquinome both within the affected (mastitis) and between the quarters (healthy) after intramammary infusion occurs adequately, and the concentration achieved was higher than the MIC_{90} against most of the mastitis causing pathogens. The dissociation constant (pKa) and lipid solubility of different chemotherapeutic agents after intramammary administration can also influence the rate of their absorption (Bajwa et al., 2007). Other factors such as the constituents of formulation and perhaps animal species can also affect the pharmacokinetic behavior. In addition, milk fraction, to a certain degree, may have an effect on the process of cephalosporins in milk (Stockler et al., 2009). Paradoxically, higher dispersion of cefquinome in to healthy quarter would protect the healthy quarter from infection; milk from such quarters will be unfit for human consumption till it reduces below the maximum permissible limits (MRL).

It was observed that cefquinome was eliminated rapidly in milk after third infusion in to mastitis quarter

($t_{1/2lz}$ = 5.24±0.51 h). Similar observation was also made previously in lactating Chinese dairy cows (Li et al., 2014). The area under the milk concentration-time curve ($AUC_{0-\infty}$) was 7915.38±1946.57 μg.mL^{-1}.h (Table 2). The mean resident time (MRT) observed in the present study was remarkably more when compared to previous studies (~ 10 h) carried out in healthy Holstein Friesian dairy cows. In relative terms, the short MRT observed in the current study can be attributable to clinical mastitis in cows subjected to study.

Cefquinome is a time-dependent antimicrobial agent. The AUC/MIC and T(%)>MIC are the two important PK/PD parameters for assessing the efficacy of antibacterial agents like cephalosporins. The most important pharmacokinetic (PK)/pharmacodynamic (PD) parameters for this type drug linked with 'time' which persists above MIC or more precisely MIC_{90}. The MIC_{90} of cefquinome against various common species of bovine mastitis bacteria viz., E. coli, Staphylococcus aureus, Streptococcus uberis, Streptococcus agalactiae and other pathogens lies in the range of 0.008-4.0 ìg.mL^{-1} (Shpigel et al.,1997; Schmid and Thomas, 2002; Ehinger et al., 2006; Tenhagen et al,. 2006).

The AUC/MIC represent the pattern of antibacterial activity of time-dependent killing and prolonged persistent effect (Andes and Craig, 2002), and it combines both time and drug concentration factors (Yu et al., 2016). We did not measured the MIC of cefquinome in the milk (mastitis) sample, nevertheless considering the hypothetical MIC value of ~0.5 ìg.mL^{-1} against mastitis causing pathogens the numerically the ratio of AUC/MIC or the T (%) >MIC was apparently much higher in the current study. The PK-parameters derived in the current study would be useful for integration with PD in clinical situations where antimicrobial resistance was observed.

CONCLUSION

The milk residue depletion kinetics of cefquinomes sulfate in crossbred Holstein Friesian cows clinically ailing from mastitis was best described by non-compartment model. The elimination half life ($t_{1/2lz}$) and mean residence time (MRT) of cefquinome in mastitic cows was relatively short. However, the therapeutic intramammary administration of cefquinome in dairy cows showed good dispersion between the quarters regardless of the pathological status of the udder. Further, the milk concentration of cefquinome at 72h post-infusion in the affected quarter was much greater than the reported minimum inhibitory (MIC) concentration against common pathogens causing mastitis.

Table 1 : The milk-concentration of cefquinome at various time intervals after last intramammary infusion of cefquinome sulfate (75 mg, Cobactan LC® x3 times at12 h interval) to mastitis affected quarter in lactating HF X dairy cows (n=8)

Time (h)	Mastitic cows								Mean ± S.E(μg.mL^{-1})
	#1	#2	#3	#4	#5	#6	#7	#8	
0	6.09	22.11	228.04	18.81	4.58	53.93	319.83	69.79	90.40 ± 41.72
6	402.24	325.05	1451.08	395.64	592.25	515.67	723.76	906.72	664.05 ± 131.17
12	159.08	41.95	753.18	21.05	380.65	156.76	414.46	94.70	252.73 ± 82.31
24	34.20	31.49	296.57	5.58	27.1	47.86	74.77	54.15	71.47 ± 30.81
36	6.79	7.48	65.22	5.15	7.28	6.08	37.48	38.99	21.81 ± 7.49
48	5.57	5.44	11.04	5.05	5.69	4.43	17.05	27.16	10.18 ± 2.68
72	5.01	4.17	7.35	3.68	4.47	3.47	5.08	9.04	5.28 ± 0.64
96	ND	ND	ND	ND	ND	ND	ND	ND	ND
120	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: '0'h = before third infusion of cefquinome sulfate; LOD = 0.052 ìg.mL^{-1}; ND= Not detected

Table 2 : Pharmacokinetic parameter (mean±S.E) of cefquinome in milk after three consecutive intramammary infusions of cefquinome sulfate (75 mg, Cobactan LC® consecutively x 3 times at 12 h interval) in mastitic quarter of lactating HFx dairy cows (n=8)

Parameter	Unit	Mastitic cows								Mean ± S.E
		#1	#2	#3	#4	#5	#6	#7	#8	
lz	h^{-1}	0.01	0.02	0.09	0.01	0.01	0.01	0.06	0.04	0.031±0.01
$t_{1/2lz}$	h	5.11	6.05	7.99	3.17	3.89	5.44	5.39	4.91	5.24±0.51
t_{max}	h	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00±0.00
$C_{max.}$	$\mu g.mL^{-1}$	402.24	325.05	1451.08	395.64	592.25	515.67	723.76	906.76	664.06±131.24
$C.last_{(Obs.)}/C_{max.}$	Ratio	0.0124	0.0128	0.0051	0.0093	0.0075	0.0067	0.0070	0.0099	0.0088±0.001
$AUC_{0\text{-}72\ h}$	$\mu g.mL^{-1}.h$	4497.42	2943.45	20113.5	2827.11	754.97	5273.52	9782.32	8007.66	6774.99±2164.77
$AUC_{0\text{-}¥}$	$\mu g.mL^{-1}.h$	5134.08	3212.57	20198.30	3255.76	7890.52	5507.86	9877.70	8246.28	7915.38±1946.57
$AUMC_{0\text{-}¥}$	$\mu g.mL^{-1}.h^2$	180330.97	73581.24	270142.16	108684.47	133072.49	91428.67	135769.27	130064.41	140384.21±21744.57
MRT	h	35.12	22.90	13.37	33.38	16.86	16.60	13.75	15.77	20.97±3.08

Note: lz = the terminal rate constant; $t_{1/2lz}$ = elimination half-life; t_{max} = time to reach maximum concentration; C_{max} =maximum drug concentration; $AUC_{0\text{-}t}$ = the area under the milk concentration-time curve from time '0' to '72 h'; $AUC_{0\text{-}¥}$ = the area under the milk concentration-time curve from time '0'to '¥'; $AUMC_{0\text{-}¥}$= area under movement curve for time '0' to '¥'; MRT = Mean Residence Time

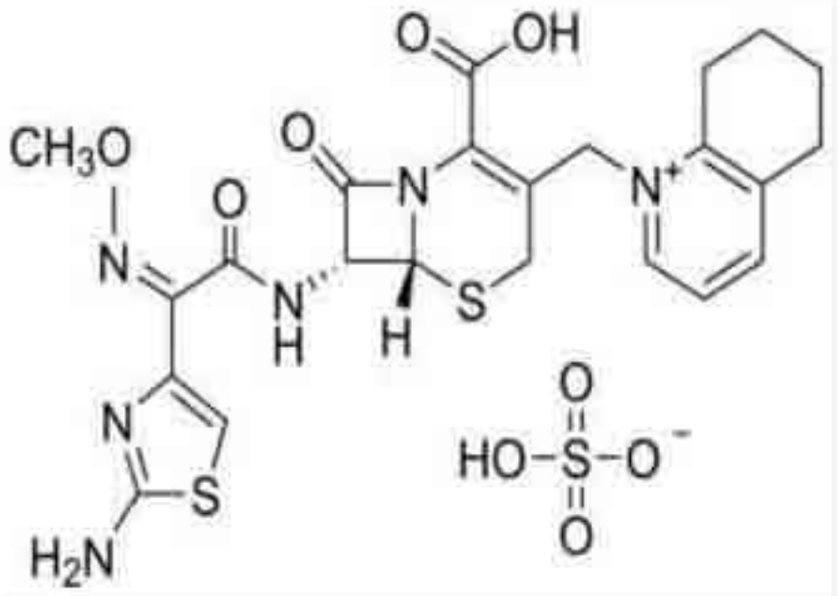

Fig.1 Structure of cefquinome sulphate

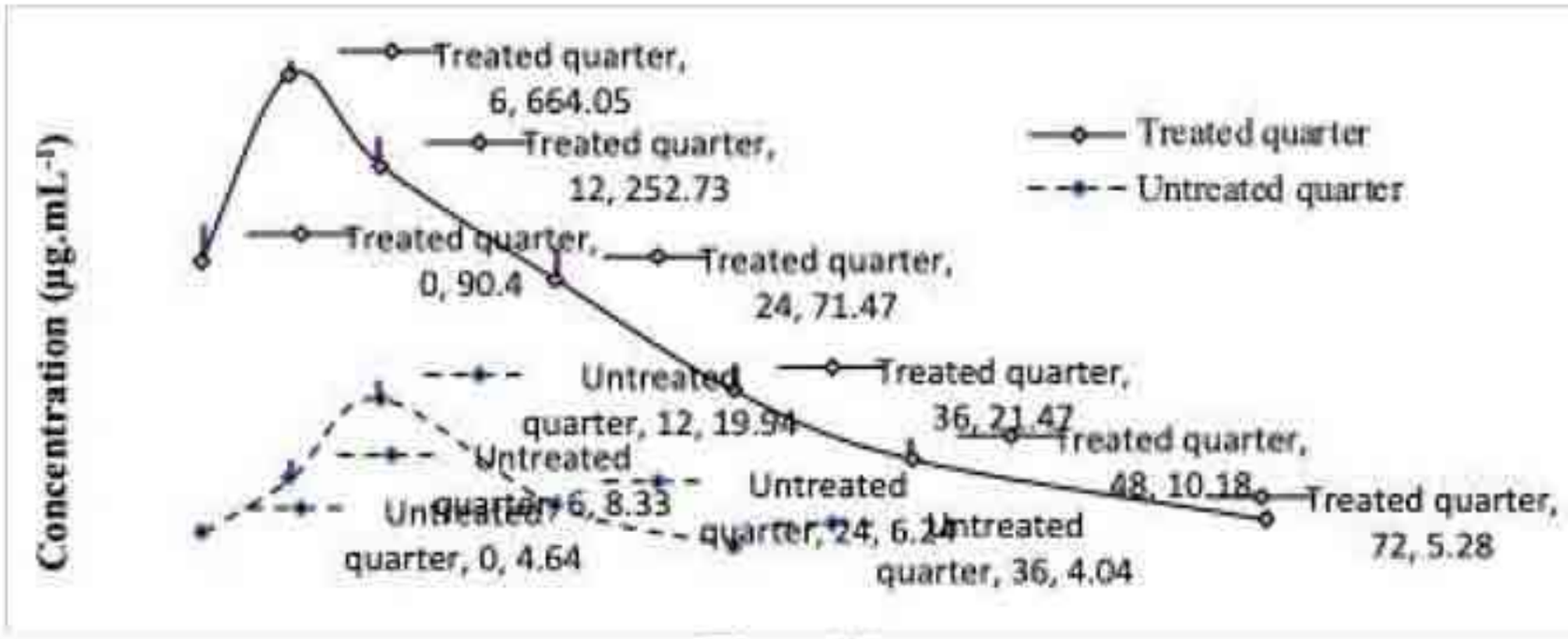

Fig. 2: Semi-logarithmic plot of time vs milk-concentration of cefquinome in treated and untreated quarter after three consecutive intramammary administrations of cefquinome sulfate (75 mg; Cobactan LC® at 12 h interval in crossbred HF x dairy cows (Mean ± S.E; n=8)

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A Study on Certain Haemato-biochemical Parameters and Screening of Gastro-intestinal Parasites of Asian Elephants (Elephas maximus) at Mathigodu Elephant Camp, Kodagu, Karnataka

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ABSTRACT

Data on wildlife animals pertaining to their physiological and pathological aspects is scanty. Asian elephants are endangered wildlife species and there is an urgent need to obtain the necessary data regarding health parameters to enhance the chances to conserve the Asian elephant population. The present study was aimed at analyzing fecal screening, RBC, WBC and Hb% of the whole blood and serological parameters like ALT, ALP, TP, CRT and BUN of the Asian elephants in the Mathigodu Elephant Camp, Kodugu, Karnataka. The study reflected the effectiveness of deworming schedule followed in the camp and also helped in understanding that there is no influence of sex on the hematological and biochemical values of serum in Asian elephants.

Keywords : Asian elephant, hematology, serology, wildlife, conservation

Wildlife conservation was given least importance when it comes to animals, considering the Veterinary public health and animal health aspects as primary concern. This was based on the economics and perceived needs of any country. Least importance is being given to the efforts for wildlife health and conservation when organizations make resource allocations. There exist only a scanty information about wildlife health values in the Indian subcontinent (Halpem et al., 2006, Maciosek et al., 2009, Miller et al., 2015., Wilson et al., 2006). Asian elephants (Elephas maximus) an endangered wildlife species needs special attention in conservation economics. (CITES, 2013). Asian elephants face severe conflicts with humans due to their habitat destruction, diseases and other natural challenges (Sukumar, 2006). Taking this scenario into consideration and also to conserve the population of these animals, Mathigodu Elephant Camp has taken initiative to document the health parameters of the elephants in the camp by conducting fecal, hematological and biochemical analysis. The data thus generated will help in future studies on Asian elephants in the Indian sub-continent and provide basic health parameters to be evaluated as part of wildlife health and there by contribute to wildlife conservation.

MATERIALS AND METHODS

A total of 25 elephants (20 males and 5 females) were included in the present study. Fecal samples from all elephants were collected fresh and transferred to the labs in sterile container. Aliquot samples of serum and blood were obtained from all elephants from the routine samples collected for their general health check up by the camp Veterinarian.

Fecal examination: Samples were examined for the presence of parasitic eggs by direct examination, floatation technique (Lane's method) and sedimentation technique by following the standard procedures.

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Hematological Examination :

The blood was tested for RBC and WBC counts using Neubauer counting chamber. The hemoglobin (Hb) concentration in the blood was analyzed using Sahli's method.

Biochemical analysis :

Plasma Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Blood Urea Nitrogen (BUN), Creatinine (CRT) and Total Protein (TP) were assayed using ERBA® biochemical kits with the help of a semi auto-analyzer.

The results obtained were statistically analyzed by unpaired 't' test.

RESULTS AND DISCUSSION

The fecal sample screening gave negative results for any of the parasitic eggs or oocysts exhibiting good deworming practice in the camp. The average RBC, WBC and Hb of male and female elephants are shown in Table 1. Likewise the average ALT, ALP, BUN, CRT and TP of male and female animals are shown in Table 2. Both the data were analyzed statistically using unpaired 't' test and no significant difference was observed between male and female elephants, thus exhibiting no significant influence of sex on the hematological and biochemical parameters. These observations were on par with the results obtained by Janyamethakul et al. (2017) except for the higher BUN, AST and ALP in male elephants which was not noticed in this study. The reason could be due to variation in the nutrition, agro climatic condition or physical activities carried out by the elephants. Further, on examination of the biochemical values in individual animals, it was observed that some of the elephants showed a very low TP level and few other elephants exhibited a very high CRT value. These changes in some elephants could be due to some inherent physiological changes and hence the Veterinarian in charge was advised to take nutritional expert advice for further action.

CONCLUSION

The present study based on fecal, hematological and serological evaluation of the elephants in Mathigodu Elephant Camp gives an indication of some of the tests which can be relied upon pertaining to the health status of asian elephants in the Indian sub-continent. More of this kind of studies are needed in all the captive/ domesticated elephants and other wild animals of this country to establish a strong data base which can become an authentic record for future research to be carried upon.

Table 1 : Average values of hematological analysis of elephants of the camp

	Total RBC (millions/ mlof blood)	Total WBCcells/µl	Hb (g/dl)
Male elephants	3.7±0.21	4732.3±810	11±0.20
Female elephants	3.23±0.10	2650±936.6	10.68±0.29

Table 2 : Average values of biochemical parameters of elephants of the camp

	ALT(U/L)	ALP(U/L)	BUN(mg/dl)	CRT(mg/dl)	TP(g/dl)
Male elephants	2.9±0.75	13±5.62	37.8±3.87	1.59±0.43	1.59±0.43
Female elephants	2.54±0.87	21.7±8.59	45.67±9.85	0.8±0.33	0.81±0.33

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Gross and Morphometrical Studies of the Ligaments of Quadriceps Tendon in Pre and Post Natal Stages of the Buffalo (Bubalus bubalis)*

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ABSTRACT

Tendon of quadriceps femoris which is very crucial structure of stifle joint for the stability and locomotion of the buffaloes. This tendon passes over the patella and insert to tibial tuberosity as patellar ligaments. Morphological and morphometry of the patellar ligaments namely medial, lateral and middle ligaments are studied from prenatal to postnatal stages which were divided into four groups. Group I was prenatal stage, Group II comprised of young animals (0-3yrs) while group III was adult animal group (3-6yrs) and Group IV had older animals aged more than 6 years. Grossly ligaments in group I was shiny, smooth and reddish in nature. Group II (young) and III (adult) was slightly whitish, hard and very tough but was much thicker in nature in group IV (older). Medial ligament was very thin among ligaments with its counterpart as lateral ligament due to enforcement of fibers from biceps femoris muscle. Length and width of lateral and middle patellar ligament was increased significantly from group I to IV but medial ligament length increase was seen only from I to III groups only. Thickness increase seen in two shells from group I to II and Group III to IV with no significant difference from II to III.

Key Words : Patellar ligaments, Prenatal, Postnatal, Buffalo and Stifle joint

Upward fixation of patella or pseudo luxation of patella hampers the normal locomotion and thereby adversely affects the performance of the buffaloes. This clinical condition is commonly encountered in Indian subcontinent and is mainly attributed to anatomical peculiarities of the straight patellar ligaments of quadriceps tendon. A thorough knowledge of these ligaments is highly essential for a clinician to bring about surgical correction of the condition. The distal part of the tendon of insertion of the main extensor muscle of the stifle i.e., quadriceps femoris is divided into three parts which pass over the patella forming the anterior part of joint capsule under which the synovial cavity extends proximally (Bland and Ashhurst, 1997) in domestic animals. An elaborate twisting movement of the patella allows the stifle to 'lock' in extension when the medial portion of the tendon is 'hooked' over the bulbous medial ridge of femoral trochlea.

Available literature shows that incidence of upward fixation of the patella is higher in buffalo than in cattle (Semieka and Misk, 1997). Hence this study was conducted to acquire a thorough knowledge of the morphometric changes of ligaments of quadriceps tendon in buffaloes during prenatal and postnatal periods.

MATERIALS AND METHODS

Intact stifle joint specimens of twenty four (24) apparently healthy buffaloes and fetuses irrespective of their breed, sex and nutritional status were procured from either hind limb by cutting at the level of distal and proximal thirds of femur and tibia respectively.

*Part of the M.V.Sc. thesis of first author submitted to SVVU, Tirupati.

Age of the buffaloes from which post natal specimens collected was determined from the dentition pattern as per FAO (1994) while that of the fetuses by employing the formula derived by Soliman (1975). The specimens collected were divided into four groups as Group I (Prenatal), Group II (young 0-3yrs), Group III (3-6yrs) and Group IV (>6yrs).

Stifle joint specimens of all groups were carefully dissected out to study gross morphological features of constituent ligaments and morphometrical values were recorded by using thread, scale and digital Vernier caliper.

RESULTS AND DISCUSSION

In group I, the patellar ligaments were observed as part of the insertion of the huge quadriceps femoris muscle of the thigh. In fresh state they were reddish brown colored, shiny and three in number. As per their position they were identified as medial, middle and lateral ligaments. These ligaments passed over the patella and inserted to anterior tibial tuberosity (Figs. 2 and 3). Similar reports regarding straight patellar ligaments was given by Sisson (1975), Nickel et al.(1986), Konig and Liebich (2004) in ox and Dyce et al. (2010) in ox and buffalo and Supriya (2010) in buffalo calves. Among the three ligaments, the middle one was strongest and well developed (Figs. 3 and 5). The lateral patellar ligament was comparatively better developed and slightly thicker (Figs. 2, 4 and 5) than the medial and the latter was very thin (Figs. 2, 3 and 5). The former ligament also received fibres from biceps femoris muscle (Figs. 3 and 4). Middle and medial ligaments were separated by a wide space filled with fat and fascia, whereas the middle and lateral ligaments were closer with a narrow gap (Figs. 2, 3 and 5). According to Uddin et al. (2009) straight patellar ligaments enhanced the clinical importance of the stifle joint in cattle. They noticed that the medial patellar ligament was distinctly weaker than the other two and was widely separated from the middle one at both ends. These reports are in close agreement with the present findings also. The middle ligament was reinforced with fibres from biceps femoris muscle, thus became comparatively stronger and thicker than the thinnest medial ligament.

The three straight ligaments of patella in postnatal specimens (Groups II, III and IV) appeared increased in their length, width and thickness as age advanced. They were tougher in adult and aged specimens. The medial ligament was thin and broader at proximal end, separated from the middle ligament at the origin and its narrow insertion joined the tibial tuberosity (Figs.1 and 5). The muscle tibialis anterior originate lateral aspect of tibial tuberosity close to the insertion of patellar ligaments (Fig. 5). Conspicuously in the anterior part of the stifle joint, tendon of common origin of long digital extensor and peroneus tertius was seen crossing between middle and lateral patellar ligaments (Figs. 1, 3, 5 and 6).

The central or middle patellar ligament was straight in position from patella to the tibial tuberosity (Fig.1, 3 and 5). It was the strongest of all the three ligaments and also received fibres from the parapatellar fibro cartilage of patella below its proximal end. Two collateral ligaments of femorotibial articulation were attached from respective postero-lateral and medial aspects of tibia, and were stretched across the joint to be attached at the postero- lateral and medial aspect of corresponding femoral condyles (Figs. 4, 5, and 6). Collateral ligaments helped in keeping the articulating ends of femur and tibia together.

Length of the lateral and middle patellar ligaments increased significantly ($P \leq 0.01$) from Group I (4.30 ± 0.51 and 4.39 ± 0.69 cm) to Group IV (12.73 ± 0.52 and 14.67 ± 0.17 cm) specimens respectively, whereas the length of medial ligament showed a significant increase from Group I (4.13 ± 0.58 cm) to Group III (13.29 ± 0.24 cm) only. Beyond this stage the increase in its length was insignificant (Table. 1). Similar opinion was given by Uddin et al.(2009) who mentioned that the medial patellar ligament was the weakest of all three ligaments. They reported that the

middle patellar ligament was thick and strong. Its length and width was 11.580 ± 0.724 cm and 2.330 ± 0.205 cm in indigenous animal and was 10.230 ± 0.382 cm and 2.019 ± 0.258 cm in cross breed cattle respectively.

The width of the lateral and medial patellar ligaments increased significantly from Group I (1.55 ± 0.16 and 0.85 ± 0.30 mm) to Group IV (5.27 ± 0.17 and 3.17 ± 0.24 cm) respectively while that of medial ligament showed a significant increase from Group I to II and from Group III to IV. Between age Groups II and III, change in thickness was insignificant (Table 1).

The thickness of the lateral and medial patellar ligaments showed a significant increase in two spells i.e., from Group I (1.17 ± 0.49 mm and 0.61 ± 0.21 mm, respectively) to Group II (4.84 ± 0.72 mm and 1.26 ± 0.06 mm, respectively) and Group III (05.71 ± 0.91mm and 1.52 ± 0.27 mm, respectively) to Group IV (8.80 ± 0.50 mm and 2.05 ± 0.08 mm, respectively) (Table 1).

CONCLUSION

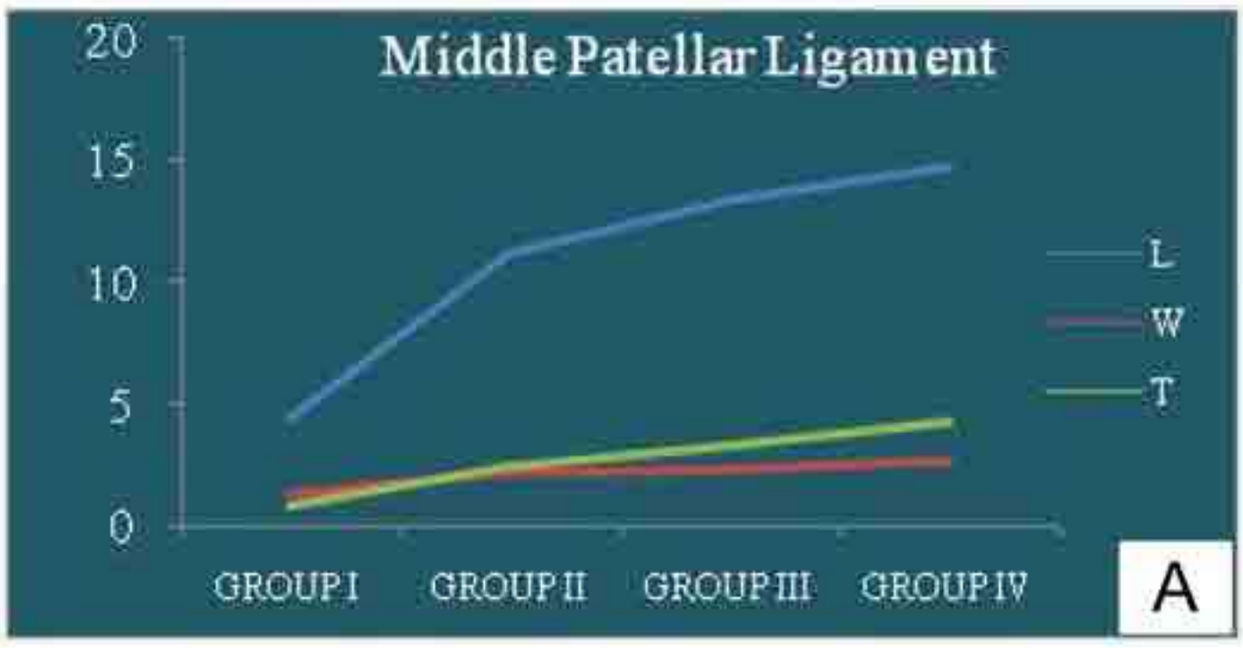
Tendon of quadriceps femoris is a very crucial structure of stifle joint which passes over the patella and insert to tibial tuberosity as patellar ligament. Length and width of lateral and middle patellar ligament was increased significantly from group I to IV but medial ligament length increase was seen only from I to III groups. Medial patellar ligament was very thin as compared to lateral and middle wherein the former receives fibers from neighboring muscles. The thinness of medial patellar ligament along with incongruity of femoral trochlea may be crucial for the pseudoluxation of the stifle joint.

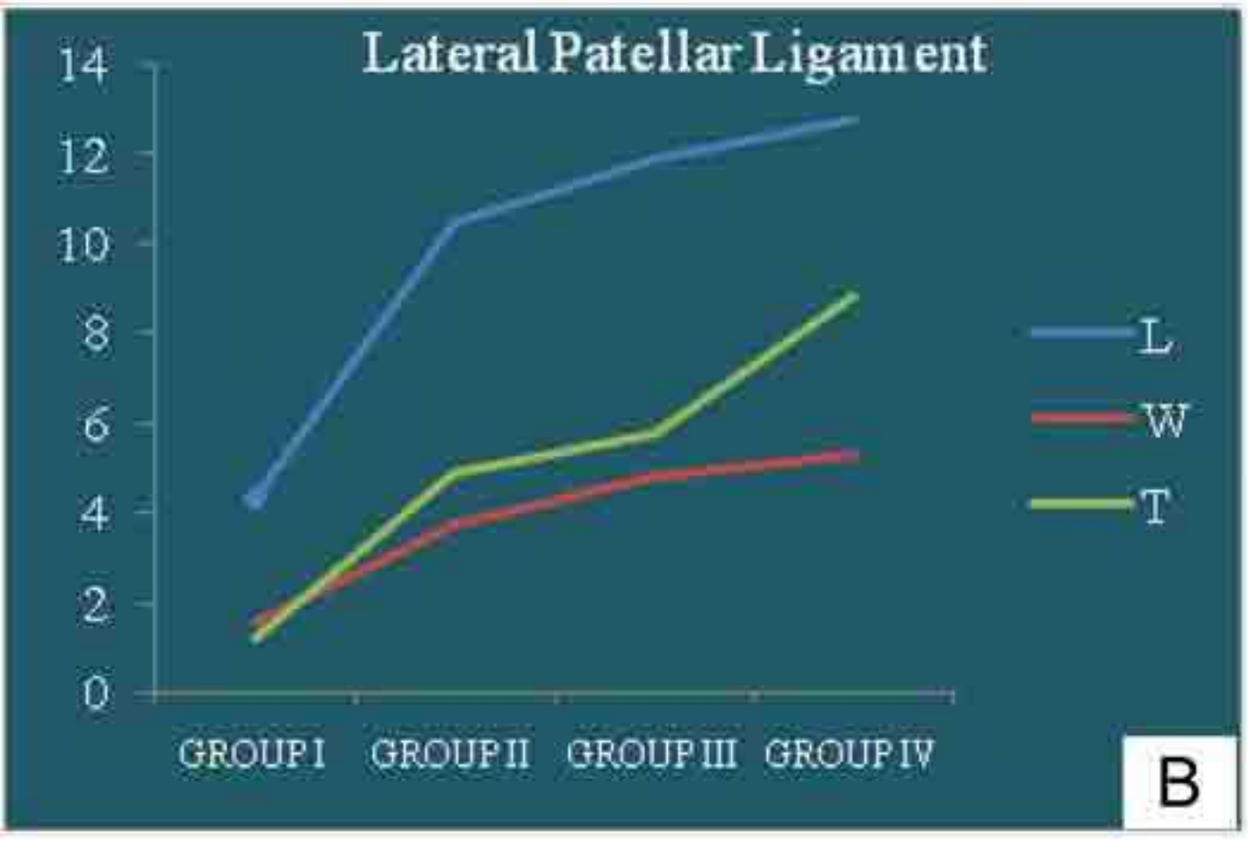
Table. 1 : Morphometric growth of patellar ligaments from prenatal (Group I) to postnatal (Groups II, III and IV) stages.

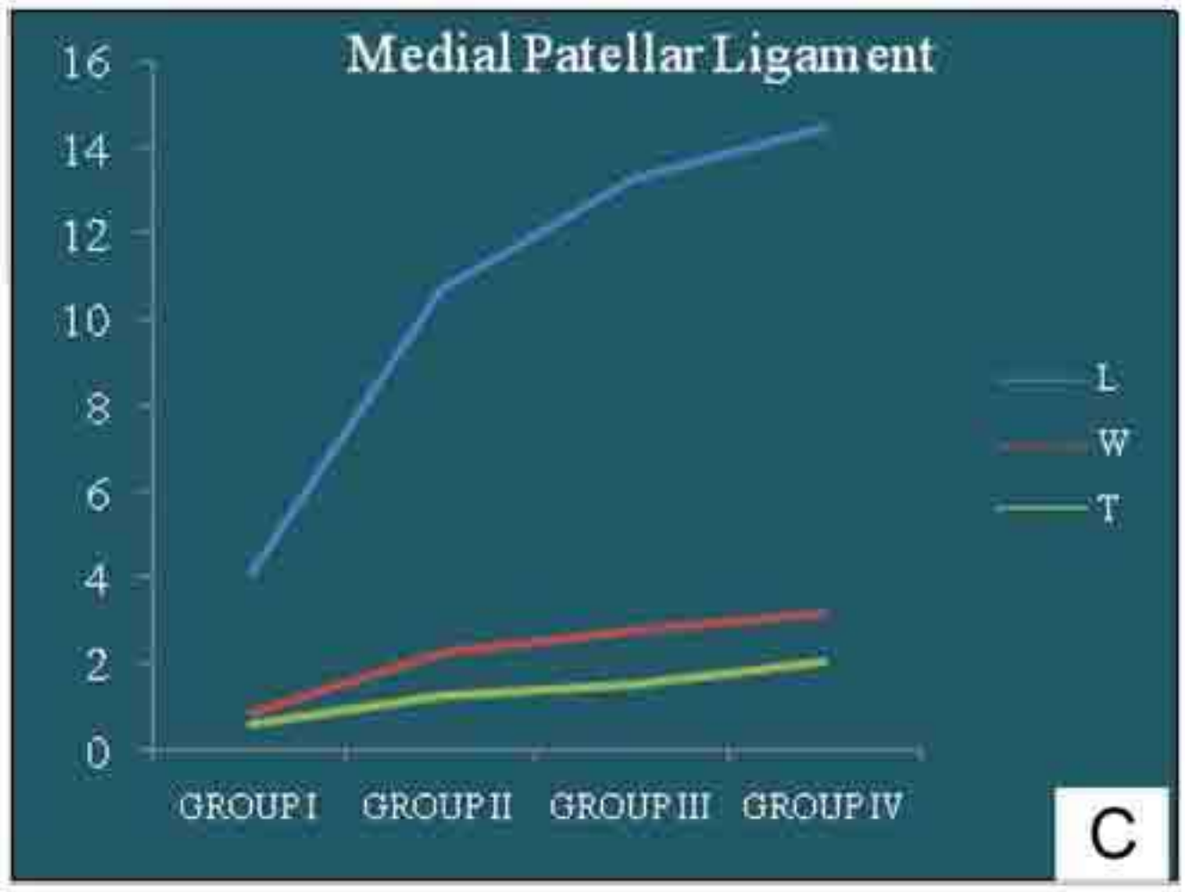
Sl.No.	Parameter		Group I		Group II		Group III		Group IV	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	Middle Patellar Ligament	L	4.39[a]	0.69	11.17[b]	0.81	13.33[c]	0.49	14.67[d]	0.17
		W	1.37[a]	0.24	02.21[b]	0.21	02.28[b]	0.15	2.68[c]	0.13
		T	0.87[a]	0.35	02.37[b]	0.19	03.25[c]	0.19	4.23[d]	0.22
2	Lateral Patellar Ligament	L	4.30[a]	0.51	10.43[b]	0.36	11.87[c]	0.57	12.73[d]	0.52
		W	1.55[a]	0.16	03.70[b]	0.12	04.83[c]	0.37	5.27[d]	0.17
		T	1.17[a]	0.49	04.84[b]	0.72	05.71[b]	0.91	8.80[c]	0.50
3	Medial Patellar Ligament	L	4.13[a]	0.58	10.69[b]	0.13	13.29[c]	0.24	14.44[c]	1.50
		W	0.85[a]	0.30	02.22[b]	0.14	02.74[c]	0.19	3.17[d]	0.24
		T	0.61[a]	0.21	01.26[b]	0.06	01.52[b]	0.27	2.05[c]	0.08

L – length, W – width (in cm) and T – thickness (in mm)

* Means with similar superscript within a row do not differ significantly ($p \leq 0.05$)







Growth pattern of medial (A), lateral (B) and middle (C) patellar ligaments from prenatal to postnatal stages

L- Length (in cms)

W- Width (in cms)

T- Thickness (in mms)

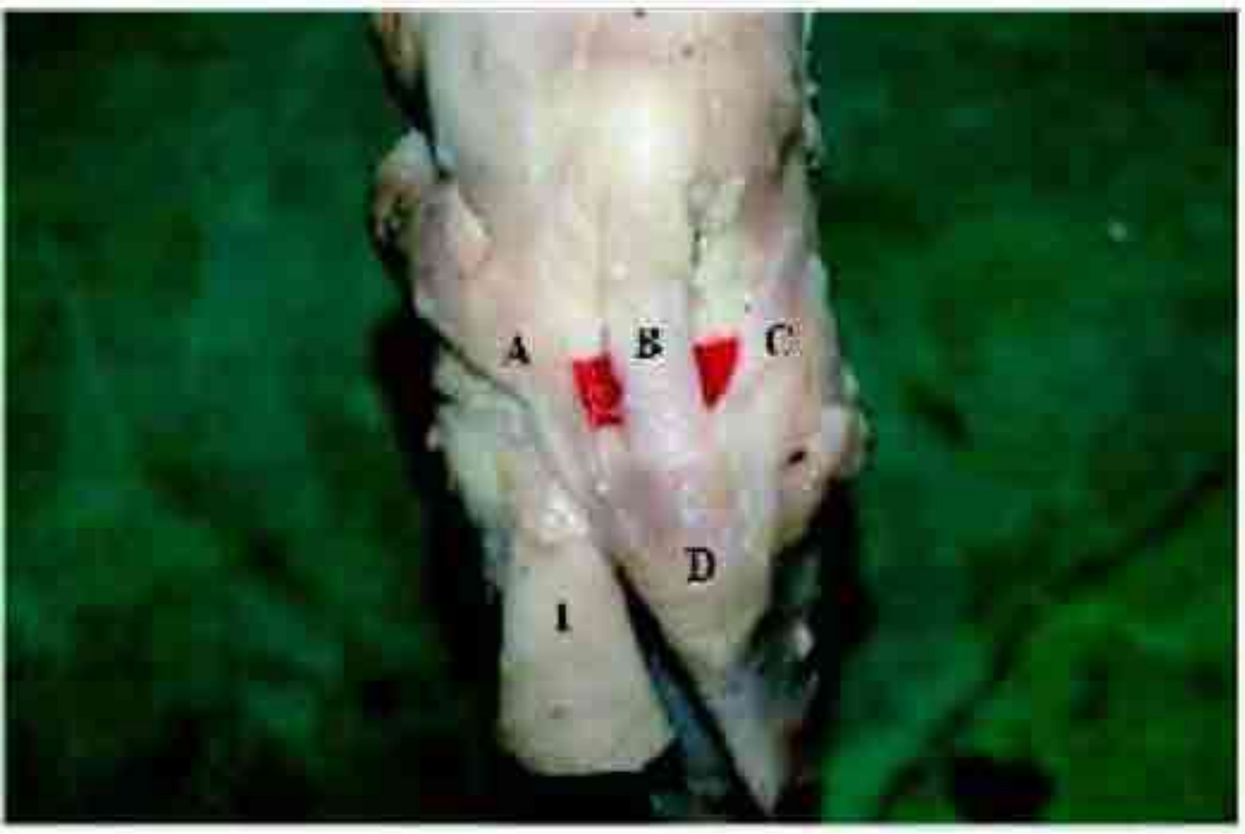


Fig.1. Anterior view of stifle of Group I aged 189 days showing Medial patellar ligament (A), Middle patellar ligament (B), Lateral patellar ligament (C), Anterior tibial tuberosity (D), Common origin of long digital extensor and peroneus tertius (1)

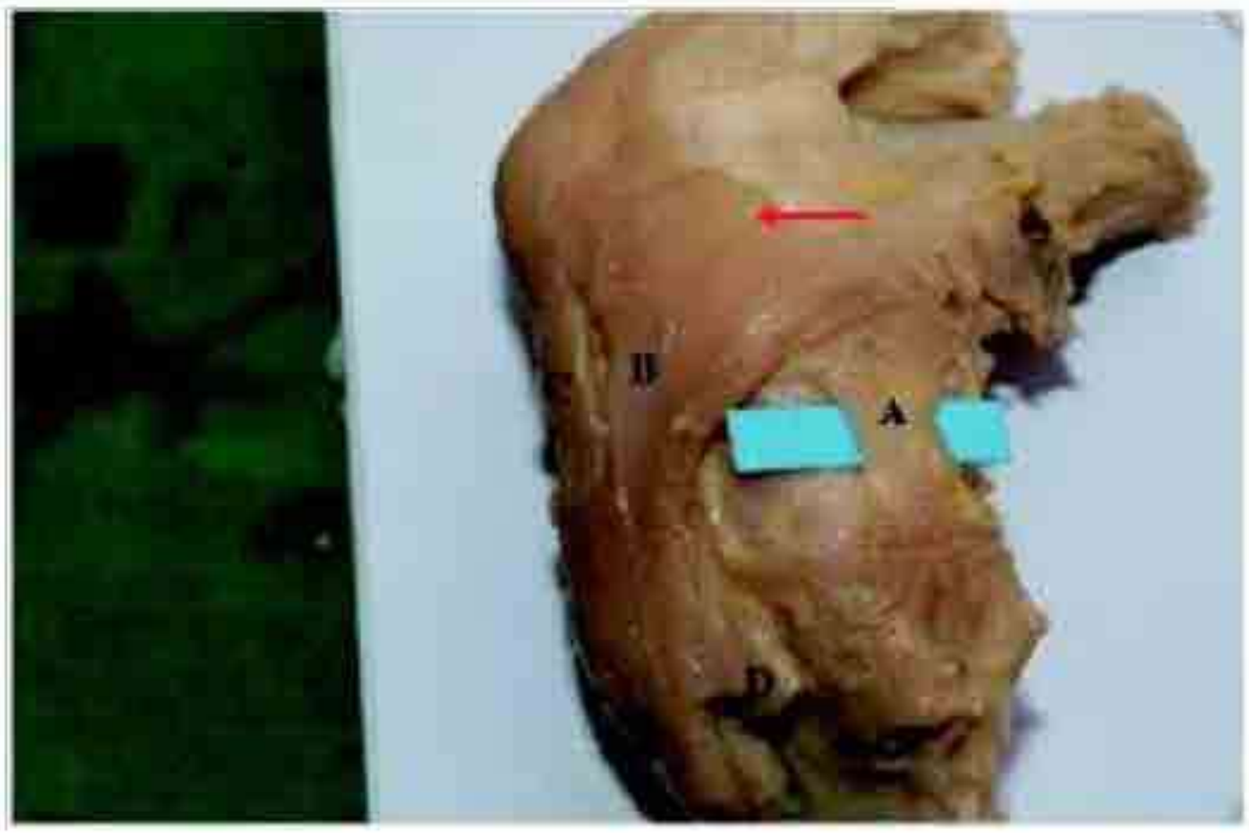


Fig. 2. Lateral view of left stifle of Group I aged 180 days showing the lateral collateral ligament (A) Lateral patellar ligament (B) Middle patellar ligament (C), Common tendon of long digital extensor and peroneustertius (D)

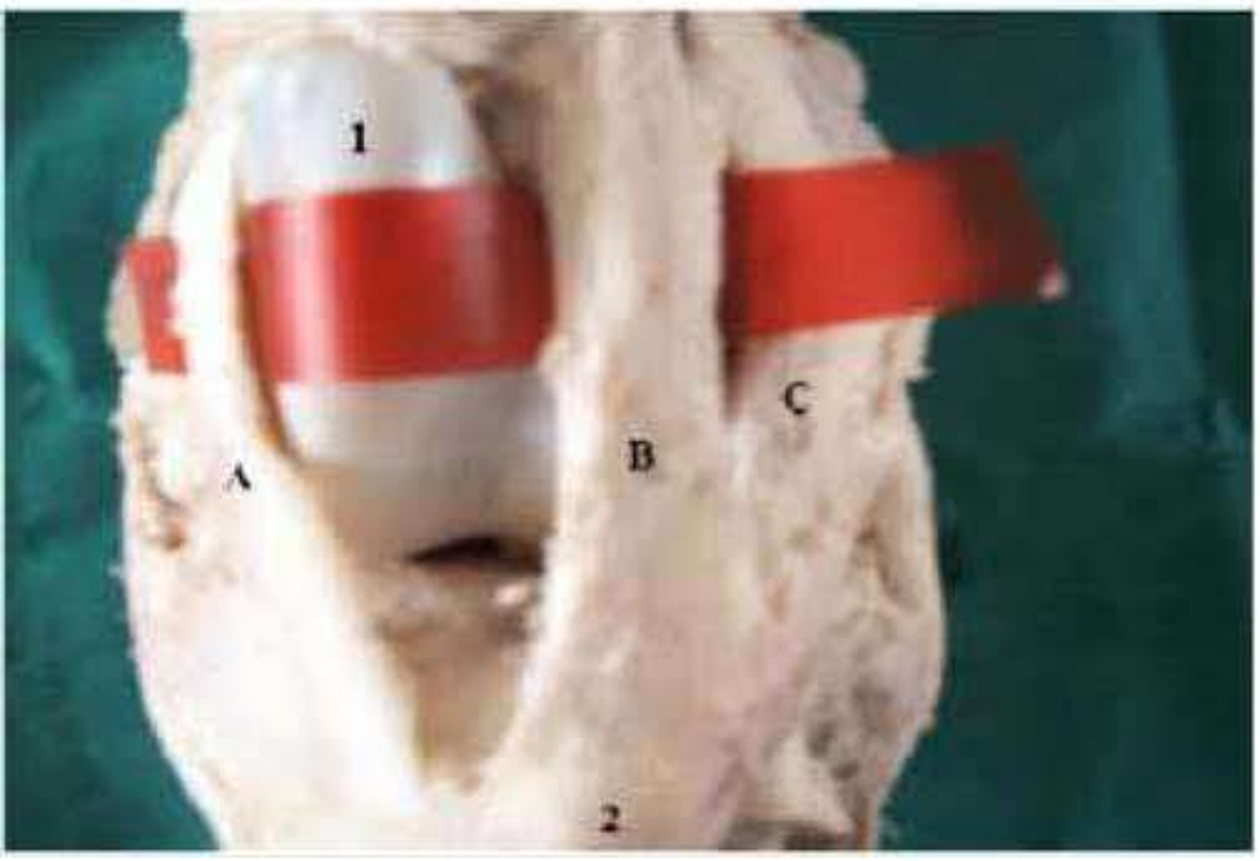


Fig. 3. Anterior aspect of stifle joint Group IV specimens
Medial patellar ligament (A),
Middle patellar ligament (B),
Lateral patellar ligament (C),
Medial ridge of femoral trochlea (1),
Anterior tibial tuberosity(2).

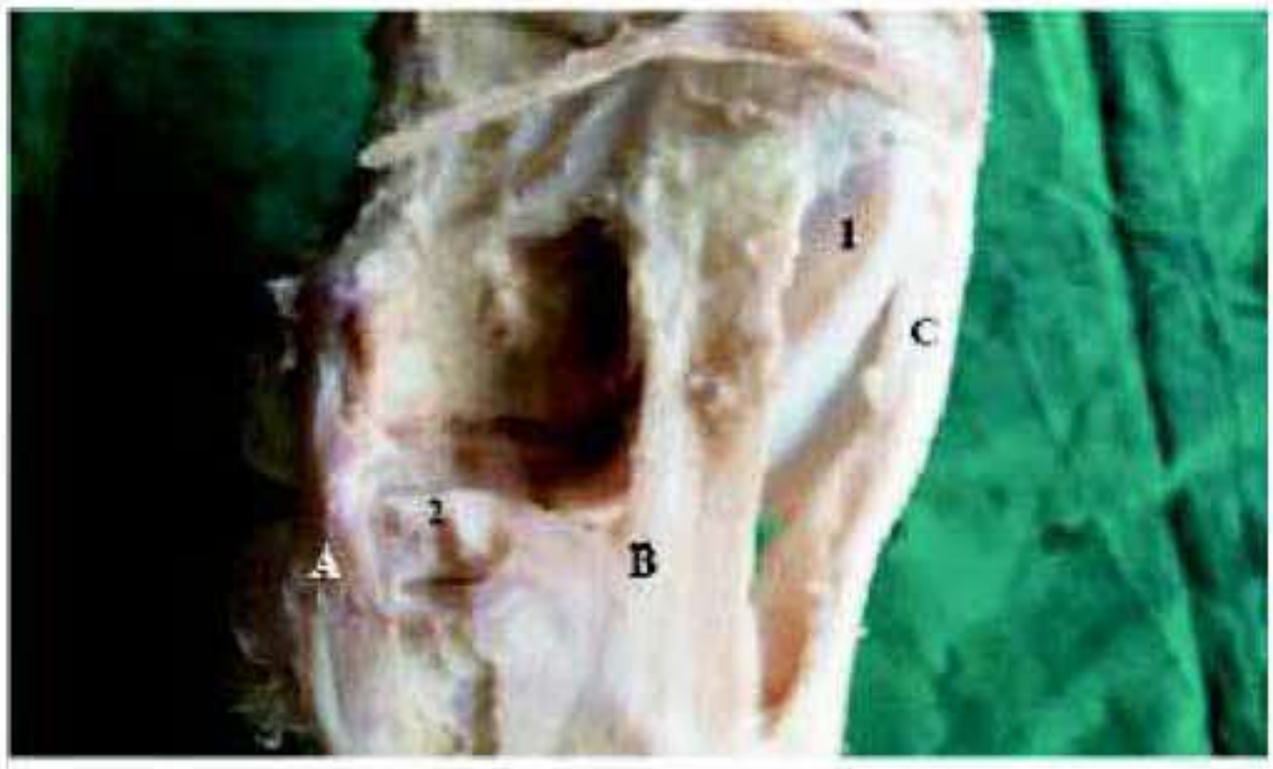


Fig. 5. Medial view of dissected stifle joint (Group IV)
showing medial collateral Ligament (A),
Medial patellar ligament (B),
Middle patellar ligament (C),
Medial trochlear ridge (1),
Medial meniscus (2).

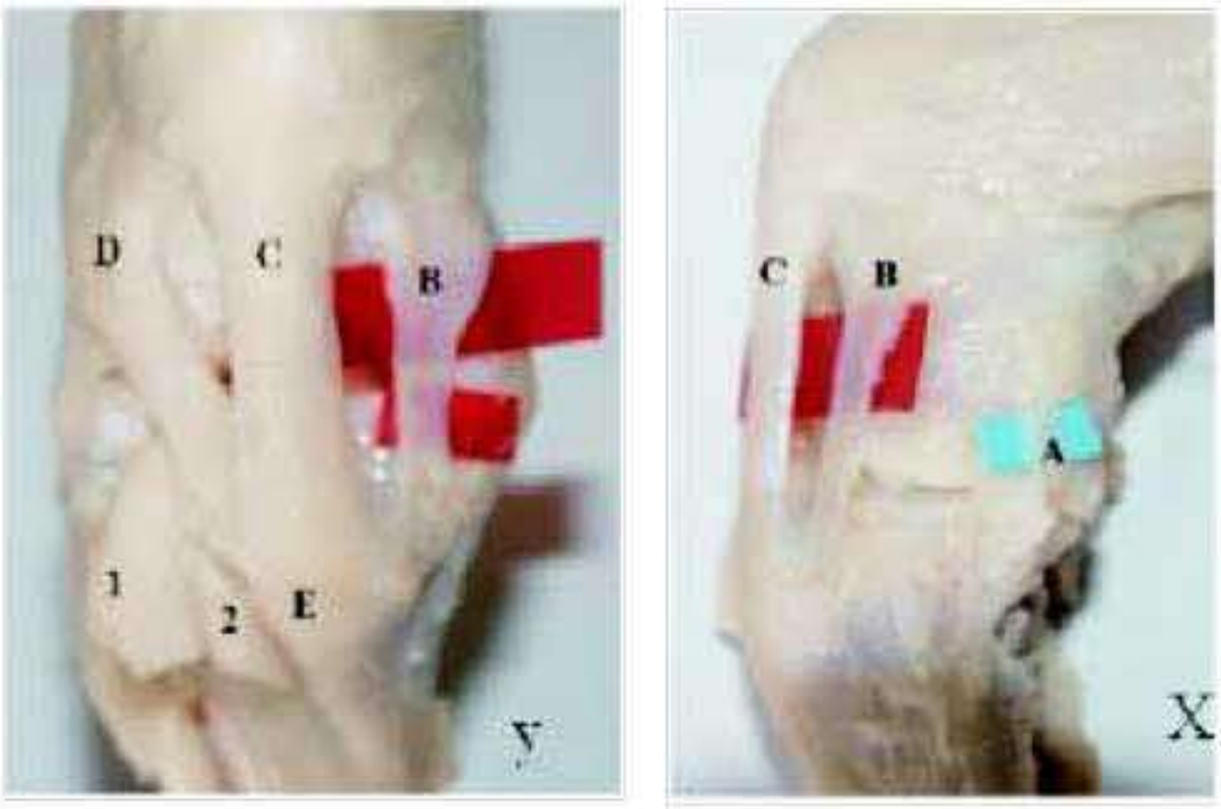


Fig. 4. Medio lateral view(x) and anterior view(y)
of stifle joint (Group I aged 189 days)
showing Medial collateral ligament (A),
Medial Patellar ligament (B),
Middle patellar ligament (C),
Lateral patellar ligament (D),
Common origin of long digital extensor and
peroneustertius (1)
Origin of tibialis anterior (2).

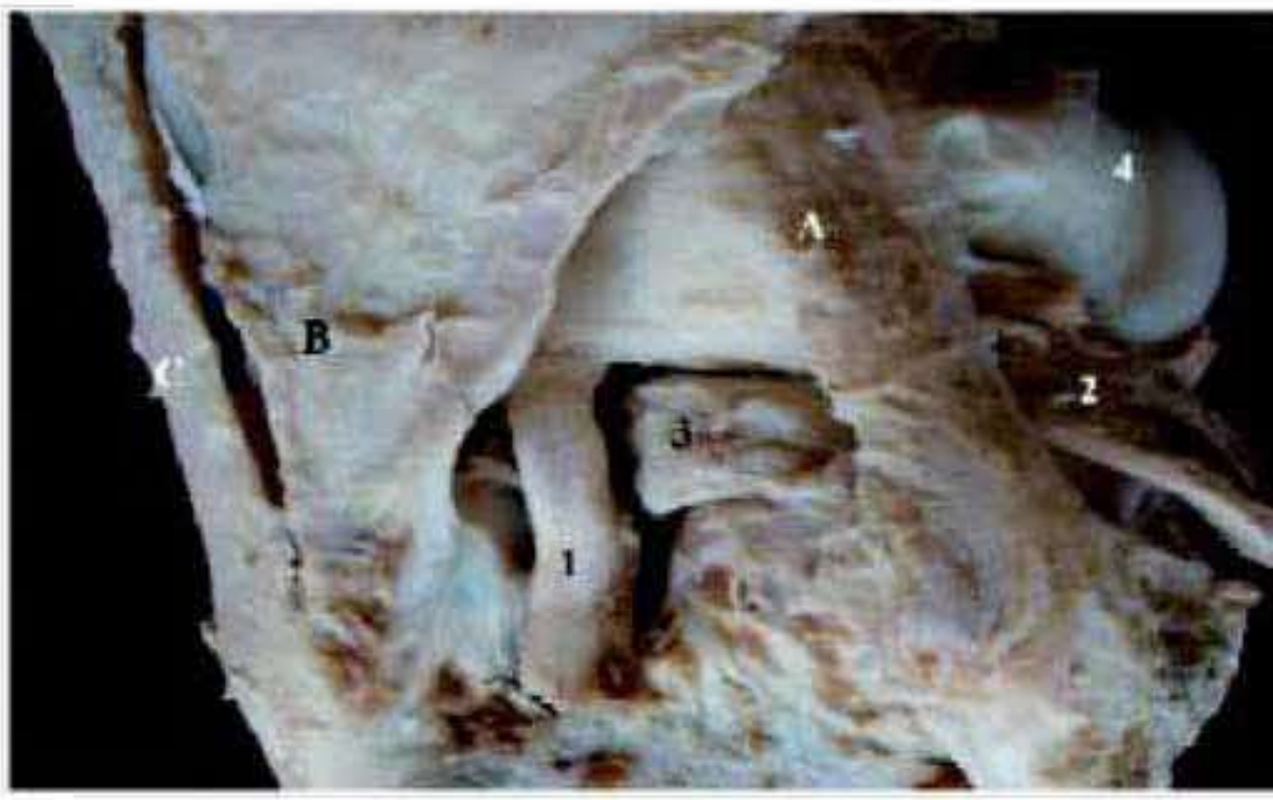


Fig. 6. Lateral view of stifle of Group IV
specimen showing lateral collateral ligament (A),
Lateral patellar ligament (B),
Middle patellar ligament (C),
Tendon of common origin of long digital extensor and
peroneus tertius (1),
Tendon of popliteal muscle (2)
Lateral meniscus and Lateral femoral condyle (3).

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An Incidence of Lantana Toxicity among Sheep in Uttara Kannada District of Karnataka

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ABSTRACT

A flock of 1500 sheep was presented with the history of 70 deaths, in Nyasargi village of Mundagod taluk of Uttara Kannada district. Mortality was noticed in the age group of 2-4 years. As per the history, animals became sick and succumbed within 2-3 days. Clinical symptoms among 15 ailing sheep included general body discomfort, dullness, depression, hurried respiration, anorexia, photophobia, swollen ears and frequent urination. The body temperature ranged between 104^0 F - 106^0 F. Mild to moderate diarrhoea in few of the ailing sheep and constipation and desiccation of faeces among others was noticed. Aimless running was a peculiar symptom noticed in few of the animals. Examination of mucus membranes revealed icterus. Blood smears were negative for any specific pathogen. Haematology revealed anaemia and leucocytosis. Serum biochemistry revealed elevated BUN, Creatinine, SGOT, SGPT, ALP, Bilirubin (Total and direct) and reduced Glucose and Albumin levels. No alteration was observed in Calcium, Phosphorus and Magnesium levels. Post-mortem lesions included icterus and hemorrhages in all internal organs and mesentery and necrotic foci on liver. Kidneys were soft, pale and swollen. Intestinal contents were mucus coated. The condition was diagnosed as lantana plant toxicity based on circumstantial evidences as lot of lantana shrubs noticed in the area where the sheep were grazed. The owner was advised to change the grazing area and confine ailing sheep in dark place. Ailing animals were treated with activated charcoal @ 5mg/kg body weight and magnesium sulphate @ 50 gms in lukewarm water, administered orally along with 500 ml of DNS I/V and liver tonics (Belamyl) 5 ml I/M, for five days. Recovery was noticed in a span of ten days.

Key words : Lantana, toxicity, ovines

Lantana (Lantana camera) is one of the most popular shrubs used for fencing the agricultural land. It is also an ornamental plant grown in gardens. It has both medicinal and toxic properties. It has anti-inflammatory, antipyretic, antispasmodic and antibiotic properties (Sharma et al., 2007). It causes cholestasis, hepatotoxicity, photo-sensitization, and even fatality in cattle, horses, sheep, dogs and humans (Brito et al., 2004). Stocks bred on lantana infested country tend to avoid it unless forced to eat it due to lack of food (Holm et al., 1979). Lantana is one of the obnoxious weeds in certain parts of India. Cattle generally avoid foraging on them but stocks newly introduced to the area may feed on them and suffer from toxicity (McSweeney and Pass, 1983). Lantana toxins are the triterpene acids, lantadene-A (Rehmannic acid), lantadene-B and their reduced forms (Sharma et al., 1998). Lantana poisoning in sheep leads to gross ruminal microbial inactivity (Subhash, et. al, 2014).

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MATERIALS AND METHODS

Clinical history

As per the history obtained from the local Veterinary doctor and the shepherd, 70 animals were reported dead in a span of about one week in a sheep flock of about 1500 animals at Nyasargi village in Mundagod taluk of Uttara Kannada district of Karnataka. The flock was nomadic in nature. Shepherds were grazing their flock in the countryside and nearby forest. As per the history, animals became sick and succumbed within 2-3 days. Mortality was noticed in all age groups but more so in the age group of 2-4 years. They were vaccinated against HS and ET about 6 months back and recently vaccination against ET was also done about two days back after the onset of the current episode.

Clinical observation

At the time of investigation fifteen ailing sheep were presented for clinical examination. All the fifteen were examined and clinical symptoms included anorexia, dullness, hurried respiration, discomfort and photophobia, body temperature of ailing animals ranged between 104° F to 106° F. Depigmentation around the eyes was observed. Conjunctival mucous membranes showed congestion and sclera was icteric. Edema of ears was seen in four animals.

Collection of blood samples

About 10 ml of blood sample was collected aseptically from jugular vein of these animals in sterile vacutainer tubes containing EDTA and were immediately transported to the laboratory on ice. Guidelines laid down by the International Animal Ethics Committee (IAEC) and prevailing local laws and regulations were followed during blood collection.

Ten ml of blood sample was also collected aseptically from the jugular vein of each of the fifteen ailing animals, in a sterile vacutainer tube. Blood samples were immediately transported on ice to the laboratory for analysis. Samples were allowed to clot for three hours. Serum was separated (Sharma et al., 2007) and stored in – 20°C deep freezer.

Microscopic examination

Blood smears were prepared from the blood samples collected aseptically and stained using Giemsa stain and observed under microscope for haemoprotozoan parasites.

Haematological analysis

Blood samples in EDTA were subjected to estimation of Haematological parameters viz., Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet Count (PLT) using ERMA PCE -210(N) Haematology analyser (Erma Inc, Tokyo), following the instructions of the manufacturer.

Biochemical analysis

Serum samples were subjected for estimation of Serum Glutamate Oxalate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), Albumin(ALB), Total Protein (T.P.), Calcium (Ca), Phosphorus (P), Magnesium (Mg), Total Bilirubin (T. Bil), Direct Bilirubin (D. Bil), Glucose (Glu), Blood Urea Nitrogen (BUN) and Creatinine (CRE), using ERBA Chem-5 plus V2 semi automatic biochemical analyzer, Transasia Biomedicals Ltd., and standard biochemical were used in the study.

Post mortem examination

Post mortem examination was conducted on 14 sheep and gross lesions were noted. Heart blood, tissue samples and rumen contents were collected for laboratory analysis.

RESULTS AND DISCUSSION

Microscopic examination of the stained blood smears did not reveal any specific pathogen

(hemoprotozoa / bacteria).

Results of the haematological analysis is presented in Table.1 and serum biochemical analysis in Table. 2.

Haematological analysis revealed leucocytosis and macrocytic hypochromic anaemia. Serum biochemistry revealed elevated liver enzymes such as SGOT, SGPT, ALP, bilirubin (Total and direct), creatinine and BUN and reduced glucose and albumin levels.

Post-mortem examination revealed generalized haemorrhages on all internal organs and mesentery, liver was pale and had lot of necrotic foci suggestive of toxicity. Both the kidneys were soft.

Ruminal content was negative for nitrates, nitrites and cyanides. Lot of lantana (Lantana camara) plants were seen in the area where sheep had grazed. The owners also confirmed that the sheep were grazing on these plants also.

Hence based on the history, clinical signs, post-mortem lesions, results of laboratory analysis and circumstantial evidence of grazing on lantana plants, it was concluded that it was case of lantana toxicity.

Treatment

All the affected animals were treated with the following :

i) Administration of Activated charcoal @ 5 gm kg^{-1} body weight orally for 2 to 3 days as per Pass and Stewart, 1984.

ii) Magnesium sulphate @ 50 grams orally in luke warm water 2 times on alternate days.

iii) Liver tonic (Livotas) 20 ml orally and Inj. Belamyl(R) @ 3 ml intramuscular for 5 days.

iv) 5% DNS - 500 ml intravenous for 3 days and 50 ml Mifex intravenous once

v) Inj. Chlorpheneramine maleate (Avil) - 2ml intramuscular for 3 days.

The treatment was administered and the remedial measures were suggested such as to isolate the affected animals and keep them in dark without exposing them to sunlight. It was also advised to change the grazing area where no lantana plants were available. Oral multivitamins, liver stimulants, purgatives and rumenotorics advised were similar to those given by Kumar et al. (2009), wherein charcoal absorbed the lantana toxins and purgatives helped to remove the toxic materials from gastrointestinal tract. The DNS and liver tonic helped to boost up the liver functions. Keeping the animals in dark place helps in detoxifying the photosensitising agents. Treatment is generally carried out according to the symptoms and have limited success (Sharma et al., 2007).

CONCLUSION

It was concluded from the study that lantana ingestion leads to photophobia and death among sheep. Clinical management with activated charcoal, fluid therapy, rumenotorics and liver tonics was successful in managing the condition. Farmers were advised not to feed their sheep on lantana plant.

Table 1 : Mean and SE of haematological parameters

Sl. No	Blood parameters	Mean ± SE
1	Total leukocyte count x 10^3/cmm	50.48 ±4.31
2	Total erythrocyte count x 10^6/cmm	11.72 ±0.731
3	Haemoglobin (g/dl)	8.9 ±0.41
4	Packed cell volume (%)	35.33 ±1.93
5	Mean corpuscular volume (fl)	30.12 ±1.03
6	Mean corpuscular hemoglobin (pg)	18.37 ±10.73
7	Mean corpuscular hemoglobin concentration (%)	25.17 ±0.36
8	Platelet x 10^3/cmm	237.5 ±45.42

Table 2 : Mean and SE of serum biochemical parameters

Sl. No	Serum Biochemical Parameters	Mean ± SE
1	Glucose (mg/dl)	54.97 ±20.00
2	Blood urea nitrogen (mg/dl)	25.85 ±4.44
3	Creatinine (mg/dl)	2.17 ±0.21
4	Serum glutamate oxalate transaminase (IU/L)	335.73 ±33.68
5	Serum glutamate pyruvate transaminase (IU/L)	114.57 ±22.26
6	Alkaline phosphatase (IU/L)	392.92 ±74.41
7	Total protein (g/dl)	4.83 ±0.29
8	Albumin (g/dl)	1.81 ±0.20
9	Calcium (mg/dl)	8.78 ±0.20
10	Phosphorus (mg/dl)	3.8 ±0.27
11	Magnesium (mg/dl)	2.42 ±0.06
12	Total bilirubin (mg/dl)	3.59 ±1.03
13	Direct bilirubin (mg/dl)	2.12 ±0.81

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Antiparasitic Activity of Allium sativum and Tinospora cardifolia against Gastro Intestinal Parasites in Pigeons.

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ABSTRACT

Prevalence of gastrointestinal parasites in pigeons and their successful treatment using a combination of Allium sativum (Garlic) and Tinospora cardifolia (Amrutha balli) was studied in Veterinary College, Hassan. Out of one hundred domestic pigeons screened for the presence of gastrointestinal (GI) parasites, 62 were found positive. Among the positive birds, 50 birds had mixed infection with Ascaridia columbae and Eimeria oocysts, 12 had mixed infection with Capillaria species and Eimeria oocysts. The birds infected with Ascaridia columbae were divided into 5 different groups. P1 served as control, P2 received a combination of Levamisole and Amprolium, P3, P4 and P5 received the above-mentioned herbal combination. The results proved that the herbal medicine was equally effective as Levamisole and Amprolium in treating the GI parasites of pigeons.

Key words : Allium sativum, Tinospora cardifolia and Pigeons

The rock pigeons from which the domastic pigeons were derived are the world's oldest domesticated birds. Research suggests that domestication of pigeons occurred as early as 10,000 years ago. Pigeons are reared as a source of food, hobby, for racing purpose, as messengers and also for experimental purposes based on their capacity and age. If these pigeons are affected by gastro intestinal parasites, their performance will be affected. Parasitic diseases adversely affect the health of the bird with loss of body weight, retarded growth, unthriftiness, damage to the gut epithelium, emaciation and death in young birds. Parasites affect birds which are mainly under stress and unhygienic conditions. Hence the treatment should be aimed both at elimination of the parasite and also boosting of the immune system. The present study details about successful treatment of the gastrointestinal parasites using herbal extracts.

MATERIALAND METHODS

Study Area : A pigeon farm located near the Veterinary College, Hassan was selected for the present study. The farm had 100 birds and the birds were reared for meat and competition purpose. The farmer came with complaint of weakness, decreased body weight and reduced reproductive performance in pigeons. The birds were reared on cage system and had no history of deworming for more than one year.

Collection of material : Fecal swabs were collected from individual bird and brought to parasitology laboratory. The swabs were processed as per the method (Soulsby, 1982) and examined. Five gram of faecal sample was collected both pre and post treatment of the pigeons showing positive for GI parasites. Egg per gram of faeces (EPG) was recorded using McMaster counting technique.

Among the positive birds, 50 were randomly selected and made into 5 groups of 10 each and the treatment was given. P1 served as control without any drug administration, P2 received an oral dose of Piperazine and Amprolium combination at 1g/gallon of water and 1 tsp/gallon of water for 1 and 3 days respectively. P3, P4 and P5 received herbal

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combination of AT (Allium sativum and Tinospora cardifolia).

Preparation of herbal extract : Ten grams of Allium sativum and 10gm of Tinospora cordifolia were crushed, made into fine paste and mixed with 10ml of water. This was given to birds @ 0.05 g/ml/bird, 0.1 g/ml/bird and 0.2 g/ml/bird to P3, P4 and P5 respectively in water daily for 5days.

Drug efficacy : The efficacy of the antiparasitic agents used in the study was calculated as per the below mentioned formula

Efficacy percentage = 100{EPG (Pre-medication)- (Post medication)/ EPG (Pre-medication)}

During the trial period all the birds were maintained as per standard managemental practice and provided feed and water ad libidum. The birds were observed for any abnormal symptoms and fecal samples were routinely screened for parasitic eggs.

RESULTS AND DISCUSSION

The faecal samples were subjected to only sedimentation and revealed the presence of Ascardia columbae eggs, Capillaria spp egg, and Eimeria oocysts. Total prevalence of parasitic ova /cysts was 62 per cent. Out of these 50 per cent had mixed infection with Ascardia columbae eggs and 12 per cent of birds had mixed infection with Capillaria spp egg, and Eimeria oocysts. The birds were examined on monthly basis for 3 months and the fecal samples were found negative for any parasitic cyst /ova after the treatment.

It was observed that the formulated mixture AT @ 0.2 g/ml/bird caused complete reduction of Ascaridia columbae eggs and Eimeria oocysts followed by 0.1 g/ml/bird, 0.05 g/ml/bird and Piperazine - Amprolium combination respectively. However, there was an increase in the EPG and OPG of the control group (Table 1). The performance of birds improved immediately after 1 week, in terms of increased food intake and gain in the body weight.

Efficacy of garlic in the treatment of pigs against Ascaris suum has been established (Valkosen, 2001). The anthelmintic efficacy of Allium sativum against nematodes using in vitro trials has been proved (Iqbal et al., 2001; Ahmed et al., 2012). Garlic was found very effective in reducing the burden of coccidia and enhancing the performance of adult goats (Mulumebet et al., 2009). The anticoccidial effect of garlic powder on Eimeria spp. infected chicken has been reported (Pourali et al., 2014). Garlic supplementation also increased the average daily weight gain of the birds. Tinospora cordifolia has been known to promote longevity and increase the body's resistance against various diseases (Kapur et al., 2008). The efficacy of anthelminitic activity of Tinospora cordifolia against earth worms by in vitro trials has been confirmed (Shraddha et al., 2014).

Prevalence rate of internal parasites as 60% and 55% in wild and domestic pigeons respectively has been recorded by Basit et al. 2006 the prevalence rate of 62% corresponds with the same. Among the parasitic eggs recorded Ascaridia columbae was highest followed by Capillaria spp. This is in accordance with the reports of Patel et al. (2000) who found Ascaris in 20.75% of birds and Capillaria spp. in 13.2% of birds. The difference in prevalence rate could be attributed to the varied geo- climatic conditions.

Prevalence of Eimeria oocysts was found to be 50% in the present study. This may be due to the fact that coccidian infections are a common problem in pigeons especially during winter season as the birds are under stress and the level of infection may increase.

CONCLUSION

From the present study it can be concluded that Ascaridia columbae, Capillaria Spp and Eimeria oocysts are common gastro intestinal parasites of pigeon. The herbal formulation AT (Allium sativum and Tinospora cardifolia) can be used effectively in pigeons as an antiparasitic agent along with safety and economic benefits.

Table 1 : Efficacy of the preparations in reducing the faecal egg count

GROUPS	NO. OF BIRDS	DRUGS	0 DAY		14TH DAY		OVER ALL EFFICACY	
			EPG	OPG	EPG	OPG	EPG	OPG
P1	10	NIL	3000	20000	4000	30000	-	-
P2	10	Piperazine (2g/litre) + Amprolium (0.6g/litre)	3100	9000	100	300	96.77	96.66
P3	10	AT 0.05g/bird	4200	13000	100	200	97.61	98.46
P4	10	AT 0.10g/ bird	2900	15000	0	0	99.5	99
P5	10	AT 0.20g/ bird	1600	18000	0	0	100	100

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Detection of Anthelmintic Resistance Against Fenbendazole and Ivermectin in GI Nematodes of Horses by FECRT

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ABSTRACT

The present paper reports on the efficacy of two commonly used anthelmintics (fenbendazole and Ivermectin) against two common gastrointestinal parasites of equines (Strongyles and Parascaris equorum) by faecal egg count reduction test. In the present study, Fenbendazole was 90% effective in reducing the egg counts of strongyles and 81% effective in reducing the faecal egg counts of Parascaris equorum, indicating the development of resistance to the drug. Ivermectin against strongyle and Parascaris equorum infection showed 97 and 96 per cent of faecal egg count reduction thereby indicating its efficacy against both the commonly prevalent nematodes.

Key words : Horse, Fenbendazole, Ivermectin and FECRT

Helminth infections caused by nematodes, trematodes and cestodes are the common in horses. Especially worms such as large and small strongyles and ascarids have proven to be problematic to horses (Brady et al., 2009). All have been associated with poor health, loss of vigor and reduced work capacity and clinical disease. Efforts have been made to control these parasites by developing parasite control programmes, based on regular use of anthelmintic drugs (Andersen et al., 2013). Billions of dollars have gone into researching different anthelmintics and determining different methods of use for the anthelmintic that produces positive results in eliminating internal parasites while managing the growing resistance problem.

In horses the anthelmintic resistance is determined for all available drug classes in vivo by faecal egg count reduction test (FECRT). This in vivo test has been used for many years based on procedures outlined in the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) published by Coles et al. (1992) and Duncan et al. (1988).

Faecal egg count reduction test (FECRT), based on enumeration of pre and post treatment faecal egg counts (FEC) in the same horses or the efficacy in a treatment group is compared with an untreated control group. At present, the gold standard for defining the anthelmintic susceptibility of both Parascaris equorum and Cyathostome infections in the field is FECRT. In Karnataka, status of anthelmintic resistance in strongyles of horses is unknown. Hence this work was taken up to study the status of anthelmintic resistance using in vivo test.

MATERIALS AND METHODS

Study area

Horses of either sex naturally infected with gastrointestinal helminths (strongyles and Parascaris equorum) or having faecal egg count more than 500 eggs per gram (EPG) were identified for the trial to evaluate the efficacy of commonly used anthelmintics. Animals in and around Bangalore irrespective of their age, were screened in the present study. Faecal samples were collected from horses including both males and females. The animals were not treated at

least 30 days before the study. History, anthelmintic usage, drugs used, frequency of treatment, record of anthelmintic usage over period of three years and commonly occurring parasitic problems were noted.

Procedure

Examination of the samples was done within four days of collection of faecal samples (under anaerobic conditions) for parasitic infection by sedimentation and floatation method with saturated sodium chloride solution with specific gravity of 1.20 as floatation fluid. The eggs per gram (EPG) were determined by the modified McMaster's method as per Coles et al. (1992).

Faecal egg count reduction test in organsied farm : The procedure detailed by Coles et al. (1992) was followed with some minor modifications. In the present study this test was carried out in one farm under study. The anthelmintics, {fenbendazole (Panacur® vet 10% suspension, Intervet India Pvt. Ltd, Pune) and Ivermectin 0.1% solution (Itin vet) MMC Health care Ltd., Chennai} were administered at the dose rate of 7.5mg/kg body weight and 200ìg/kg body weight respectively to two different groups each containing five animals.

The faecal samples for the study directly collected from the rectum of horses from all the farms were screened for the presence of strongyle and Parascaris ova. Thirty horses with EPG 500 or more were selected in which the mean EPG±SE ranged between 633.33±33.33 and 1466.7±33.33 for Strongyle ova and 833.3±33.33 and 2117±16.67 for Parascaris equorum and a minimum of 10 grams of faeces was collected directly from the rectum on the day of dosing. The egg count was estimated by modified McMaster's technique. Group A animals were positive for Strongyle ova and group B had Parascaris equorum infection. Group A was divided in to sub groups containing 15 animals, five in each subgroup T1, T2 and C. T1 was treated with fenbendaole, T2 with Ivermectin and C served as a control group without the drug. Similarly Group B was divided in to sub groups containing 15 animals, five in each subgroup T1, T2 and C. T1 was treated with fenbendaole, T2 with ivermectin and C served as a control group without the drug. The faecal samples from horses in both groups were examined on the 7th day and 14th day post treatment and the eggs per gram was recorded

Interpretation of the data: Reduction in the egg count was calculated by using the following formula. Percentage reduction in egg count= 100 (1-Xt/Xc) where X is the arithmetic mean 't' is the treated group egg count on 7/14th day and c is the control group egg count on 7/14th day and resistance was calculated with RESO.EXE software (Coles et al.,1992)

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

Susceptible, ≥ 95%: moderately resistance, ≤ 94-80%: high resistance, ≤ 79%

RESULTS AND DISCUSSION

The drug fenbendazole reduced the strongyle egg count by 90 per cent in the organized farm under study, with lower 95 per cent confidence limit of 85 per cent and upper 95 per cent confidence limit of 93 per cent. The results of FECRT are given in Table 1 and Figure 1. A less than 95 per cent reduction in strongyle egg counts indicated the development of resistance to the drug. In the present study there was 90 per cent reduction in the egg count hence indicated the existence of resistance to fenbendazole in the study.

The drug ivermectin reduced the strongyle egg count by 97 per cent in the study, with lower 95 per cent confidence limit of 94 per cent and upper 95 per cent confidence limit of 98 per cent. The results of FECRT are given in Table 1. In the present study susceptibility to ivermectin was observed.

The drug fenbendazole reduced the Parascaris equorum egg count by 81 per cent in the farm under study with lower 95 per cent confidence limit of 72 percent and upper 95 per cent confidence limit of 87 per cent. The results of FECRT are given in (Table 2). A less than 95 per cent reduction in Parascaris equorum

egg counts indicated the development of resistance to the drug.

The drug ivermectin reduced the Parascaris equorum egg count by 96 per cent in the farm under study with lower 95 per cent confidence limit of 94 per cent and upper 95 per cent confidence limit of 98 per cent. The results of FECRT are given in Table 2. In the present study susceptibility to ivermectin was observed.

In the pre sent study the percentage of reduction in egg count was 90 per cent with Fenbendazole indicating resistance and 97 per cent with ivermectin indicating susceptibility to the drug. The results of the present study are in agreement with findings of Varady et al. (2000) in Slovak Republic, FECR values ranging from 65.1 to 86.3 per cent indicating resistance in 14 farms under study. Varady et al. (2004) reported 84.4 – 89 per cent faecal egg count reduction with fenbendazole indicating resistance to strongyles.

Garcia et al. (2013) from Texas observed increased trend in parasite resistance in younger animals (P=0.81) which is in agreement with the present study as the animals tested were in the age group ≤2 years of age.

The findings of Singh et al. (2012), Shahardar et al., (2006), Mohsinuddin et al., (2013) and Yadav et al., (1993) who had observed 96, 100,100 and 96.8 to 99.3 per cent reduction in facecal egg counts indicated effectiveness of the ivermectin, similar to the present study.

In farmed horses following treatment with fenbendazole and ivermectin for Parascaris equorum infection, the percentage of reduction in egg count was 81 per cent with Fenbendazole indicating resistance and 96 per cent with ivermectin indicating susceptibility to the drug in contrary to Slocombe et al. (2007) from Canada and Relf et al. (2014) from UK who reported 97.6 and 97.5-99.9 per cent overall efficacy in Parascaris infected horses dosed with fenbendazole and ivermectin respectively. This may be attributed to indiscriminate usage of this drug since the introduction of the drug in the market and dosing the horses every time with combination of other drugs.

The overall efficacy of ivermectin in farm in the present study for Parascaris infection showed 96 per cent reduction in the faecal egg count. It was in agreement with Visser et al. (2001) and Boersema et al. (2002) who reported good efficacy of ivermectin and Larsen et al. (2011) who reported 96.9 per cent reduction in the egg count after 10-14 days of treatment and reported no signs of development of resistance in Parascaris equorum.

Nareaho et al. (2011) from Finland, Slocombe et al. (2007), Craig et al. (2007) Relf et al. (2014) from UK and Boersema et al. (2002) reported resistance of P.equorum to ivermectin. However, since ivermectin was introduced into the Indian market more recently and its use were for a lesser period, its efficacy is still good in this region.

CONCLUSION

On the basis of present study it could be summarized that horses of organsied farms have developed resistant to the fenbendazole compound and fairly good efficacy is achieved when dosed with ivermectin. Judicious use of this compound with the combination of other anthelmintic is the need of the hour to avoid the problems of anthelmintic resistance.

Table 1. Analysis of FECRT in strongyle infected horses with RESO computer programme

	AM Zero day (EPG)	AM 14th day (EPG)	Per cent reduction	Lower 95% confidence limit	Upper 95% confidence limit	Results
Control	1007	1133	-	-	-	-
Fenbendazole	863	103	90	85	93	Resistance
Ivermectin	897	33	97	84	98	Susceptible

Table 2. Analysis of FECRT in Parascaris equorum infected horses with RESO computer programme

	AM Zero day (EPG)	AM 14th day (EPG)	Percent reduction	Lower 95% confidence limit	Upper 95% confidence limit	Results
Control	1007	1313	-	-	-	-
Fenbendazole	1600	246	81	72	87	Resistance
Ivermectin	1727	103	96	94	98	Susceptible

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Pharmacoepidemiological Studies on Usage Pattern of Antibacterial Agents in Veterinary Hospitals across the Karnataka State*

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ABSTRACT

The present study was undertaken to analyse the prescription pattern (N=1005) data collected from Veterinarians (voluntary) in the State of Karnataka. Analysis of prescriptions with respect to livestock species (cattle, sheep and goats; N=912) revealed that among the major therapeutic segment of drugs belonging to antibacterials (28.37%), > non-steroidal agents (NSAIDs;15.70%), > antihistaminic (H_1 blockers;12.42%) classes were prescribed most in livestock species. Further, among the antibacterial agents cephalosporins (19.39%) > tetracyclines (16.24%) > β-lactam + β-lactamase inhibitors (11.92%) were most prescribed. Analysis of number of drugs per prescription given by Veterinarians were in the order of three or more (64.80%) > double (24.23%) > single (10.97%). Among the poly-drug therapy undertaken by Veterinarians 46.39% of prescriptions had antibacterial agents with one or the other NSAIDs, while 11.60% of perceptions had antibacterial agents and both glucocorticoides and NSAID's. The survey revealed that cefquinome sulphate was most preferred antibacterial agent in the treatment of (intramammary) mastitis in dairy cows in the State.

Keywords: Prescription pattern, Antibacterial agents, Polydrug therapy, Mastitis, Livestock

Monitoring of prescription pattern provide a bridge between areas like rational use of drugs, pharmacovigilance, evidence based medicine, pharmacoeconomics, pharmacogenetics and ecopharmacovigilance (Jain et al., 2015). It is generally accepted that at least, analysis of current Veterinary drug prescriptions among and/or within the State would yield meaningful statistics on usage pattern of drugs and thereby favour rational use of drugs in livestock species. Periodical assessment of prescribing behaviour of Veterinarians can identify the defects related to their scientific merit of the prescription in order to undertake corrective measures effectively, thus favour rational use of drugs.

Indiscriminate use of antibacterial agents is one of the most important reason for the development of antimicrobial resistance today. There is a general consensus that the wide spread use of antimicrobial agents has imposed a strong selection pressure leading to multiple drug resistant (MDR) microorganisms. Food borne bacteria including known pathogens and commensal bacteria display an extensive and diverse range to antimicrobial agents of human and Veterinary importance (Alatossava and Alatossava, 2007). Further, presence of antimicrobial residues in foods of animal origin, viz., meat, egg and milk or their products has adverse consequences not only on public health but also domestic or international trade.

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The relationship between antimicrobial use and resistance is likely to be related to frequency of prescription of the drug, the dose and the duration of treatment (Chauvin et al., 2002). In order to determine the compliance of veterinarians with indiscriminate use of antimicrobials, it is important to document whether actual prescriptions correspond to standard recommendations. Monitoring antimicrobial usage is also a pre-requisite for risk assessments of the impact of animal antimicrobial use on bacterial resistance relevant to humans, as well as evaluating the effect of interventions such as restriction of use of critical antimicrobial class (Nicholls et al., 2001). However, there are no systematic studies to document the usage or prescription pattern of chemotherapeuticals, especially prescription of various classes of Veterinary antibacterial agents in the country or in the respective states.

In this scenario, there is a need to analyze and evaluate the prescription pattern of drugs used in the Veterinary practice to determine the scientific merit of prescription with respect to selection of antibacterial agents, drug-combinations made during polydrug therapy. Similarly, it is necessary to identify the most frequently employed antibacterial agents in the treatment of mastitis in dairy cattle which could be contributing either for the presence of drug residues in milk or development of antimicrobial resistance.

MATERIALAND METHODS

The Veterinarians serving in Department of Animal Husbandry and Veterinary Services (AH & VS), Government of Karnataka in four different administrative divisions of the State viz., Belagavi, Kalburagi, Bengaluru and Mysuru who were voluntarily willing to participate in the study were identified. They were asked to share the prescription data (retrospectively) during the period 1st April, 2016 to 31st March, 2018 through a well structured format developed for the purpose. Out of 1005 prescription data received only the prescriptions data belonging to bovine, ovine and caprine species (N=912) were considered for the current study.

Therapeutic segments of drugs prescribed: The Veterinary prescriptions pertaining to livestock species were classified according to their therapeutic segments. Therapeutic classification included antibacterial agents, non-steroidal anti-inflammatory drugs, glucocorticoides, antihistaminics, antiparasitic drugs, vitamins (injections), diuretics, replacements fluids, health supplements, drugs for topical use and hormones. The vitamins prescribed for per oral use, minerals and herbal supplements were included under the health supplements. Rest of the therapeutic agents were grouped under miscellaneous class.

Selection of class of antibacterial agents: The prescription containing one or more antibacterial agents given by Veterinarians to various livestock species across State were classified according to chemical class they belong, viz., sulphonamides, penicillins, cephalosporins, combination of â-lactam antibiotics with β-lactamase inhibitors, tetracyclines, combination of sulphonamide with trimethoprim, aminoglycosides, macrolids, fluoroquinolones. Rest of the antibacterial agents including herbal remedies prescribed by Veterinarians were grouped under miscellaneous class. Further, the number of prescriptions of drugs meant for human use (extra-label) were also recorded.

Preference of antibacterial agents for outpatients: The preference of prescriptions of antibacterial agents for the treatment of various ailments in livestock species reported to medicine, surgery and gynaecology outpatients was analysed. Further, the preferences for use of various antibacterial agents in lactating and non-lactating animals were also determined.

Usage pattern according to system involved: The class of antibacterial agents preferred by Veterinarians for the treatment of ailments affecting different systems (irrespective of definitive or tentative diagnosis) in livestock species was examined. They were grouped under various systemic ailments, viz., nervous system, ear-nose-throat (ENT), mastitis or udder related ailments, uro-genital system, respiratory system, gastrointestinal system, dermatology,

musculoskeletal, haematopoietic system and metabolic disorders etc.,

Polydrug therapy in livestock species: The number of drugs prescribed by veterinarians per prescription irrespective of spectrum was analysed. The segregation of prescription was carried out irrespective of the aetiology of disease or disorder or the system involved.

Pattern of antibacterial drug combinations: The prescription of drugs with antibacterial agents was further analysed to determine the type of combinations with (i) non-steroidal anti-inflammatory drugs or (ii) steroids (glucocorticoides) or both (iii) co-administration of another bacteriostatic or bactericidal agent(s) and (iv) any plant or homeopathy based system of medicines. The type of combination of bacteriostatic or bactericidal agents was analyzed irrespective of their administration by oral, local (topical) or systemic administration in a livestock species.

Most favoured antibacterial agents for mastitis in dairy cows: The various antibacterial formulation preferred by Veterinarians in the clinical management of clinical mastitis in dairy cattle were examined. The mode of delivery of antibacterial agents against mastitis was classified vis-à-vis systemic, intramammary or both of the routes of administration irrespective of their usage as -per the labelled directions or extra-label use were also analysed.

RESULTS AND DISCUSSION

Therapeutic segments: Analysis of prescription pattern revealed that among the therapeutic segments, antibacterial agents were prescribed most (28.37%) followed by non-steroidal anti-inflammatory drugs (NSAIDs) (15.70%), antihistamines (H_1-blockers) (12.42%) and health supplements (9.35%). A notable number of prescriptions were also made towards injectable vitamins (8.30%). Among the other segments, the usage pattern of antiparasitic drugs accounted respectively, 1.43, 3.24 and 0.17 per cent for oral, parenteral and topical use in the livestock species. Among the livestock species, maximum number of prescriptions was delivered towards bovines (93.79%) when compared to either ovine (3.38%) or caprine (2.83%) (Table 1).

Antibacterial class: The analysis of prescription data with respect to selection of class of antibacterial agents chosen by Veterinarians across the Veterinary institutions in the State revealed that cephalosporins (19.39%) were most widely employed in livestock species, followed by tetracyclines (16.24%), penicillins (15.42%) and aminoglycosides (12.38%). The least preferred antibacterial classes includes diaminopyramidines (sulphonamide (s) + trimethoprim combinations) (0.82%) and macrolides (0.47%). The antibacterial agents classified under miscellaneous group viz., polymixins, amphenicols, nitrofurazones, nitroimidazoles, antiseptics and herbal antimicrobial remedies together constituted 7.24% (Fig. 1).

Prescription for outpatients:

Analysis of the data showed that the top most four class of antibacterial agents prescribed towards outpatient animals (livestock species) classified under general medicine, surgery (and radiology) or gynaecology (and obstetrics) included â-lactam group of antibiotics including combinations with β-lactamase inhibitors (52.84%) > tetracyclines (15.02%) > aminoglycosides (12.12%) > fluoroquinolones (8.01%). Livestock species presented to general medicine alone (lactating and non-lactating) received one or the other antibacterial agents (82.98%) when compared to surgery (and radiology) (8.79%) and gynaecology (and obstetrics) (8.23%). Further, lactating animals received β-lactams including combinations with β-lactamase inhibitors (n=355) (Fig.2).

Usage pattern as per system involved: The class of antibacterial agents employed by Veterinarians in the State for the treatment of ailments affecting different systems (irrespective of definitive or tentative diagnosis) in livestock species was more in β-lactams or their combination with β- lactamase inhibitors (53.33%) followed by tetracyclines (14.55%) and aminoglycosides (11.23%). Relatively, prescriptions

towards mastitis/udder related ailments were maximum (50.52%) when compared to other systems, viz., haematopoietic (11.75%), musculoskeletal (10.19%) and gastrointestinal tract (9.15%) (Fig. 3). It is worthwhile to note that a remarkable number of antibacterial agents were delivered to animals (bovines) due to failure on the part of Veterinarian to diagnose haemoprotozoal diseases early.

Polydrug therapy : Drug therapy with three or more therapeutic agents at a time prescribed accounted to 64.80% when compared to either double (24.23%) or single (10.97%) in livestock species (Fig. 4).

Pattern of drug combinations : The prescription of antibacterial or antibiotic alone irrespective of their spectrum was employed in 24.67 % of prescriptions when compared to its combination with either bacteriostatic or bactericidal agents (1.31%). However, combination of antibacterial agent (or antibiotics) with NSAIDs and glucocorticoides were 47.39 and 9.97 %, respectively. Interestingly, prescription analysis revealed that 11.60 % prescriptions made by Veterinarians included combination of all the three aforesaid classes of therapeutic agents. Further, the analysis also revealed that the antibacterial agents were used concomitantly with plant base traditional remedies (2.94%) or even homeopathy (2.12%) (Fig. 5).

Most favoured in mastitis: The antibacterial prescriptions (irrespective of label or extra-label use) when subjected to analysis for preference of route of administration indicated that intramammary route was most (50%) by Veterinarians in the treatment of clinical mastitis in dairy cows. Among the various veterinary antibacterial formulations (meant for systemic or local use) cefquinome sulfate was most favoured prescription in the clinical management of mastitis in dairy cows. Co-incidentally a commercial antibiotic formulation containing 75 mg of cefquinome sulfate indicated for intramammary infusion was employed in the treatment of mastitis in the State (Fig. 6).

Analysis and evaluation of prescription or usage pattern of Veterinary drugs is lacking in India or in member States unlike western countries. Indiscriminate use of drugs in veterinary medicine world over is a serious issue not only from the point of safety of animals but also its consequences on public health according to pharmacoepidemiological survey on usage of veterinary medicinal formulations carried out on regular basis. In the present study, an attempt has been made to analyse the prescription pattern of veterinary medicinal products. Further, a special emphasis on prescription pattern of antibacterial agents in livestock species was made in view of widespread antimicrobial resistance observed in the country (MHFW, GOI, 2011)

Analysis of the data showed that maximum prescriptions were made against common ailments in cattle and buffaloes (bovines) when compared to other livestock species presented to Veterinary institutions in the State. In a study conducted in teaching Veterinary hospital in Nigeria revealed that ovine, caprine and canine species were presented most for Veterinary intervention unlike other species (Agaie et al., 2016). Relatively, the State has more number of bovines as compared to other livestock species and therefore such observation made in the present study may not have much significance.

Prescription data analysis undertaken to identify the Veterinary therapeutic segments were drugs employed most revealed that antibacterial agents (28.37%) followed by non-steroidal anti-inflammatory drugs (NSAIDs) (15.70%) were prescribed. Further analysis indicated that cephalosporin (β-lactam) class (19.39%) of antibacterial agents were more frequently used in livestock species in the State. According to Regula et al, (2009) penicillins and cephalosporins (both are β-lactams) were most frequently prescribed in the Veterinary practices in Switzerland. Similarly, such practices were also recorded in pet animals (dogs and cats) in Sweden and Norway (Odensvik et al., 2001) as per the data based on drug wholesaler's statistics.

Preference of antibacterial agents in livestock species (cattle, buffaloes, sheep and goats) presented as outpatients in Veterinary institutions in our study showed that outpatients (lactating animals) presented

to general medicine received maximum number of prescriptions containing antibacterial agents, among which β-lactams including combinations with β-lactamase inhibitors (52.84%) were more frequently prescribed when compared to other outpatients category. This may due to the fact that majority of the dairy animals often presented to Veterinary institutions in the State included mastitis or udder related ailments which required mostly Veterinary intervention involving antibacterial chemotherapy. Analysis of prescription pattern of antibacterial agents according to system involved also confirmed that 50.52 % of them were prescribed towards mastitis or udder related ailments followed by haematopoietic system (11.75%) irrespective of the validity of the clinical diagnosis (tentative or definitive diagnosis). Examination of prescription data also revealed that often Veterinarians use antibacterial agents even in cases of haemoprotozoal diseases in livestock species due to failure to diagnose during initial phase of the infection. Although, it is unfair to compare the present usage pattern of antibacterial agents observed in the current study with the data pertaining to African countries, where small ruminant population was more dominant and receives 37.30 per cent of drugs towards ailment of gastrointestinal diseases in Ethiopia (Beyene et al., 2016).

Polydrug therapies by using three or more drug formulations were found remarkably more (Fig. 4) when compared to two or single drug therapy undertaken in livestock species. Similar prescription pattern was also recorded by Beyene et al. (2015).

Further examination of the prescription in our studies, it was evident that often multiple drug therapies included extra-label usage of therapeutic agents meant for human use. Polypharamcy practices increases the risk of drug-interactions in medical practices in the State of Goa (Upendra and Bhounsule, 2017), however such comparisons should be restricted to drug interactions alone, but not to compare the circumstances in which they are prescribed. Karande et al. (2005) suggested interventions to rectify over prescription of antibiotics and syrup formulations, inadequate labeling of drugs and lack of access to an essential drugs list so as to further improve rational drug usage in medical practice. Gopalakrishnan et al. (2013) also reported that the practice of poly-pharmacy, low usage of generic drugs, injudicious usage of antibiotics and injections and low usage of drugs prescribed from essential drugs list were responsible for unsuccessful drug therapy.

The present study revealed that combinations of antibacterial agents with other class of drugs used in livestock species is alarmingly high (Fig.5), and such combinations included NSAIDs (47.39%), glucocorticoides (9.97%) or both of them (11.60%). Therefore, in similar lines with that of medical profession Gopalakrishnan et al. (2013) there exists a vast scope to restrict poly-pharmacy prescription in veterinary practice in order overcome overuse or abuse of drugs in the State. Further, irrational use of antibacterial agents in conjunction with drug combinations of NSAIDs with glucocorticoides indicate either overuse of drugs on account of lack of proper clinical diagnosis or misused for alleviating clinical symptoms rather than 'bacterial cure'. Additionally, drug combinations of NSAIDs and glucocorticoides can aggravate gastro-intestinal ulcer (Johnston and Budsberg, 1997). Hence, it necessitates the importance of pharmacovigilance in the veterinary field. Present studies also indicated that antibacterial agents were also delivered with inappropriate combination with bactericidal (antibiotics) agents (1.31%), thus the final outcome would be antagonistic as per Jawetz's law rather than synergistic in nature on most occasions. Interestingly, but a matter of serious concern that the veterinarians often succumb to homeopathic medicinal substances in livestock species even when the animals were receiving allopathic drug therapy concurrently.

Our survey revealed that among the various antibacterial formulations cefquinome sulfate, a fourth generation cephalosporin was most preferred for the treatment of clinical mastitis in cows. The PK- studies of cefquinome in mastitic crossbred HFX dairy cows are lacking, and such studies are clinically important as PK-feature likely to vary according to agroclimatic conditions as well as between the cross breeds of the

country of origin due to variation in body size and milk production (Zhai et al., 2007).

CONCLUSION

The present study for the first time documented the usage pattern of Veterinary drugs in the livestock species in the State of Karnataka. The study revealed that antibacterial agents (cephalosporins > tetracyclines > penicillins > aminoglycosides), NSAIDs and antihistaminics (H_1 blockers) are most prescribed among veterinary drugs in livestock species in the State of Karnataka. β-lactams > Aminoglycosides > Fluoroquinolone group of antibacterials constitutes major share with respect to prescription towards udder related ailments like mastitis. Prescription of fluoroquinolones and aminoglycosides in lactating animals presented to general medicine is a serious concern. Polydrug therapy consisting of three or more drugs is relatively more when compared to use of two or single. Cefquinome (sulfate) is most popularly prescribed antibiotic for the control of mastitis in dairy cattle in the State. The data gathered in the present study can be used by policy makers to regulate judicious drug prescribing practice among practising veterinarians. Further, the current study necessitates an integrated national databases system to coordinate rational use of antibacterial agents in food animals, which would not only improve the prescription standards but also to ensure safety of foods of animal origin.

Table 1 : Classification of prescribed drugs to various livestock species based on their therapeutic segments

Therapeutic segments	Bovine	Ovine	Caprine	Total (%)
Antibacterial agents	750	34	29	28.37
Non-steroidal anti-inflammatory drugs (NSAIDs)	420	17	13	15.70
Glucocorticoides	156	4	12	6.00
Antihistamines (H_1-blockers)	335	12	9	12.42
Antiparasitic				
a. Oral use	32	7	2	1.43
b. Parenteral use	90	2	1	3.24
c. Topical	5	0	0	0.17
Injectable Vitamins	226	7	5	8.30
Diuretics	16	0	0	0.56
Replacement fluids	182	1	2	6.45
Health supplements#	261	5	2	9.35
Topical use	61	5	4	2.44
Hormones	35	0	0	1.22
Others*	119	3	2	4.33
Total (%)	93.79	3.38	2.83	100

Note: # = includes vitamins (per oral) / and minerals, herbal supplements, if any; * = cardiovascular drugs and respiratory related drugs

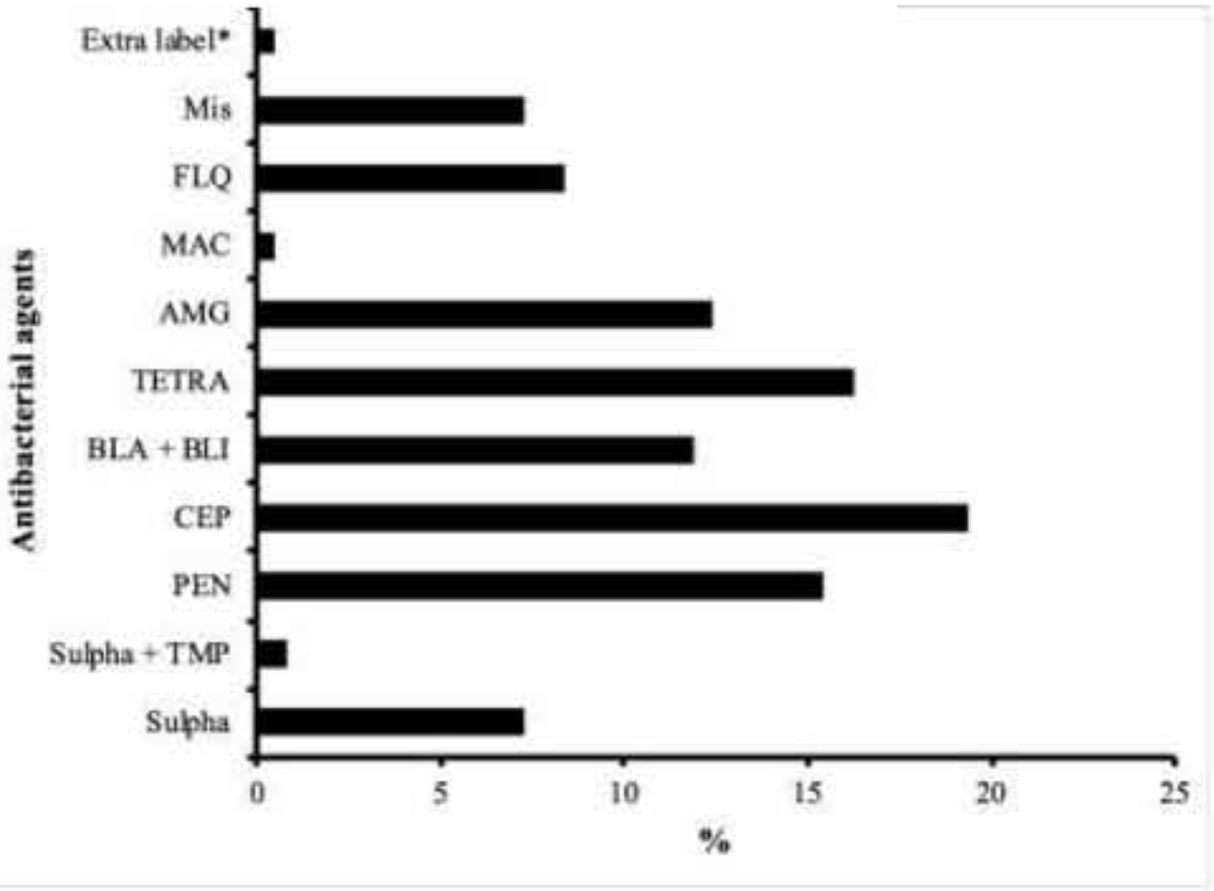

Fig. 1: Prescription preferences for antibacterial agents used in various species across the veterinary institutions (Note: Sulpha = Sulphadimidine; TMP = Trimethoprim; PEN = Penicillins; CEP = Cephalosporins; BLA = β-lactam antibiotics; BLI = β-lactamase inhibitors; TETRA = Tetracyclines; AMG = Aminoglycosides; MAC = Macrolides; FLQ = Flouroquinolones; Mis (Miscellaneous) = includes polymyxins, amphenicols, nitrofurans, nitroimidazoles, antiseptics and herbal antimicrobial remedies; * = Use of antimicrobial agent intended for human use)

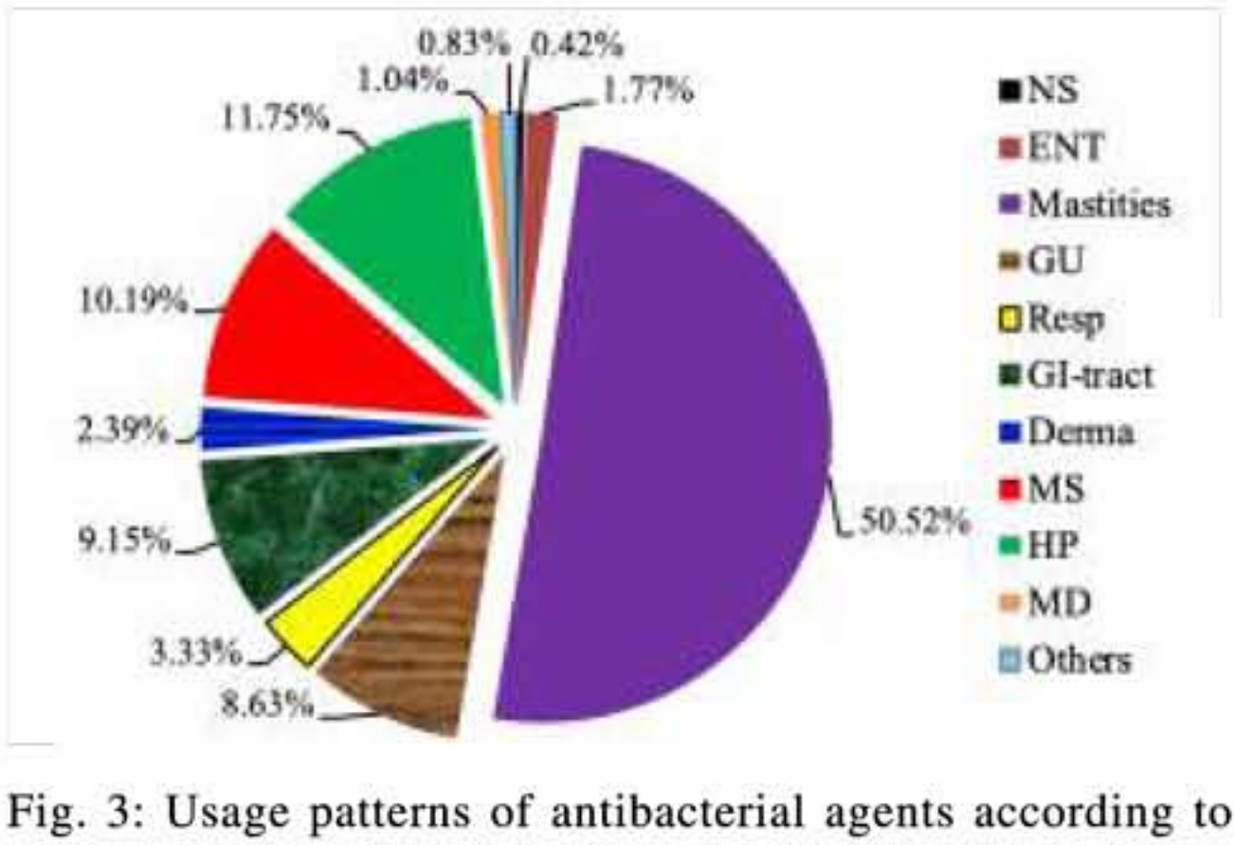

Fig. 3: Usage patterns of antibacterial agents according to system involved irrespective of definitive or tentative diagnosis in livestock species (Note: NS = Nervous system; ENT = Ear, Nose and Throat; GU = Genito-Urinary; Resp = Respiratory; GI = Gastro-intestinal; Derma = Dermatology; MS = Musculoskeletal; HP = Haematopoietic; MD = Metabolic disorder).

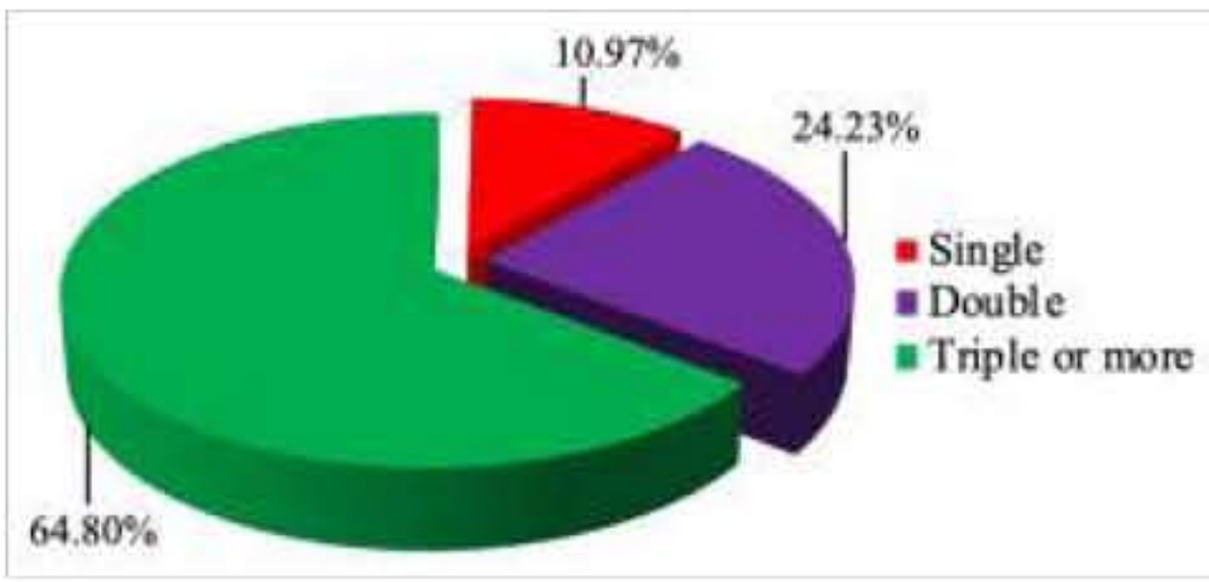

Fig. 4: Number of drugs per prescription given by veterinarians across the State
(Note: Polydrug therapy also includes usage of drugs during vaccinations/external therapeutic or cosmetic application of chemicals and herbal medicines, supplements, if any but excluded fluid therapy).

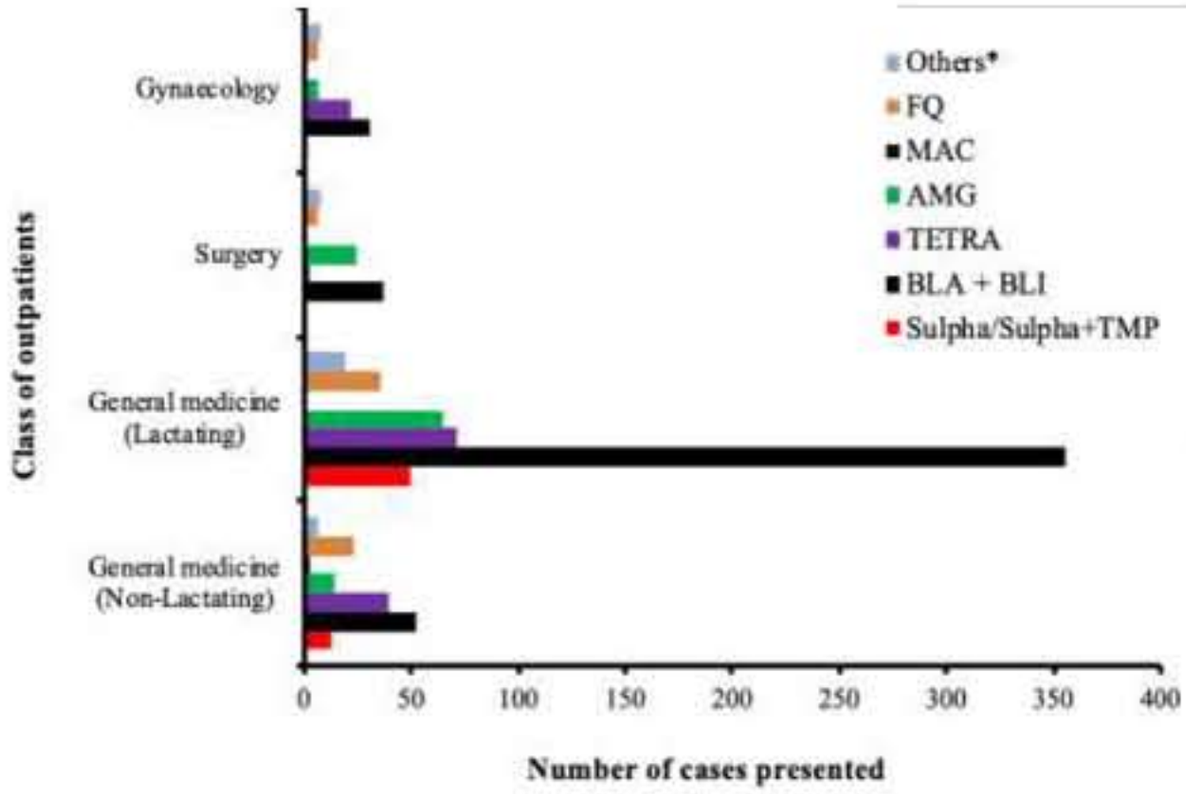

Fig. 2: Preference of antibacterial agents in livestock species presented as outpatients in veterinary institutions (Note: Sulpha = Sulphadimidine; TMP = Trimethoprim; TETRA = Tetracyclines; AMG = Aminoglycosides; MAC = Macrolids; FLQ = Fluorquinolones; BLA = β-lactam antibiotics; BLI = β-lactamase inhibitors; * = Includes nitroimidazoles, amphenicols or any other bacterial/antiseptic agents)

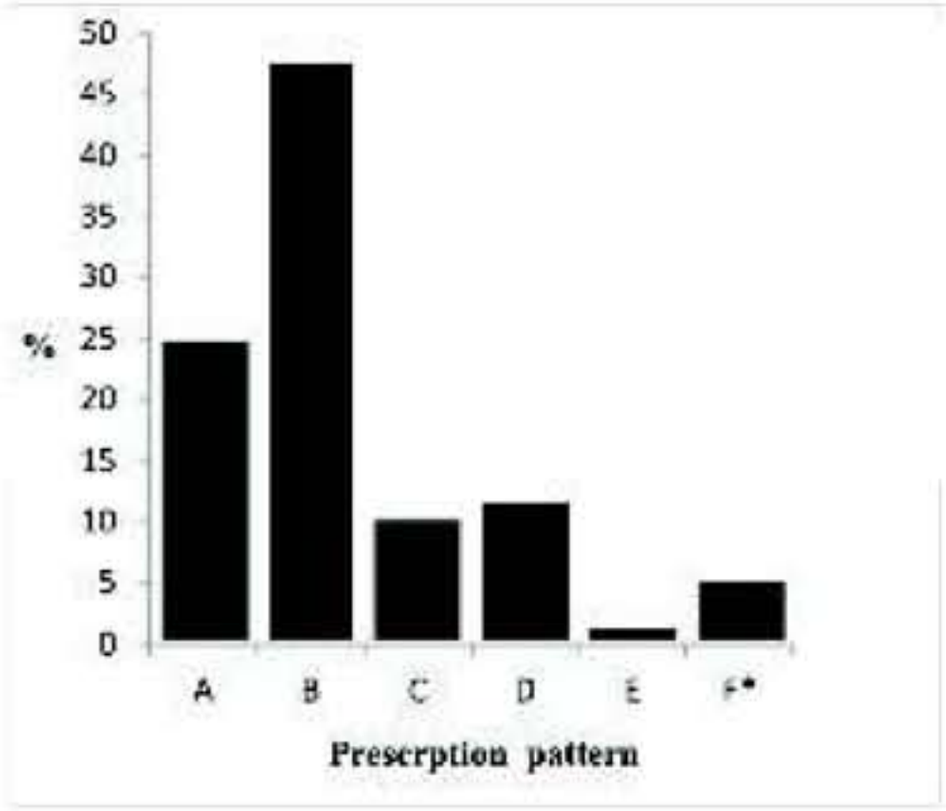

Fig. 5: Pattern of prescription of antibacterial agents with other therapeutic agents in the livestock species (Note: NSAIDs= Non-steroidal anti-inflammatory drugs; GLC= Glucocorticoids; *= Includes herbal remedies with commercial preparations or traditional remedies using plants undertaken by local healers or farmers themselves)

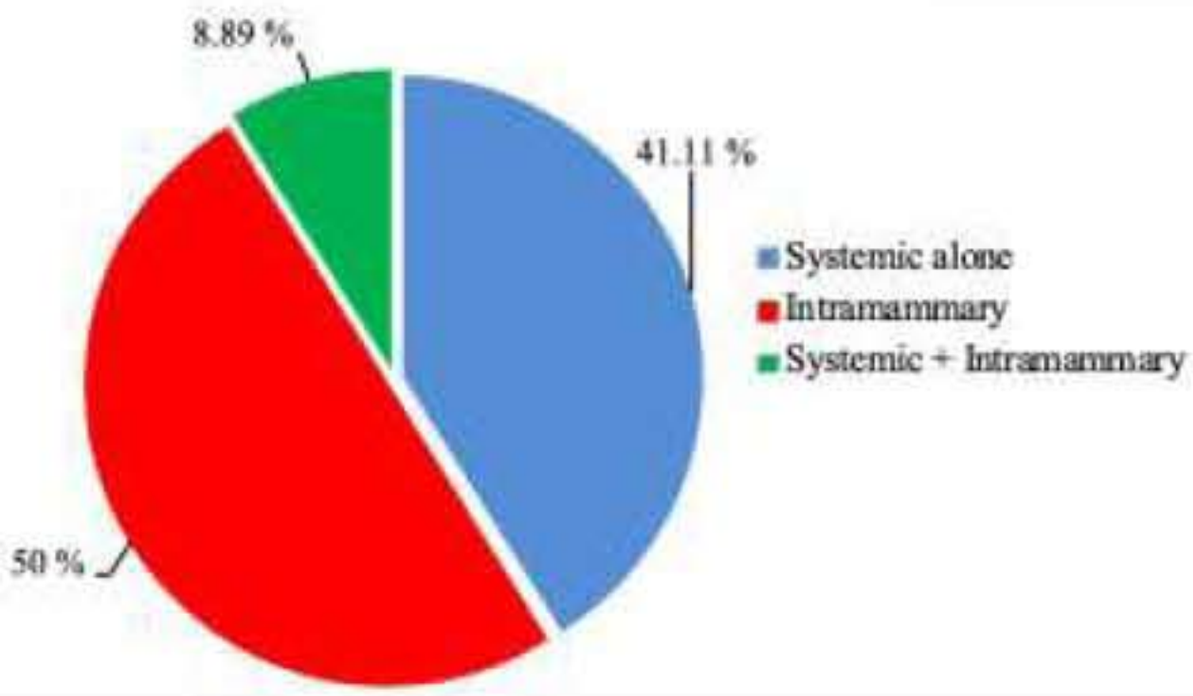

Fig. 6: Most preferred mode of delivery of antibacterial agents in clinical mastitis affected dairy cows

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Effect of Supplementation of Zinc Oxide and Zinc Methionine on Growth Performance in Piglets

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ABSTRACT

The experiment was conducted to know the effect of feeding organic and inorganic sources of additional zinc on growth incidences of gut microbial status in piglets. Sixty graded (Large White Yorkshire) suckling piglets were randomly divided into three treatment group of twenty piglets each on the basis of litter size, parity and live birth weight. Treatment I (T_1) served as control and these piglets were fed with deionized water daily as oral suspensions. Treatment II (T_2) and Treatment III (T_3) were fed with Zinc oxide (ZnO) and Zinc methionine (ZnM) daily (50 ppm) as oral suspension. The average weekly body gain in piglets during the experiment period in T1, T2 and T3 was 0.855, 0.964, 1.024 kg respectively. The average daily gain (ADG) in T_1, T_2 and T_3 did not show significant statistical difference even though the gain in piglets fed with T_2 and T_3 were higher than that of the T1. The piglets supplemented with T1 gained 122.2 gms of weight per day whereas 137.8 gms and 146.4 gms of gains were recorded in the supplemented group T_2 and T_3 respectively. The study indicated that the supplementation of Zinc has a positive impact on growth performance of young pigs.

Key words : Zinc, growth performance, body weight, piglets.

Minerals play an important role in growth, health, and well being of the piglets, even though constituting a small percentage of swine diet (NRC, 1998). Zinc (Zn) is an essential micro mineral for swine and plays an important role in immunity, wound healing, normal growth and development, reproduction, and several metabolic processes (Ensminger, 1991).

The zinc requirement for nursery piglets given in the Nutrient Requirements for Swine (NRC, 1998) is set at 100 ppm Zinc however, the addition of 2,000 to 3,000 ppm Zinc as ZnO is a common recommendation of the swine feed industry. The use of high concentrations of inorganic Zinc has raised some environmental concerns due to low Zinc retention rates and bioavailability of ZnO. Therefore, interest in using organic minerals has increased because of the reported potential of higher bioavailability than from inorganic mineral sources (Hahn and Baker, 1993). Ward et al. (1996) reported that the beneûcial effect on growth from supplementation of high concentrations of ZnO could also be achieved by feeding lower concentrations of organic Zinc (250 ppm of Zinc-methionine) with normal concentrations of inorganic Zinc (160 ppm of Zinc sulfate).

The Zinc requirement for nursery piglets (5 to 10 kg) given in the NRC (National Research Council, 2001) is set at 100 ppm Zinc. However, research studies have shown that pharmacological supplementation of Zinc (2,000 to 3,000 ppm Zinc), usually as inorganic Zinc oxide (ZnO), will decrease the incidence of post-weaning scouring, and increase average daily gain in nursery piglets (Case and Carlson, 2002).

Zinc deficient animals have suppressed immune responses, growth retardation, impaired taste and smell, and decreased spermatogenesis in males. In cases of severe zinc deficiency, severely depressed immune function, frequent infections, dermatitis, diarrhoea,

alopecia, and mental disturbances were observed (Walsh et al., 1994; Zalewski, 1996). In one of the study conducted by (Carlson et al., 1999; Hill et al., 2000) Zinc as ZnO has been widely used in nursery piglet diets by the swine industry to enhance growth performance and decrease the incidence of post-weaning scours (Holm and Poulsen, 1996). Zinc oxide is the only inorganic form of zinc known to enhance growth performance in weanling piglets when fed at pharmacological concentrations (Smith et al., 1997), but the lower concentration of an organic zinc source is found to maintain growth performance compared with pharmacological concentration of zinc as ZnO in experiment conducted by Ward et al. (1996) and Case and Carlson, (2002), but not in case of study conducted by Carlson et al. (2004)

Dietary supplementation with zinc oxide (ZnO) results in improved growth performance and reduced scours in weanling piglets (Hahn and Baker, 1993). However, the high levels of zinc excreted by supplemented piglets have raised concerns about its potential environmental pollution (Carter et al., 2002; Meyer et al., 2002). Additionally, the mechanism responsible for the growth-promoting effect of zinc remains un-known. Elucidating such a mechanism is expected to optimize the growth-promotion efficacy of ZnO while minimizing the amount of zinc supplemented to the piglet's diet. Therefore the current study was carried to determine the effect of supplementation of zinc oxide and zinc methionine at low levels on growth performance of piglets.

MATERIALS AND METHODS

The experiment was conducted at piggery Farm, Department of Instructional Livestock Farm Complex, Veterinary College, Bangalore. The experiment was conducted during the year 2008 to know the effect of feeding organic and inorganic sources of additional zinc on growth performance of young piglets. Sixty graded (Large White Yorkshire) suckling piglets with a mean average weight of 1.313 kg were used in eight weeks growth trial. The piglets were randomly divided into three treatment group of twenty piglets each on the basis of litter size (in all the experimental groups, two sows with each seven piglets and one sow with six piglets were allowed to be with the sows throughout the experiment and the remaining piglets born with these piglets were raised separately), parity and live birth weight, so that each group had comparative average initial weight. The piglets selected for the study were allowed to stay with mother till the completion of the study period (Table 1).

Treatment I (T_1) served as control and these piglets were fed with deionized water daily as oral suspensions. Treatment II (T_2) and Treatment III (T_3) were fed with Zinc Oxide (ZnO) and Zinc Methionine (ZnM) {BIOPLEX®- (Bioplex is a trace mineral supplementation product containing zinc, manganese, copper, iron and cobalt)} supplied by (Vet Care India Pvt Ltd, Bengaluru daily (50 ppm) as oral suspension. Piglets were allowed to suckle mother's milk ad libitum during the growth trial, till the completion of duration of study.

Housing and management : The experimental animals (piglets) were maintained in three groups along with their respective sows in separate concrete pens of size 4.2 x 2.8 meter. Pigs were allowed in open yard in the morning for exercise and had access to grass and sunlight. Plenty of fresh water was made available all the time. During the whole experimental period the sows along with their piglets were kept under hygienic condition. Pens were daily washed and kept dry and clean. Fifteen days prior to farrowing, deworming was carried out using pipeparzine adipate at the rate of 100 mg per kg body weight. Sows were allowed ad libitum access to feed i.e. kitchen waste which was fed in concrete feeders provided in the pens and for the entire duration of the study the piglets were allowed to suckle milk from the respective mothers without any extra supplementation.

Growth performance

Body weight gain

Piglets were weighed once in a week on the same day to record weekly gain by electronic weighing balance and the weight was recorded in kg.

Linear body measurements : Body length and chest girth measurements were taken once in a week by measurement tape, the body length was measured from the tip of snout to the base of tail and chest/heart girth was taken around the chest just behind the shoulder and elbows and the measurements were recorded in cm.

Statistical analysis: Data on growth performance, incidence of non specific scours, piglet mortality, skin coat condition and gut microbial status were analyzed by ONE WAY ANOVA using statistical software (SPSS, Version 16) for windows (2008).

RESULTS AND DISCUSSION

Body weight gain (Kg)

The average weekly body weight gain in piglets during the experiment period in T1, T2 and T3 was 0.855, 0.964, 1.024 kg respectively. The average gain in body weight of the piglets in T2 and T3 groups was more when compared to that of piglets in T1 group. However, the average gain in weight of piglets fed with different sources of zinc such as zinc oxide (T2) and zinc methionine (T3) showed no significant statistical difference compared to control (T1) (Table 2.1a). The difference between initial and final body weight of the piglets at the beginning and at the end of each experimental period was calculated and divided by the number of days in each period to arrive at the average daily gain (ADG). The ADG was expressed in g/day. The average daily gain in T1, T2 and T3 did not show significant statistical difference even though the gain in piglets fed with T_2 and T_3 were higher than that of the T1. The piglets supplemented with T1 gained 122.2 gms of weight per day whereas 137.8 gms and 146.4 gms of gain were recorded in the supplemented groups T_2 and T_3 respectively. (Table 1 and Figure 1). The results of the present investigation have shown that supplementation of 50 ppm of zinc has increased the growth rate in T_2 and T_3 compared to T_1 (Control) group, but the results were not statistically significant. However the present study reveals that, the supplementation of 50 ppm of zinc as ZnO (T_2) and ZnM (T_3) had no significant effect on body weight gain among three groups indicating that supplementation of 50 ppm zinc is not sufficient for improvement of growth in piglets.

Reports suggest that pharmacological concentration of zinc improves growth performance via an improvement in gut morphology of their small intestine by increased height of villi in comparison with the depths of crypts Hedemann et al. (2009) and Li et al. (2001). However, the mechanism by which high levels of zinc improve growth rate in piglets is still not clear. Zinc has antibacterial properties, (Dupont et al., 1994) which may explain the growth-promoting effect but there is a lack of scientific evidence to understand the exact mode of action. Dietary zinc levels of 50 to 125 ppm are generally enough to meet the nutrient requirement in piglets. When supplied with high concentrations of Zinc (2000 to 3000 ppm), it is known to exert positive influence on growth rate (Jay et al., 2009). However, these high levels of dietary zinc are beneficial to piglets only during the early phase of their life (Selle and Ravindran, 2008).

Body measurements : The linear body measurements namely body length and chest girth were recorded and the average initial body length and chest girth of the experimental piglets among T_1, T_2 and T_3 were 42.44, 44.05, 45.45 cms and 29.18, 30.27, 30.87 cms respectively. The weekly averages of body length and chest girth (cms) recorded during the experiment is presented in Table 2 and graphically represented in Figures 2 and 3. Similarly, these results did not show any significant variations in between the three treatment groups. The results of the present experiment are in agreement with the studies conducted by Oke et al. (2006). However, the results of the present study are not in agreement with earlier observations made by (Onyimonyi et al., 2010), who has observed beneficial effect of Zinc in linear body measurements.

Table 1 : Average body weights (g) of the piglets at the beginning, Weekly average daily gain (g) and overall average daily gain (g) of piglets used in the experiment

Weeks/Treatments	T1	T 2	T 3
Average body weights (g) of Piglets	1.38±0.073	1.38±0.059	1.37±0.079
1st week	113.6±13.25	98.72±13.13	118.15±11.69
2nd week	102.9±13.344	106.72±10.95	114.85±10.22
3rd week	118.18±14.42	119.08±12.01	151.8±12.04
4th week	118.18±14.42	131.43±16.97	132.26±16.7
5th week	116.08±11.83	150.36±16.31	150.23±16.21
6th week	130.99±12.95	160.8±17.9	167.35±13.19
7th week	140.12±13.13	169.89±15.14	165.85±13.2
8th week	137.62±13.1	164.69±16.1	171±12.3
ADG (g)	122.2±12.98	137.8±11.29	146.4±13.19

F value is not significant, T_1 = (control) piglets fed with deionized water daily as oral suspensions.
T_2 = (ZnO) piglets fed with zinc oxide daily (50 ppm) as oral suspension,
$T_{3=}$ (ZnM) piglets fed with zinc methionine 50ppm) as oral suspension.

Table 2 : Average weekly body length (cm) and chest girth of the experimental piglets during the trial period

Treatment	At birth	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	Mean
Body length (cms)										
T_1	37.45	39.28	41.08	40.88	40.75	42.73	44.6	46.68	48.48	42.44±3.56
T_2	37.35	39.1	40.93	42.83	44.68	44.85	46.83	48.95	50.95	44.05±4.49
T_3	37.53	39.4	41.33	43.38	45.35	47.4	49.43	51.58	53.65	45.45±5.53
Chest Girth (cms)										
T_1	26.05	27.30	28.53	28.40	28.03	29.23	30.45	31.68	32.93	29.18±2.16
T_2	25.95	27.15	28.40	29.63	30.88	30.73	31.98	33.23	34.50	30.27±2.79
T_3	25.78	27.03	28.28	29.60	30.85	32.13	33.43	34.70	36.00	30.87±3.50

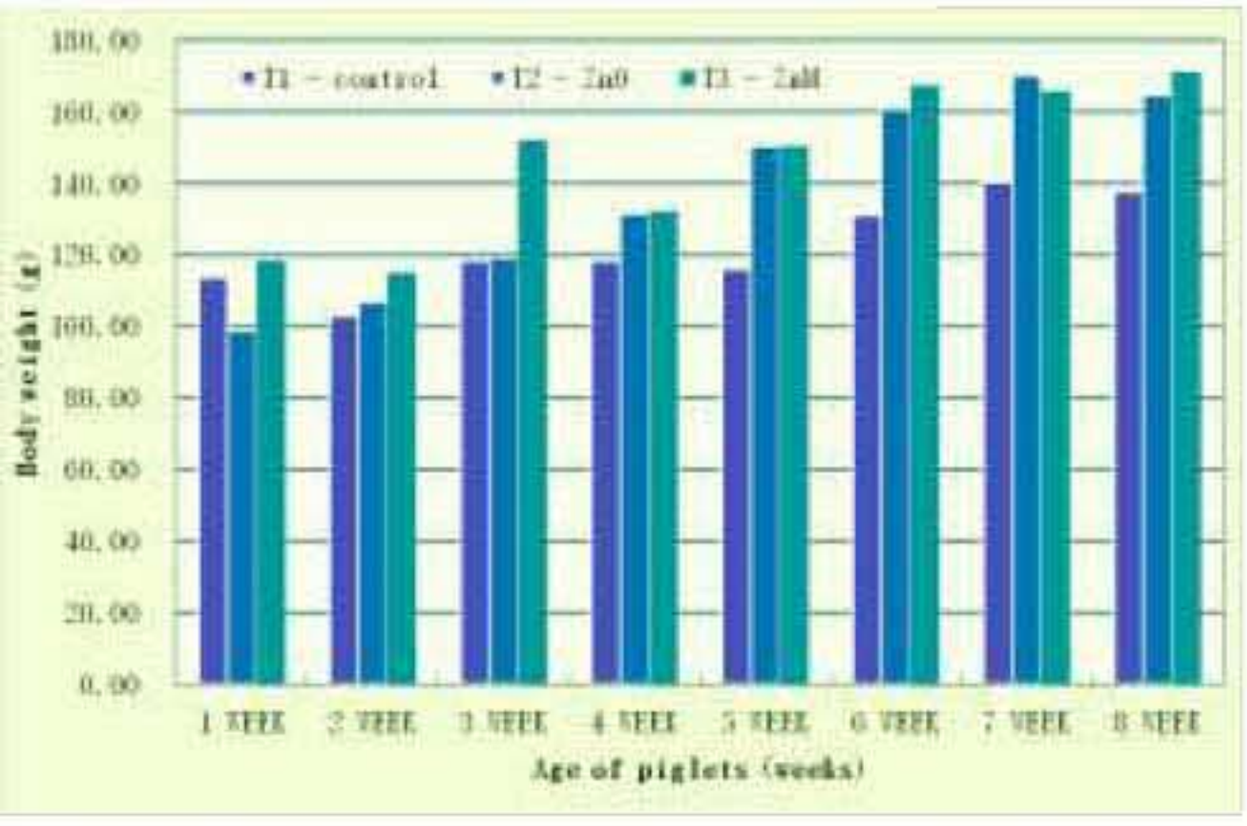

Fig. 1 : Average weekly gain in body weight (g) of the experimental piglets during the eight weeks trial period

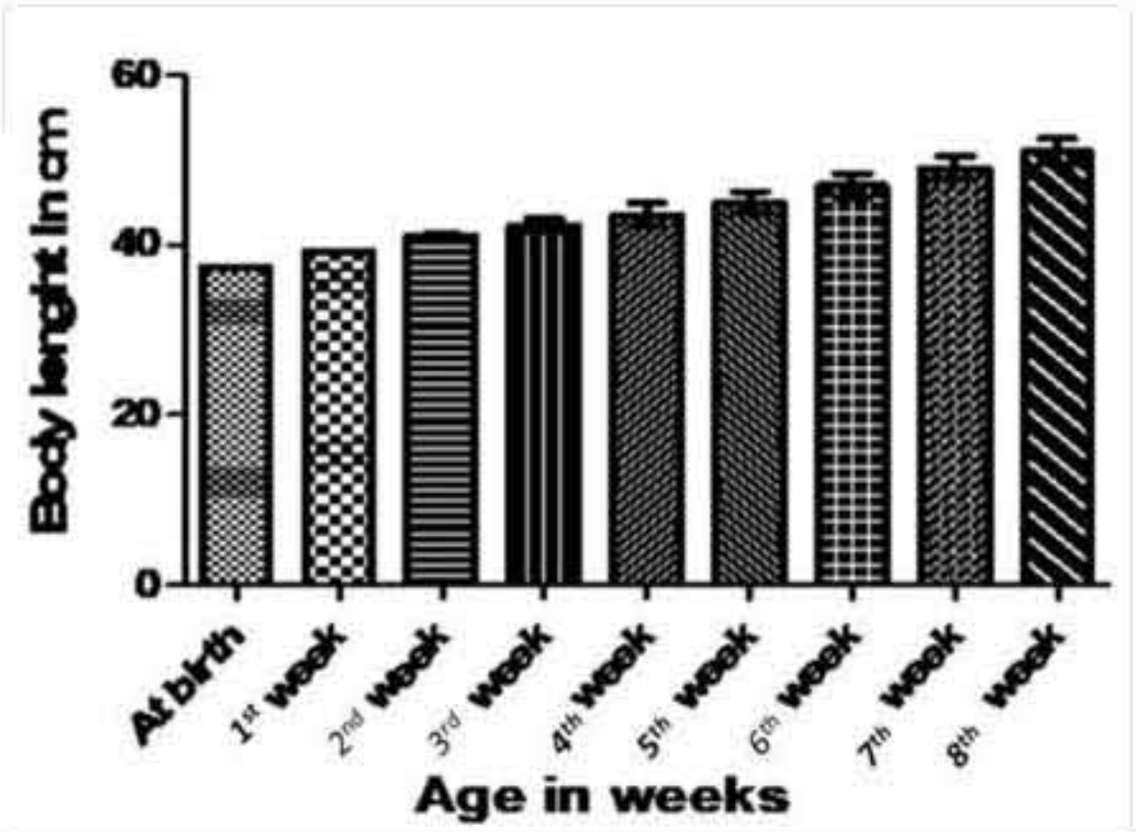

Fig. 2 : Average weekly body length (cm) of the experimental piglets during the eight week trial period

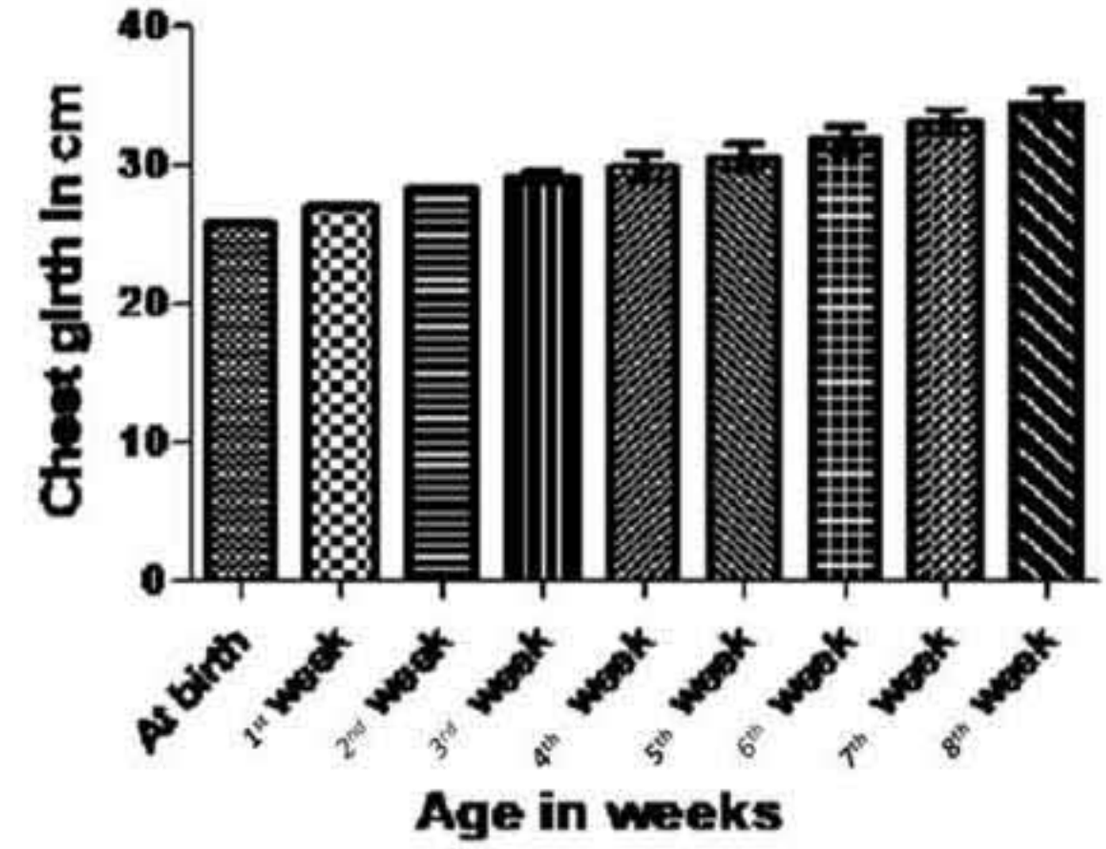

Fig. 3 : Average weekly chest girth (cm) of the experimental piglets during the eight week trial period

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Isolation and Characterization of Lactobacilli from Bovine Rumen liquor*

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ABSTRACT

A total number of thirty isolates of rumen liquor origin were isolated and screened for genus level identification. Lactobacillus spp. R11, R12, R13, R27 and R30 were subjected to a battery of biochemical tests like arginine hydrolysis, aesculin hydrolysis, nitrate reduction and sugar fermentation pattern and molecular identification through 16sRNA sequencing. The isolates R11, R27, R30 were identified as Lactobacillus plantarum and R12 and R13 as Lactobacillus fermentum.

Key Words: Lactobacilli, Biochemical tests, PCR and Rumen liquor.

Probiotic lactobacilli play important role in the animal as well as in human intestine by extending their therapeutic benefits (Corcionivoschi et al., 2010). Probiotics are living microbial feed supplements that may beneficially affect the host animal upon ingestion by improving its intestinal microbial balance (Wulansih et al., 2017). The population of lactobacilli in the rumen appears to vary with the type of diet of the animal in which lactobacilli predominated in the rumen of animals on concentrate diets, but were less numerous in grass-fed animals. Lactobacilli have found their application in areas like fermented foods, silages, fish, crab waste management and poultry by product industries (Giorgio et al., 2010). Several strains of lactobacilli (Lactobacillus rhamnosus GG, Lb. casei Shirota, Lb. paracasei CRL431, Lb. fermentum RC14) have been associated with the development of probiotic dairy foods. Hence lactobacilli have been generating a great deal of consumer, industrial and scientific interests. This study deals with the isolation and identification of lactobacilli mainly from bovine rumen fluid and further gives a way to study the probiotic attributes of the same.

MATERIALS AND METHODS

Sample Collection: Fresh rumen liquor (5 samples) from fistulated crossbred male calves (Sahiwal x Holstein-Friesian; age ≅ 3 years; weight ≅ 250 kg; fed on standard diet of wheat straw, green fodder maize and concentrate mixture) maintained at cattle yard, NDRI, Karnal was collected in thermos flask, strained through four layers of muslin cloth and centrifuged at 2000 x g/ 10 min and kept at 39°C till further processing.

After carrying out initial dilution (10^{-1}), samples (1 ml) were immediately suspended in 9 ml of sterilized 0.1% sterile peptone water and homogenized thoroughly by vortexing. Pour plating on BCP-MRS agar was carried out with 10-$^{-5}$ and 10^{-6} dilutions of samples and incubated anaerobically at 37°C-39°C/ 24–48 hrs. Typical yellow coloured colonies that appeared on MRS agar were transferred to MRS broth and further analyzed for cell morphology by microscopic examination after simple, Gram staining and catalase test (Harrigan, 1998). The isolates that were Gram positive rods with catalase negative were purified

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thrice on MRS agar. All the purified isolates were maintained in MRS broth at 4°C and sub-cultured after every 7-15 days. The stocks of all the isolates were preserved in 50% glycerol stock medium at -70°C. The cultures were activated prior to use by subculturing them twice in MRS broth.

Molecular characterization of the isolates : Based on microscopic examination, around 15 isolates were subjected to molecular characterization for definite confirmation of genus level. The genomic DNA from isolates were extracted by Pospiech and Neikmann (1995) method. The extracted genomic DNA along with the tracking dye were electrophoresed on 0.8% agarose gel with ethidium bromide (0.5 µg/ mL) (Mini/ Maxi submarine, Hoeffer, USA) at 80 V for 30 min using 1X TAE buffer. Gels were monitored on UV Transilluminator and were subsequently photographed (Gel capture, DNR Bio-Imaging System).

Identification of isolates as lactobacilli by PCR : The genus level identification of isolates as lactobacilli was confirmed by PCR using genus specific primer in Table 1 (Dubernet et al., 2002).

Agarose gel electrophoresis : PCR amplified products obtained with different DNA templates were electrophoresed on the agarose gels (1.5%) by following the standard procedure as given by Sambrook et al. (1989).

Biochemical characterization of lactobacilli for species level identification : After confirmation of genus, for the appropriate identification of isolates to species level, only 5 of the isolates were studied for different biochemical reactions discussed below were performed that included arginine hydrolysis, aesculin hydrolysis, nitrate reduction and sugar fermentation test as per Harrigan (1998).

Characterization of isolates up to species level by species-specific primers and 16s rRNA : Identification of various lactobacilli isolates at species level was carried out by following primers with their respective annealing temperature for PCR cycles (Table 2).

16S rRNA sequence analysis of isolates : 16S rRNA gene in isolates was amplified using primers after isolating genomic DNA by Pospiech and Neikmann (1995) method. The PCR products thus obtained were got custom sequenced.

RESULTS AND DISCUSSION

Isolates of 30 nos. based on colony morphology (yellow colour, oval shape and 0.5 mm in diameter) were picked up after plating rumen liquid samples on BCP-MRS agar which were of colonies of lactobacilli.

Microscopic examination: All the 30 isolates were studied for their staining and cell morphology. The isolates were found to be purple coloured Gram-positive rods of varying size under microscope using oil immersion objective. All the isolates were negative for catalase enzyme production. These characteristics of the isolates were found to match with that of lactobacilli. Hence, these primary results indicated that the isolates belonged to the lactobacilli group and paved the way for further specific tests.

For ascertaining the identity of lactobacilli, among 30 isolates obtained, 15 belonged to genus Lactobacillus spp. were subjected to PCR assay based on genus specific primer (Forward) 5' CTCAAAACTAAACAAAGTTTC3' (Reverse) 5' CTTGTACACACCGCCCGTCA 3' of 250 bp product size targeted against 16SrRNA (Dubernet et al., 2002). One of the requirements for carrying out the PCR assay is extraction of template DNA from the test cultures. In this study, the template DNA was extracted from lactobacilli cultures by following Pospiech and Neikman (1995). These 15 cultures were checked for genus identification. All the isolates resulted into the amplification of 250 bp PCR products on the agarose gel (0.8%), which was specific for lactobacilli only. On the basis of present PCR results, isolates showed the positive signal in the form of discrete and distinct 250 bp band on the gel as indicated in Fig 1, thereby establishing their identity as lactobacilli. The results in the present study in this regard are in complete agreement with those of Dubernet et al. (2002) (Fig 1).

Arginine hydrolysis: Arginine hydrolysis broth was used to check the ability of the isolates to hydrolyze arginine resulting in the production of ammonia. In the Arginine hydrolysis test all the isolates showed negative reaction with the fact that isolates could not hydrolyse arginine (Table 3) Arginine hydrolysis is used as characteristic of lactobacilli and indicated by change of colour from yellow to orange in presence of ammonia making the pH of the broth alkaline.

Aesculin hydrolysis : The aesculin hydrolysis test was used to check the ability of the isolated organisms to hydrolyse the glycoside aesculin to aesculetin and glucose in the presence of 10-40 % bile. The aesculetin combines with ferric ions in the medium to form a black complex. In this test all, the isolates were found to positive, as there was an appearance of dark brown/ black colour in the plate after incubation as shown in the Table 3. The results of aesculin hydrolysis are in sharp contrast to that of arginine hydrolysis and gas from glucose.

Nitrate reduction test : Nitrate reduction is a valuable criteria for differentiating and identifying various types of bacteria. Certain bacteria reduce nitrate to nitrite while others are capable of further reducing nitrite to form nitrogen or ammonia. In the nitrate reduction test all the isolates showed negative reactions, as there was no appearance of red/ pink colour after incubation of isolates in nitrate broth as shown in the Table 3.

Sugar fermentation pattern : The tests were performed using CHL media with the different sugar discs. The different isolates showed different types of sugar utilization patterns and some tubes containing culture and sugar turned yellow where as the other remained brown coloured indicating the positive and negative tests respectively (Table 4). When these sugar fermentation, patterns were compared with that of those given for Lactobacillus species in the "Bergey's manual of Determinative Bacteriology" (Holt et al., 1994), all the isolates were tentatively identified as Lactobacillus plantarum.

The data obtained for species identification comprising a number of biochemical and sugar utilization pattern tests were also subjected to software PIBWIN (2004) and the tentative identification was done so as to confirm the obtained results while matching with "Bergey's Manual of Determinative Bacteriology" (Holt et al., 1994). It was found that the isolates with both the methods were similar species.

Identification of lactobacilli isolates at species level

Species specific PCR : After ascertaining the identity of isolates as Lb. plantarum efforts were made to confirm them at species level by subjecting them to species specific PCR assays. When subjected to Lb. plantarum based PCR assay using L pla 3/ L pla 2, among 5 isolates, 3 isolates R9, R27 and R30 were able to amplify 248 bp product specific for Lb. plantarum (Fig. 2).

16S rRNA sequence analysis of isolates : The use of ribosomal sequence analysis in classification of microorganism is favoured because of their universal distribution among all cellular life forms and also due to their highly conserved nature during the evolutionary process. The primary structure of all ribosomal sequence including 16S rRNA sequence consists of alternating conserved and variable domain, which make them very suitable for detection and identification of microbial species and also are ideal targets for specific DNA probe. In the present study, blast analysis of sequence, R12 and R13 clearly revealed 99 per cent homology with Lactobacillus fermentum.

CONCLUSION

The present study was taken up to find the existence of species of lactobacilli in rumen liquor. Among 30 isolates obtained, only 15 were lactobacilli. Out of those selected 5 lactobacilli isolates, 3 (R11, R27 and R30) were identified as Lactobacillus plantarum while 2 (R12 and R13) as Lactobacillus fermentum.

Table 1 : Lactobacilli genus specific primer

Primer region	Primer sequence	Product size	Annealing Temp
16s	(F) 5' CTCAAAACTAAACAAAGTTTC 3'	250 bp	55 ºC
rRNA	(R) 5' CTTGTACACACCGCCCGTCA 3'		

Table 2 : Species specific primer

Lactobacillus species	Primers	Annealing temperature
L.fermentum	Lfer3/ Lfer4	62.0ÚC
L.plantarum	L pla 3/ L pla 2	60.2ÚC

Table 3 : Biochemical characterization of lactobacilli isolates

Isolates	Arginine hydrolysis	Aesculin hydrolysis	Nitrate reduction
R 9	Negative	Positive	Negative
R 12	Negative	Positive	Negative
R 13	Negative	Positive	Negative
R 27	Negative	Positive	Negative
R 30	Negative	Positive	Negative

Table 4 : Sugar fermentation pattern of lactobacilli isolates for species identification

Isolates	Ar	Mt	Xy	Ce	Ga	Rf	Tre	Ma	Mn	Sb	La	Su	Tentative spp.
R9, R13	-	+	-	+	+	+	+	+	+	+	+	+	L. plantarum
R12	+	+	-	+	+	+	+	+	+	+	+	+	L. plantarum
R27, R 30	+	+	-	+	+	+	+	+	+	+	+	-	L. plantarum

Note : Ar-Arabinose, Mt-Mannitol, Xy-Xylose, Ce-Cellulose, Ga-Galactose, Rf-Raffinose,Tre –Trehalose, Ma-Maltose, Mn –Mannose, Sb-Sorbitol, La –Lactose, Su-Sucrose

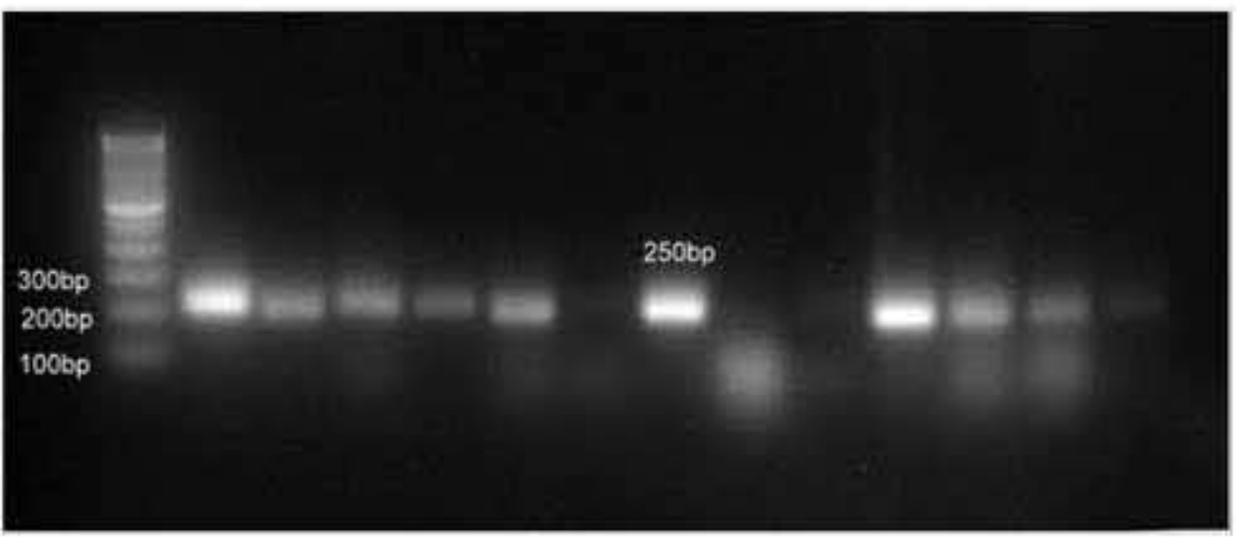

Fig 1: Genus level identification of lactobacilli isolates

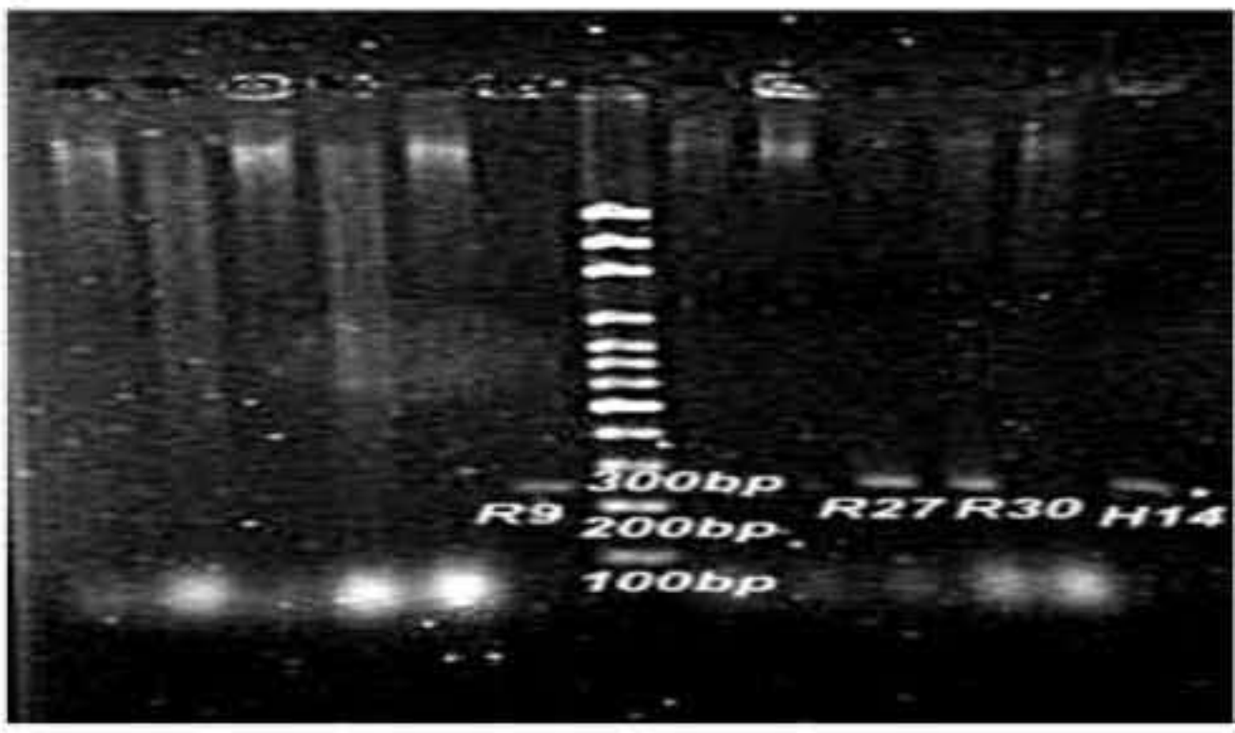

Fig 2: Identification of lactobacillus isolates at species level using L pla 3/ L pla 2 primers specific for Lb. plantarum

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Studies on the Efficacy of Different Antibacterials in the Treatment of Pyoderma in Dogs*

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ABSTRACT

Comparative studies on the efficacy of the results of ABST and in-vivo drug trials for treatment of pyoderma in dogs was carried out. In the first phase of the study, samples from lesions of dogs presented with clinical signs suggestive of pyoderma were collected and subjected to antibacterial sensitivity testing using standard protocol. In the second phase, forty dogs with clinical signs and lesions typical of pyoderma were randomly divided into 4 groups of 10 animals each. Group I was treated with the most sensitive antibacterial drug (Enrofloxacin) as per ABST,Group II and Group III were treated with second (Ampicillin) and third most sensitive (Azithromycin) antibacterials as per ABST, and Group IV was treated with the most sensitive antibacterial drug (Enrofloxacin) along with Retinoid cream. The results of clinical trials (in-vivo) did not correlate with that of ABST (in-vitro) with dogs treated with Ampicillin (Group II) showing better response as compared to Enrofloxacin (Group I) which showed maximum sensitivity on ABST

Keywords: ABST, Ampicillin, Azithromycin, Enrofloxacin, Retinoid cream

Dermatological disorders in dogs are of major concern due to multifaceted etiological factors, higher cost of treatment and long term management associated with prolonged unhealthy environment with respect to odour, hair fall and coat appearance. Of the various dermatological disorders, pyoderma is one of the most frequently observed conditions in small animal practice and yet also one of the most frustrating to treat. Around 90% of pyoderma cases in dogs are associated with bacteria which belong to Staphylococcus species. Underlying conditions such as atopy, parasitic infestations or endocrinopathies promote bacterial adherence and colonization, and their higher multiplication which leads to secondary pyoderma.

It is generally accepted that coagulase-positive staphylococcal organisms isolated from active cutaneous infections represent the major pathogens in infected dogs. Staphylococcus intermedius has been implicated in approximately 90% of cases of pyoderma in dogs. However, though until recently Staphylococcus intermedius was considered the most common organism associated with canine pyoderma, in actuality, isolates originally based on phenotypical characteristics, identified as S. intermedius have been found to be from three different species, Staphylococcus intermedius, Staphylococcus pseudintermedius and Staphylococcus delphini. For definitive identification of these species, molecular diagnostic methods, such as polymerase chain reaction (PCR) techniques are required (Bannoehr et al., 2009).The term Staphylococcus intermedius Group (SIG) is now used to refer to the three previously mentioned isolates (S.

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intermedius, S. pseudintermedius and S. delphini) as a group (Bannoehr et al., 2007 and Sasaki et al., 2007). It has now emerged that most canine isolates phenotypically identified as S. intermedius, were in fact S. pseudintermedius.

Antibiotic resistance in staphylococci is of great concern due to continuously increasing incidence of resistance to methicillin and to the other antimicrobials regularly used by veterinarians which can complicate treatment. Also, a high rate of multidrug resistance was reported among methicillin-resistant S. pseudintermedius (MRSP) strains in dogs (Schwarz et al., 2008). Staphylococcal resistance to antimicrobial agents is said to be more pronounced in urban animals with recurrent pyoderma that have undergone long-term, empirical treatment. Hence susceptibility of pyoderma causing staphylococcal organisms to different antibacterials should always be checked in vitro in order to select the best treatment (Huerta et al., 2010)

Thus, taking into consideration these aspects of pyoderma, the present study was undertaken in two phases in those animals which were confirmed as pyoderma through molecular tests. In the first phase of the study anti bacterial sensitivity pattern of the isolates was tested and this was followed with comparison of effectiveness of therapy for pyoderma based on anti bacterial sensitivity test (ABST) results in different groups of animals in the second phase in order to correlate the results of ABST with invivo testing.

MATERIALS AND METHODS

Selection of animals for study

The study was done in two phases. In the first phase, animals presented to Veterinary College, Bengaluru with clinical signs suggestive of pyoderma such as papules, pustules, erythema, alopecia, pruritus and epidermal collarettes were selected as subjects for bacterial culture and ABST. Samples were collected from the lesions using sterile cotton swabs and subjected to bacterial culture, primarily using nutrient broth or brain heart infusion broth and subculture using Mannitol salt agar. All plates were incubated aerobically at 37ÚC for 18-24 hrs.Twenty plates (isolates) suggestive of staphylococcus species based on results of culture and biochemical tests (HiStaph kit by HiMedia) were subjected to ABST using Meuller Hinton Agar as per standard protocol and according to procedure suggested by Bauer et al (1966).

In the second phase of the study, forty animals with clinical signs suggestive of pyoderma presented to Veterinary College, Bengaluru and which were confirmed by culture/molecular diagnosis were selected for therapeutic trials with the antimicrobials found most sensitive as per ABST and randomly divided into four groups of ten animals each (Group I,II, III, IV and V). The animals in each of the groups were treated for a period of two weeks, which was extended for a further one week in unresponsive cases. The animals were monitored on Day 14 following initiation of therapy and after 1 month following cessation of therapy. Scores were allotted depending on the response to therapy as excellent (5) good (4), moderate (3), marginal (2) and poor (1) based on resolution of clinical signs and lesions and on recovery.

The animals of Group I were given conventional antibiotic therapy with the most sensitive antibiotic as per ABST, animals of Group II were given the conventional therapy with the second most sensitive antibiotic, animals of Group III were given the conventional therapy with the third most sensitive antibiotic and animals of Group IV were given conventional antibiotic therapy with the most sensitive antibiotic along with a topical cream containing retinoids.

The response to therapy was monitored based on recovery and the disappearance of clinical signs and the lesions at the end of 14th day and at the end of the one month. A detailed study of the clinical signs was made on these animals prior to treatment and the lesions observed were listed. Based on the disappearance of lesions, a grading was done with scores ranging from 1-5 for each of the lesions. The lesions / clinical signs graded included papules, pustules,

epidermal collarettes, alopecia (localized and generalized), erythema, scaling and presence of erosive lesions / draining tracts / ulcers all of which correspond to primary clinical signs of pyoderma (Mason, 1997 and Deboer, 1995) (Table 1).

The scores for individual lesions were then added up and the average for each animal was taken for grading the response to treatment and also for comparison between groups using statistical analysis. Further, an overall assessment was also made on complete recovery of animals.

Statistical Analysis : The data was subjected to statistical analysis using T test and ANOVA using Graph Pad Prism Software and also as per procedure described by Gupta (1998).

RESULTS and DISCUSSION

Enrofloxacin was the most sensitive antibacterial with 85% sensitivity followed by Ampicillin at 80% sensitivity, Azithromycin at 60%, Vancomycin and Cefepime at 50%, Clindamycin and Cefoxitin at 40%, Cephalolithin at 35%, Cefixime and Cefpodoxime at 30% , Cefadroxil at 20% and Cephalexin at 15% (Table 2).

Group I : Treatment with a conventional antibacterial enrofloxacin orally : Animals of Group I were given the conventional therapy with the most sensitive antibacterial as per ABST i.e., Enrofloxacin @ 10mg / kg body weight twice daily.

Based on the resolution of clinical signs and lesions Mean ±SE score of animals of Group I after 14 days of therapy was 1.47±0.64. Mean ± SE score after 1 month of therapy was 1.63±0.72. On a percentage basis the number of the animals that showed complete recovery was 30% (3 out of 10) after 14 days of therapy. There was no recurrence in any of the recovered cases for the next 1 month (Table 3 and 4) and (Fig. 1 and 2).

Group II: Treatment with a conventional antibacterial ampicillin orally : Animals of Group II were given the conventional therapy with the second most sensitive antibacterial as per ABST i.e., ampicillin @ 15mg / kg body weight twice daily.

Based on the resolution of clinical signs and lesions Mean ±SE score of animals of Group II after 14 days of therapy was 2.18±0.77. Mean ± SE score after 1 month of therapy was 2.68±0.93. On a percentage basis the number of the animals that showed complete recovery was 60% (6 out of 10) after 14 days of therapy. There was no recurrence in any of the recovered cases for the next 1 month (Table 3 and 4) and (Fig. 1, 2, 4 and 5).

Group III : Treatment with a conventional antibacterial azithromycin orally : Animals of Group III were given the conventional therapy with the third most sensitive antibacterial as per ABST i.e., Azithromycin @ 5mg / kg body weight once daily.

Based on the resolution of clinical signs and lesions Mean ±SE score of animals of Group III after 14 days of therapy was 1.80±0.73. Mean ± SE score after 1 month of therapy was 1.97±0.79. On a percentage basis the number of the animals that showed complete recovery was 40% after 14 days of therapy. There was no recurrence in any of the recovered cases for the next 1 month (Table 3 and 4) and (Fig. 1, 2, 6 and 7).

Group IV: Treatment with enrofloxacin orally along with retinoid cream once daily : Animals of Group IV were treated with the antibacterial of Group I i.e.Enrofloxacin @ 10mg / kg body weight twice daily along with topical therapy using a cream containing retinoids.

Based on the resolution of clinical signs and lesions Mean ±SE score of animals of Group III after 14 days of therapy was 2.33±0.87. Mean ± SE score after 1 month of therapy was 2.35±0.87. On a percentage basis the number of the animals that showed complete recovery was 50% after 14 days of therapy. There was no recurrence in any of the recovered cases for the next 1 month (Table 3 and 4) and (Fig. 1, 2, 8 and 9).

The results of the response to therapy based on mean ± SE score between Groups I, II, III and IV were compared (Table 5 and Figure 3). Overall comparison of the Mean ± SE scores of the 4 groups at the end of the one month period showed statistically significant difference ($P<0.05$) between Group I and II. Statistically significant differences were not observed between Group I and III, Group I and IV, Group II and III, Group II and IV and Group III and IV.

Therapy for pyoderma is frustrating and challenging to the clinician because of the prolonged duration of therapy required, frequent recurrences and development of resistance towards the antibacterial drug. Clinical trials were undertaken to check the efficacy of treatment using three of the antibiotics that were found to be the most sensitive on ABST studies so as to check if in vivo efficacy correlates with sensitivity tests. Enrofloxacin showed maximum sensitivity with the 85% of the isolates being most sensitive followed by ampicillin and azithromycin with sensitivity 80% and 60% respectively.

In the present study, efficacy of different therapeutic regimen were evaluated (Table 3, 4, and 5 and Fig 1,2,3,4,5,6,7,8 and 9). The percentage of response to treatment varied between a minimum of 30% (Group I) to a maximum of 60% (Group II). Statistically significant differences ($P<0.05$) was observed between Group I and II but there was no statistically significant differences in the responses between Group I and III, Group I and IV, Group II and III, Group II and IV and Group III and IV.

However, in the clinical trials, therapy with Enrofloxacin showed 30% recovery on day 14 and azithromycin showed 40% recovery on day 14 of therapy. Ampcillin showed 60% recovery on Day 14 of therapy and retinoid topically along with enrofloxacin showed 50% improvement on the Day 14 of therapy. Thus it was found that enrofloxacin and azithromycin are only minimally effective in the treatment of pyoderma. Our data following clinical trials confirm relatively high resistance levels in canine S. pseudintermedius, primarily against macrolides and fluoroquinolones (Pellerin et al., 1998 and Ruscher et al., 2008).

It can therefore be concluded that ampicillin is a more effective systemic antibiotic for treatment of pyoderma. However, the findings in the study do not co-relate with the sensitivity pattern obtained prior to clinical trials. Thus, though enrofloxacin appeared to be the most sensitive antibacterial on ABST the clinical efficacy of enrofloxacin as well as the other antibiotics i.e., azithromycin and ampicillin did not correlate with the sensitivity shown in ABST. This difference and reduced efficacy could probably be due to variation in efficacy in in-vivo and in-vitro conditions. Further, lack of penetration of the antibiotics in the required concentration to the skin tissue and the presence of exudates and debris affecting the mechanism of action could be some of the other factors that resulted in reduced efficacy of these antibiotics on clinical trials as compared to in vitro tests.

Inappropriate treatment regimens, under dosing and mis-selection of the antibiotics may have aggravated the resistant gene which are normally dormant (Hillier et al., 2014). Further, susceptibility results should always be interpreted in the context of the clinical disease and current and prior history of antimicrobial use in the patient, bearing in mind that susceptibility in vitro does not always parallel clinical response in infected animals. Besides, the older generation antibiotics like ampicillin are very rarely used in the present day clinical practice for treatment of pyoderma and this could be the reason for the higher levels of sensitivity exhibited to ampicillin on clinical trials in the present study.

Further, in the present study Group IV which was treated with retinoids topically along with enrofloxacin showed better response to therapy as against Group I which was treated with enrofloxacin alone though there was no statistically significant difference between these two groups. Retinoid is a derivative of Vitamin A and it has many physiologic functions and is involved in the regulation of cellular growth and differentiation. It is also essential to maintain the integrity of epithelial tissues

and is particularly important for the keratinization process. Both deficiency and excess of vitamin A can give rise to cutaneous lesions such as hyperkeratinization, scaling, alopecia, poor hair coat and increased susceptibility to microbial infections (Scott et al., 2000). Retinoids are often used in dermatological disorders, more so in human beings and especially in refractory cases. In the present study, the improved response in animals treated with retinoids along with enrofloxacin as compared to enrofloxacin alone could be due to the ability of retinoids for modulating epidermal growth and differentiation. Thus it was found to be a useful adjunct in the therapy for pyoderma.

Thus it can be concluded that enrofloxacin and azithromycin are minimally effective in the therapy of pyoderma, whereas, the efficacy of ampicillin was moderate to good and it can be used as an initial therapeutic agent in non refractory cases. Further, retinoids can be used as a useful adjunct in the therapy of pyoderma.

Table 1 : Score card for the evaluation of pyoderma based on lesions in dogs

Sl. No.	Criteria for allocation of scores	Scores	Grading
1	Complete resolution of all clinical signs/lesions in 14 days	5	Excellent
2	Complete resolution of all clinical signs/lesions in 1 month	4	Good
3	Few lesions persisting at the end of 14th day	3	Moderate
4	More than 50% of lesions persisting at the end of 14th day	2	Marginal
5	No change/ recurrence/worsening of the condition after 1 month	1	Poor

Table 2 : Antibacterial sensitivity/ resistance pattern of staphylococcus from lesions with pyoderma in dogs (n=20)

ABST Discs	Sensitive	Sensitive (%)	Resistant	Resistant (%)
Enrofloxacin	17	85	3	15
Ampicillin	16	80	4	20
Azithromycin	12	60	8	40
Vancomycin	10	50	10	50
Cefepime	10	50	10	50
Clindamycin	8	40	12	60
Cefoxitin	8	40	12	60
Cephalothin	7	35	13	65
Cefixime	6	30	14	70
Cefpodoxime	6	30	14	70
Cefadroxil	4	20	16	80
Cefalexin	3	15	17	85

Table 3 : Mean ± SE of scores as response to therapy based on resolution of clinical signs in different groups of dogs with pyoderma (n=40)

Days of treatment	Overall Mean ±SE			
	Group I	Group II	Group III	Group IV
14 th day	1.47±0.64	2.18±0.77	1.80±0.73	2.33±0.87
1 month	1.63±0.72	2.68±0.93	1.97±0.79	2.35±0.87

Table 4 : Response to therapy in per cent based on complete recovery in different groups of dogs with pyoderma (n=40)

Days of Treatment	Animals recovered							
	Group I		Group II		Group III		Group IV	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
14 th day	3	30	6	60	4	40	5	50

Table 5 : Comparison of response to therapy based on resolution of clinical signs between the different groups of dogs with pyoderma (n=40)

Treatment groups	Mean difference	Significant (S*) / Non Significant (NS)
Group Ivs Group II	-0.88	S
Group I vs Group III	-0.335	NS
Group I vs Group IV	-0.79	NS
Group II vs Group III	0.545	NS
Group II vs Group IV	0.09	NS
Group III vs Group IV	-0.455	NS

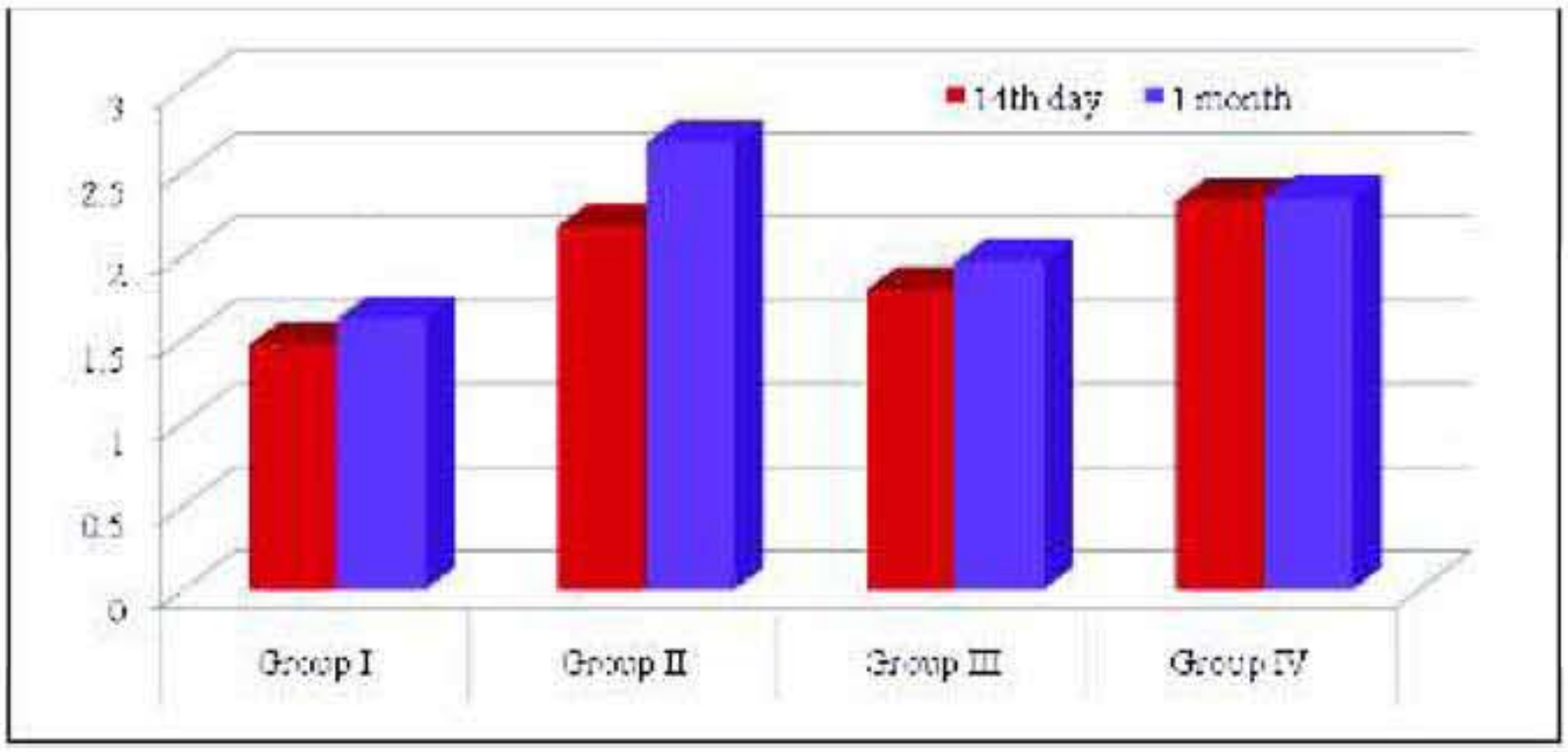


Fig. 1: Mean ± SE of scores as response to therapy based on resolution of clinical signs in different groups of dogs with pyoderma

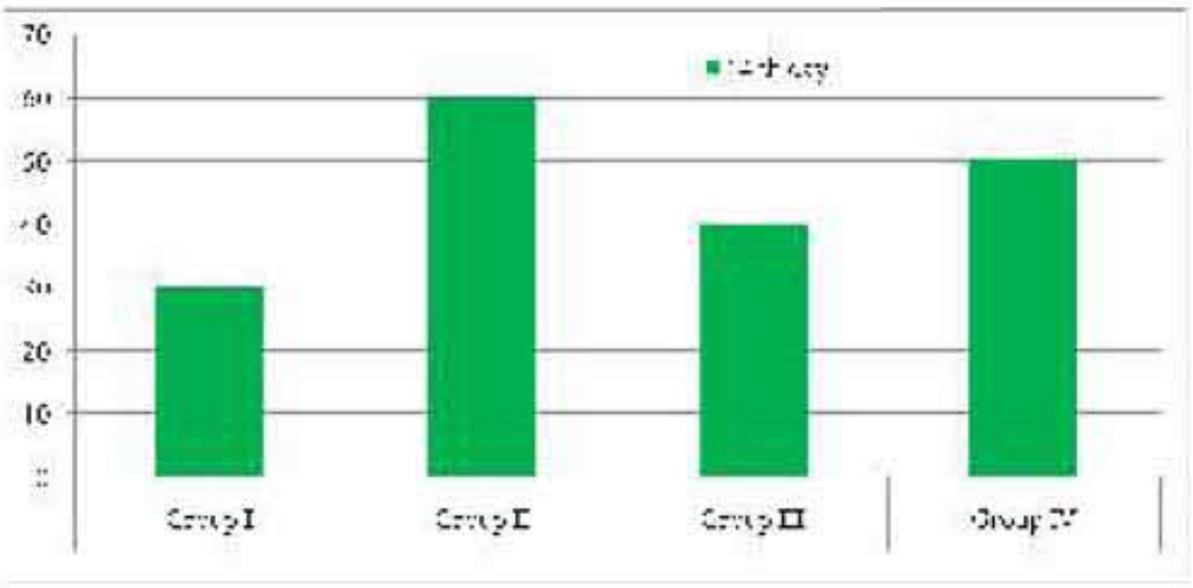


Fig. 2: Response to therapy in per cent based on complete recovery in different groups of dogs with pyoderma (n=40)

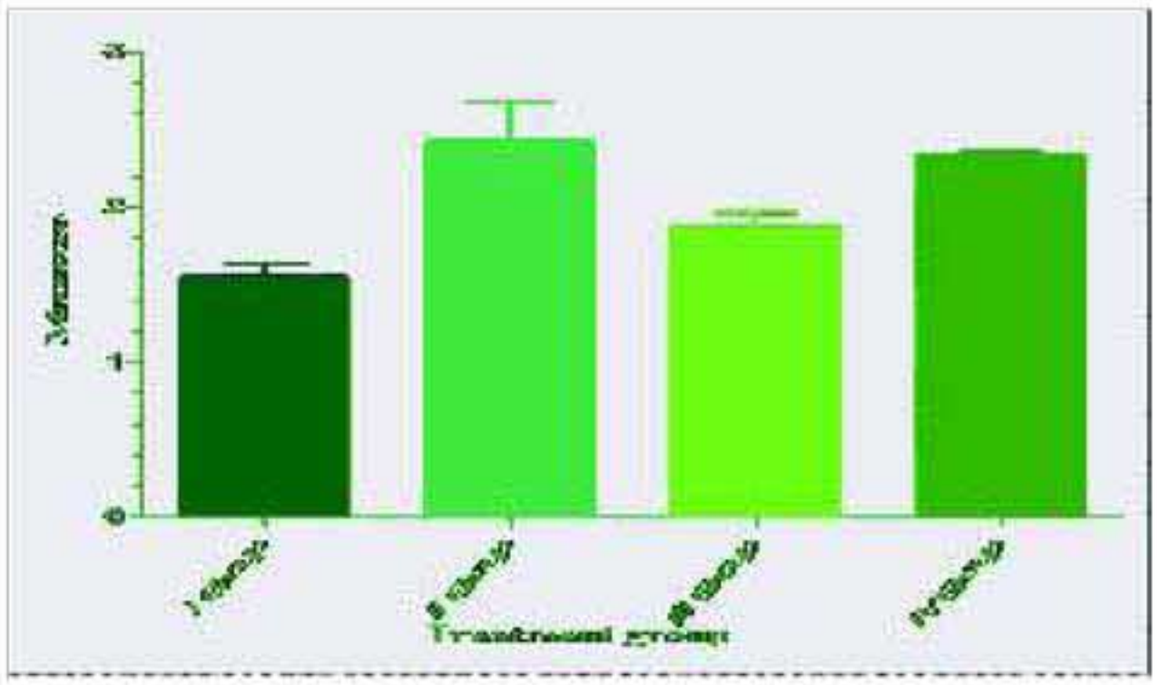
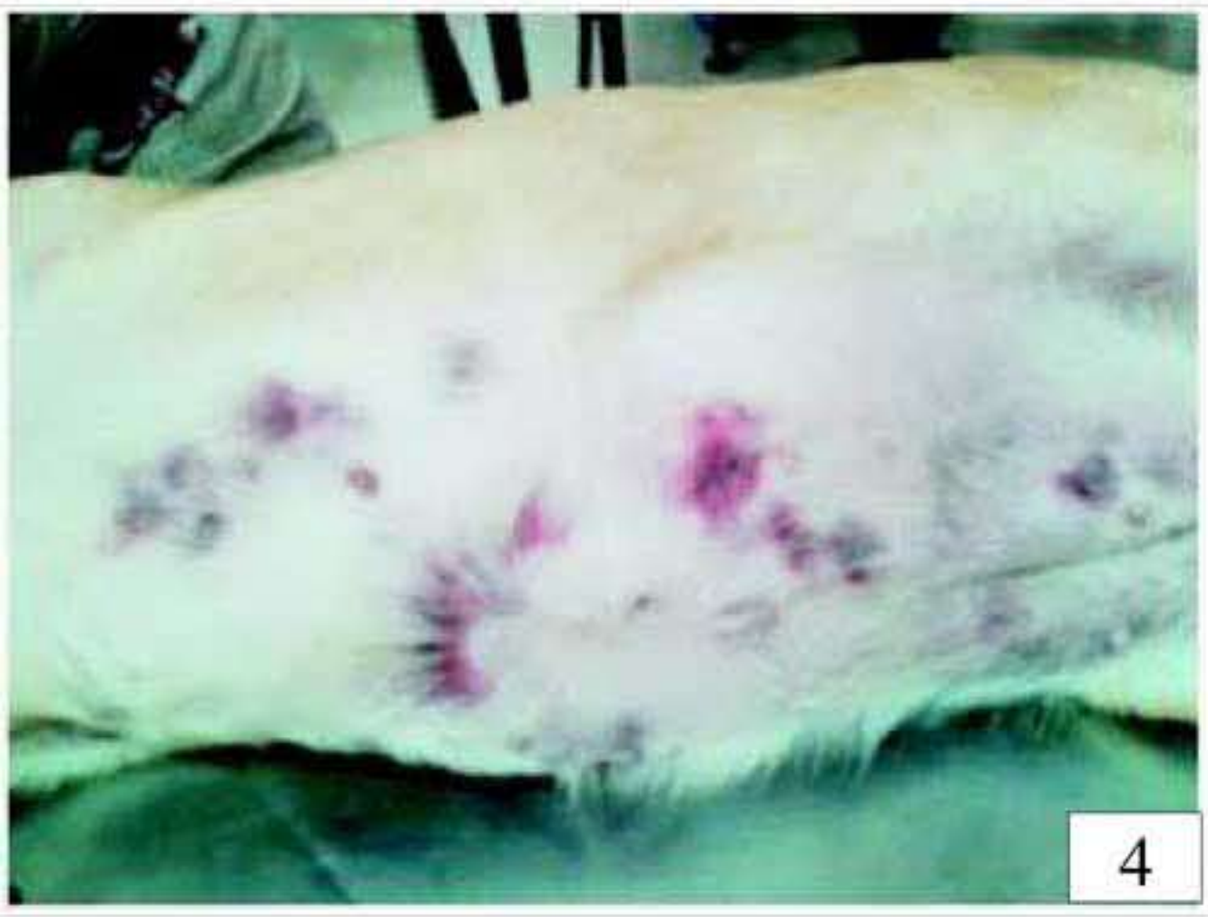


Fig. 3: Mean ± SE of scores as response to therapy based on resolution of clinical signs in different groups of dogs with pyoderma (n=40)



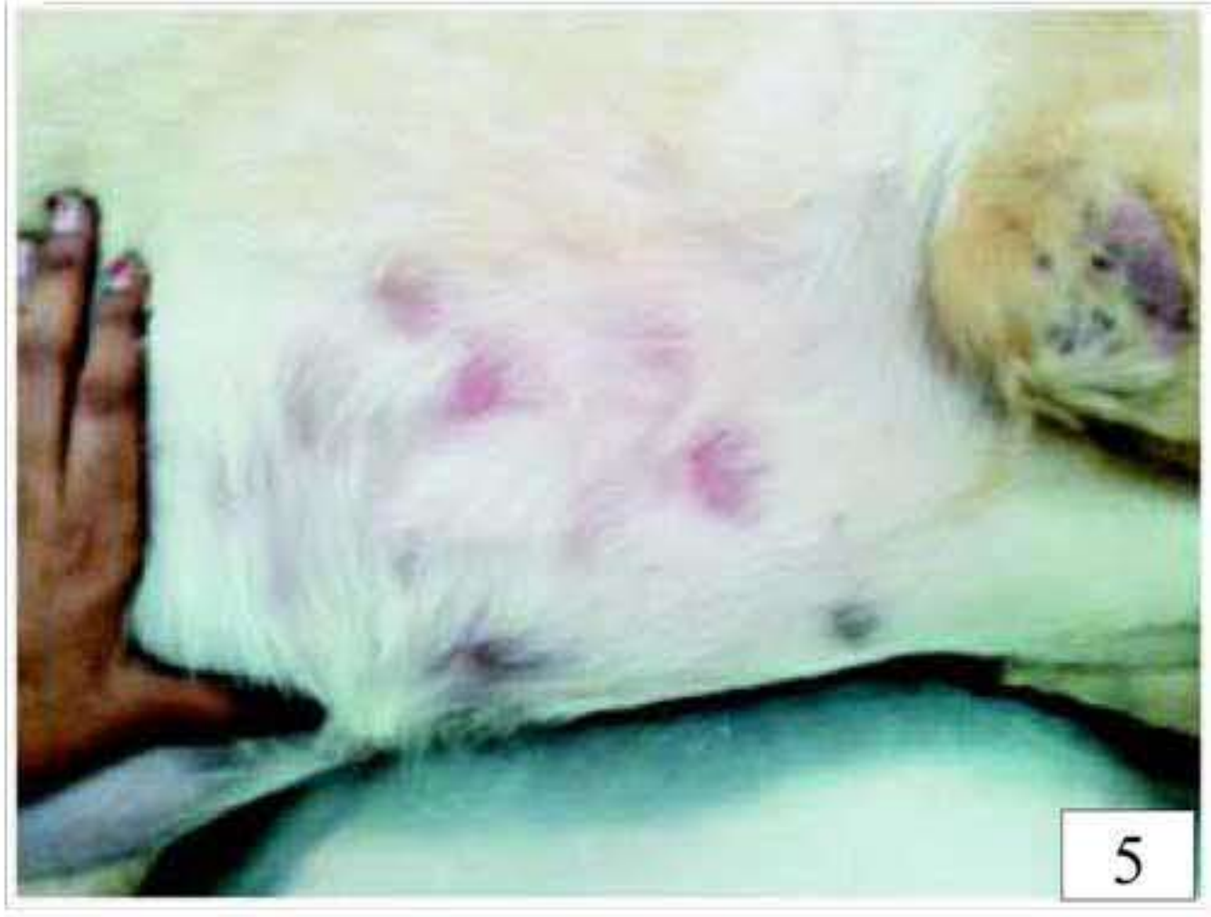
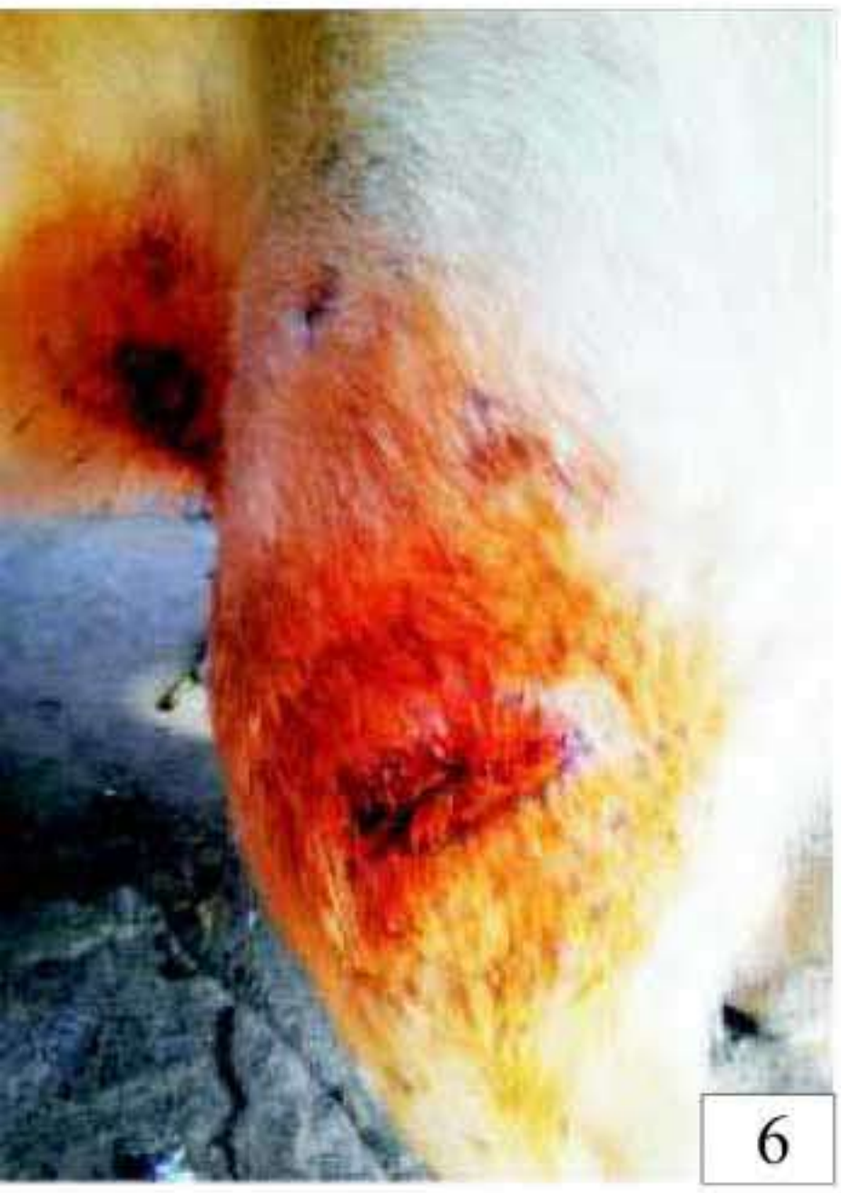


Fig 4 and 5: Multiple erythematous epidermal collarettes on the ventral abdomen of a dog with superficial pyoderma on Day 1 of clinical trial and improvement on Day 7 of therapy with ampicillin of therapy .



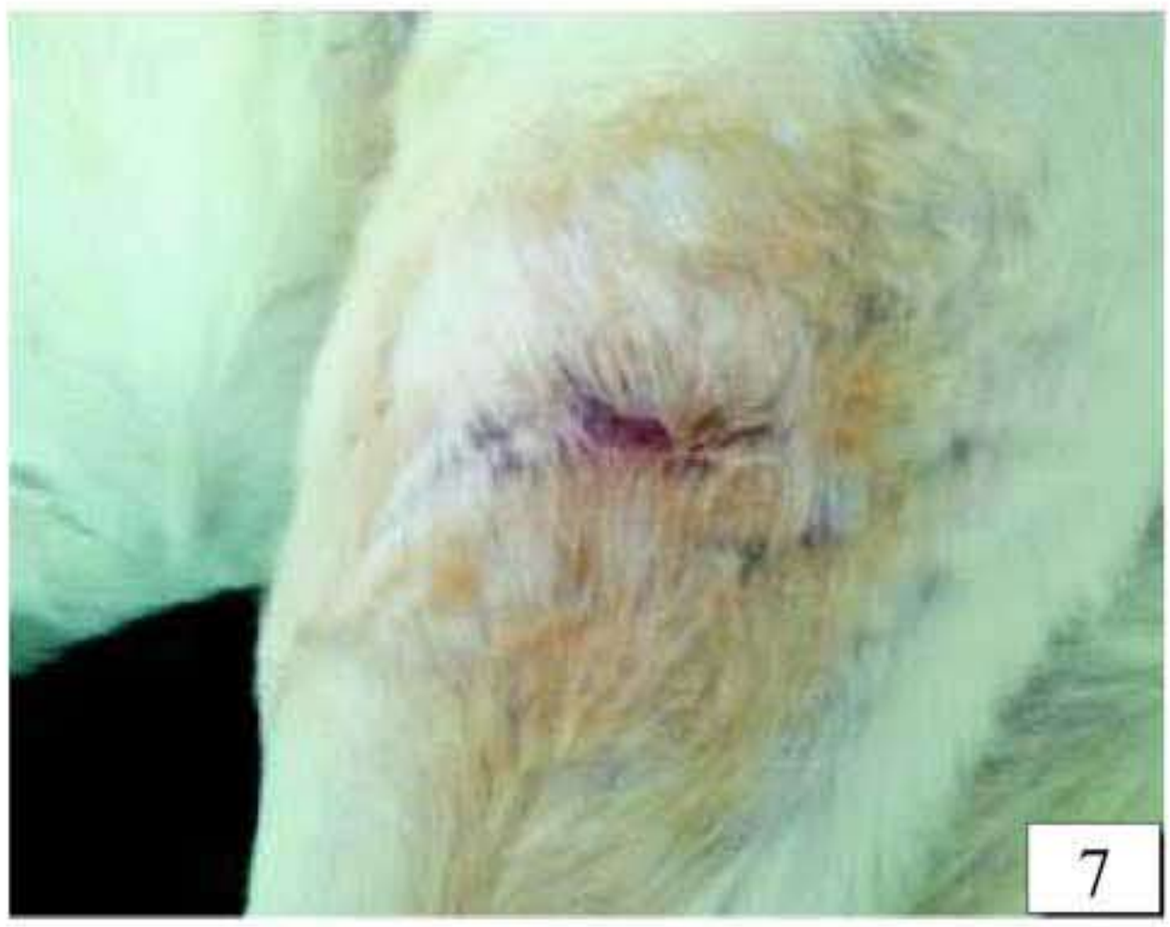


Fig 6 and 7: Pyotraumatic dermatitis on the left hind limb of a dog and improvement in condition in a dog with pyoderma after therapy with azithromycin

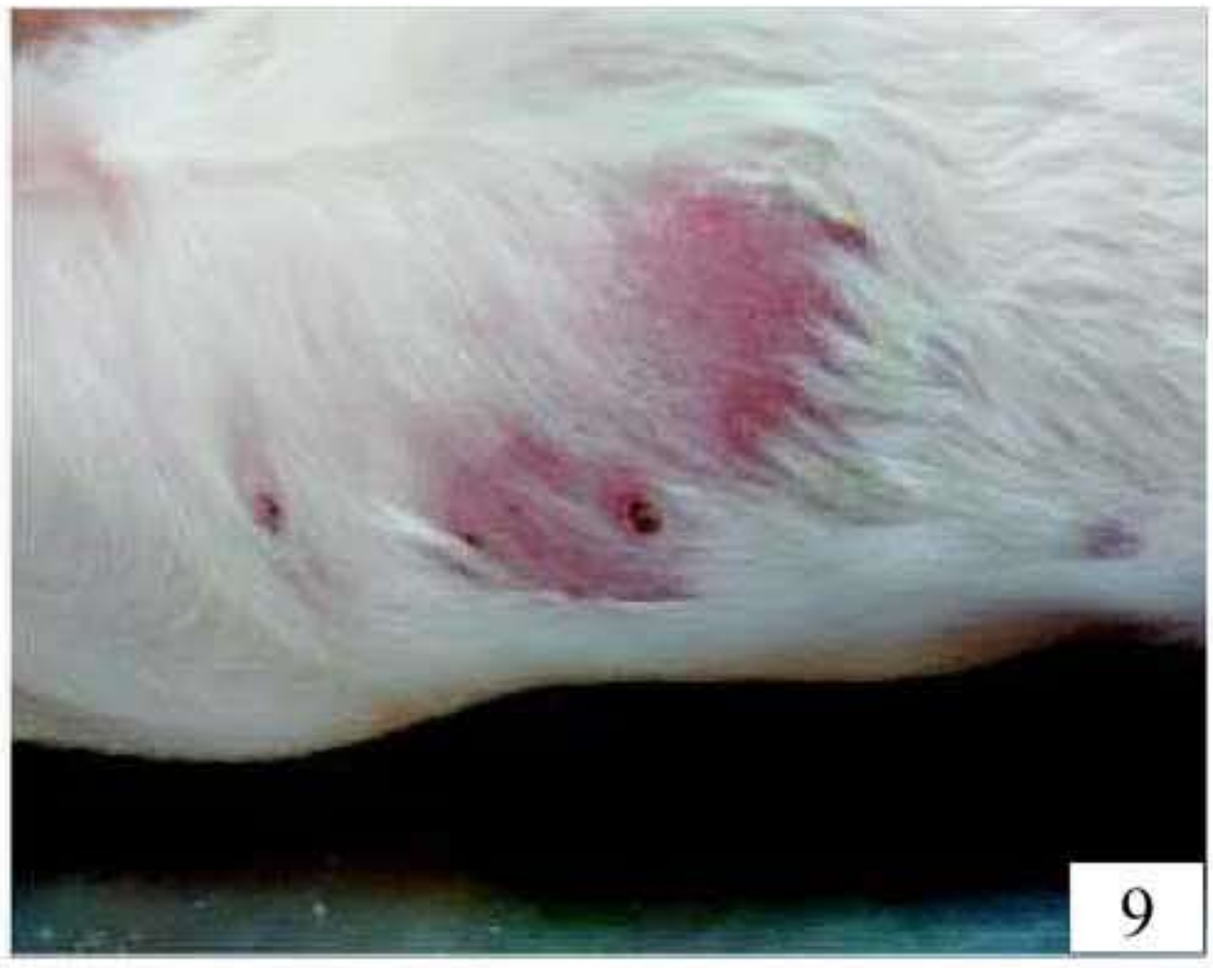


Fig 8 and 9 : A dog with superficial pyoderma showing typical epidermal collarettes on ventral abdomen and improvement in condition on day 7 of treatment with enrofloxacin and retinoid cream of therapy .

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Comparative Efficacy of Different Diagnostic Techniques in the Diagnosis of Ehrlichiosis in Dogs*

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ABSTRACT

A comparative study on the efficacy of different diagnostic tests in the diagnosis of ehrlichiosis in dogs presented to Veterinary College Hospital, Bengaluru was carried out. Fifty dogs with clinical signs suggestive of ehrlichiosis were utilized for the study. Blood was collected from the animals and subjected to different diagnostic tests such as blood smear examination, buffy coat smear, Enzyme Linked Immuno Sorbent Assay (ELISA)-Rapid Assay Kit and Polymerase Chain Reaction (PCR). It was found that PCR was the most reliable diagnostic test with high sensitivity and specificity followed by ELISA Rapid Assay Kit method. Blood and buffy coat smears were found to be not very reliable in the diagnosis of ehrlichiosis in dogs.

Keywords : Ehrlichiosis, Diagnosis, ELISA Rapid Assay Kit, PCR, Ehrlichia canis

Ehrlichiae are one of the several kinds of obligate intracellular pathogens first described at the Pasteur Institute in Algeria (Donatien and Lestoquard,1935). It was initially classified under ricketsial organisms but now considered a tick borne bacterial disease belonging to the family Anaplasmataceae and genera Ehrlichia (Mavromatis et al, 2006). There are several species of Ehrlichia infecting man and animals (Dawson and Ewing, 1992). In India , Ehrlichia canis is the most common species reported and it causes Canine Monocytic Ehrlichiosis (CME), a potentially fatal tick borne disease (Bindu et al., 2007a). Besides E.canis, other ehrlichiae encountered in canines include E.ewingi (Anderson et al., 1992), E.risticii (Kakoma et al., 1994) and E. chaffeensis (Dawson and Ewing, 1992).

Diagnosis of ehrlichiosis frequently seems difficult, as the clinical signs are non specific and the pathognomonic signs described are not always manifested. Hence the greatest challenge in battling ehrlichiosis is detecting and accurately assessing the clinical signs. An early diagnosis of the disease is imperative to ensure successful treatment and good prognosis. (Bindu and Lalitha, 2007)

Currently definitive diagnosis of ehrlichiosis is mostly based on hematological, biochemical and serological tests. Microscopic demonstration of typical intracytoplasmic morulae of E.canis in leukocytes is not a very reliable diagnostic technique as the organism is not readily demonstrable in blood smears (Woldehivet and Ristic, 1993 and Waner et al., 1999).

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Serological tests fail to distinguish a current infection from either previous infection or exposure without establishment of infection. Furthermore, serologic cross-reactivity between ehrlichial species poses a serious problem in differentiating the infecting ehrlichia species (Waner et al., 2001). Isolation by cell culture, although sensitive and specific, takes a long time to produce results, thus limiting its use as a rapid diagnostic tool (Waner et al., 2001).

Sensitivity, specificity, ease of use, rapidity, and the ability to analyse a large number of samples at the same time make nested PCR a superior option for detection of early as well as persistent canine ehrlichiosis. Only limited attempts have been made in India to diagnose canine ehrlichiosis using PCR (Bindu et al., 2007a). Hence the present study was undertaken to find out the best and the most rapid technique for the diagnosis of ehrlichiosis and to compare the efficacy of these techniques.

MATERIALS AND METHODS

The animals utilized for the present study were clinical cases of dogs with signs suggestive of tick borne diseases presented to Veterinary College Hospital, Bengaluru. In the present study, a total of 50 clinical cases were selected based on history and clinical signs such as pyrexia, lethargy, anorexia, presence or history of tick exposure, anemia, hematuria, epistaxis, congested/pale/icteric mucous membranes, lymphadenopathy, lameness, petichial hemorrhages on ventral abdomen and weakness.

Sample collection : Blood was collected into EDTA vacutainers using sterile syringes from the saphenous or cephalic veins of dogs with clinical signs suggestive of ehrlichiosis .

Blood and buffy coat smear examination : Blood smears and buffy coat smears were made from blood of affected dogs and stained with Giemsa stain (Himedia) as per the standard procedure for demonstration of hemoprotozoan organisms (Schalm et al.,1975)

ELISA rapid assay kit method : SNAP-4DX test kit manufactured by IDEXX, USA was used for screening of ehrlichiosis and other tick borne infections. According to manufacturer's instructions serum, plasma or anti-coagulated whole blood (e.g., EDTA, heparin), either fresh or stored at 2-8° C for up to one week, could be used for the assay. In the present study whole blood and plasma were used for the test. The test kit consisted of conjugate, SNAP device, transfer pipette, sample tubes and reagent rack and the protocol as described by the manufacturer was followed. The result was read after 8 minutes after conducting the test.

DNA extraction and PCR : DNA was extracted from all the blood samples using the kit procured from QIAGEN, GmbH, Germany (DNA Blood Mini Kit) by following the manufacturer's protocol.

PCR for the detection of E. canis in the blood samples was carried out based on the method of Murphy et al. (1998) with some modifications. Primers were selected based on research work of Murphy et al. (1998). Genus specific primers used for the amplification of ehrlichial DNA were ECC (5' -AGA ACG AAC GCT GGC GGC AAG C- 3') and ECB (5'- CGT ATT ACC GCG GCT GCT GGC A -3').

Extracted DNA (4.0 µL) was used as a template to amplify a fragment of the 16S rRNA gene in 25 µL of reaction mixture containing 12.0 µl of Taq DNA Polymerase Master Mix RED (2X), 1.0µl of primer Ehrlichia F (20 pmol/µl) , 1.0µl of primer Ehrlichia R (20 pmol/µl) and 7.0µl of sterile Nuclease free distilled water.

PCR was carried out in a thermal cycler (Eppendorf, Germany). The thermocycle profile consisted of initial denaturation at 94 °C for one min, followed by 29 cycles of denaturation at 94°C for one min., annealing at 65 °C for 2 min. and extension at 72 °C for 2 min. This was followed by a final extension at 72 °C for 5 min. The amplicons obtained were subjected to nested PCR for confirmation of the species of ehrlichia. Nested reactions were performed using 1 µL of this amplicon as a template with species specific

primers of E. canis, namely, E.canis-F (20 pmol/µl) (5'- CAA TAA TTT ATA GCC TCT GGC TAT AGG A- 3') and E.canis-R (20 pmol/îl) (5'- TAT AGG TAC CGT CAT TAT CTT CCC TAT - 3') under the same reaction conditions as described above.

RESULTS AND DISCUSSION

Blood and Buffy Coat Smear Examination : The blood smear and buffy coat smears were prepared from all the 50 cases and examined under oil immersion objective of microscope. All the fifty samples examined were negative for ehrlichia organisms in direct blood smear as well as buffy coat smear examination. This might be due to the fact that the percentage of E. canis infected cells in the peripheral blood is low as indicated by Iqbal et al. (1994) and Harrus et al. (1998). The stage of infection and level of parasitemia is important while demonstrating the organisms by blood smear examination and it requires more number of blood smears to be examined. Further, faster the processing and examination of the smears, greater are the chances of getting positive results as there are chances of disintegration of parasites in the collected blood samples and this could be the reason for the lack of success rate in finding morula in blood and buffy coat smears. Iqbal et al. (1994) and Harrus et al. (1998). Woody and Hoskins (1991) and Bindu et al. (2007b) reported that success rate of the examination of morulae in blood smears was only about 4% and that the technique was difficult and time consuming and requires immediate processing.

ELISA Rapid Assay Kit Method : All the 50 samples collected from suspected clinical cases were subjected to ELISA Rapid Assay test using the SNAP 4DX kit. Eleven (22%) cases were found positive for canine ehrlichiosis by ELISA rapid assay test kit (Table 1 and 3) and (Plate 1 and 2)

This is in accordance with the findings of Wise and Tarlinton (2011) who observed 19% cases of ehrlichiosis using ELISA Rapid Assay kit. Similar findings with lower sensitivity were reported by Mircean et al. (2012) who found 2.1% positive for ehrlichiosis in dogs from Romania. Dziegiel et al. (2016) found 1.5% cases positive for ehrlichiosis in eastern Poland and Ebani et al. (2014) found 7.07% positive for ehrlichiosis in rural and urban dogs in Central Italy.

PCR : A total of 50 blood samples collected from dogs suspected for ehrlichiosis were subjected for detection of ehrlichia DNA by nested PCR with genus specific and species specific primers. Ehrlichia genus was identified based on the presence of 477 bp DNA fragment produced which was also obtained with positive control using genus specific primers, which was confirmed by sequencing. Of the 50 samples, 41 were positive for the presence of ehrlichia DNA with genus specific primers. Ehrlichia canis species was identified based on DNA fragment of 387 bp band obtained using species specific primers which was also obtained with positive control, which was confirmed by sequencing (Table 2 and 3) and (Plate 3, 4, and 5).

All the 41 samples which were positive for ehrlichia genus were positive for E. canis species when subjected to nested PCR with E. canis specific primers (Table 2 and 3) and (Plate 3, 4 and 5).

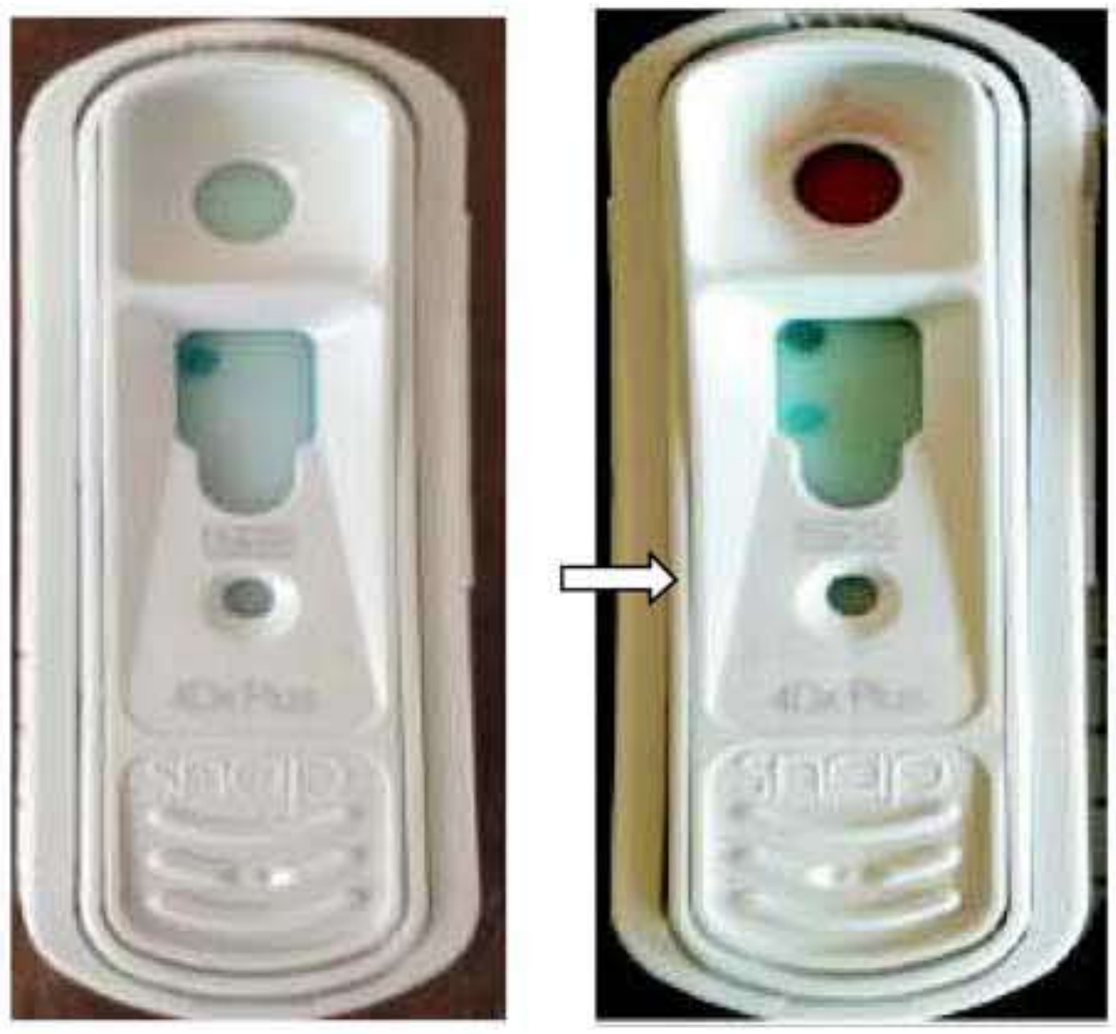
Of 50 samples, 41 (82%) were positive for the presence of ehrlichia DNA with genus specific primers by PCR and were also positive for E. canis species with nested PCR. This is similar to the findings of Harrus et al. (1998), Murphy et al. (1998), Asha et al. (2004), Bindu et al. (2007b), Carvalho et al. (2008), Nakaghi et al. (2008), Saira Banu et al. (2009), Sunita Choudhary (2009) and Arun (2015) who also used the primers and detected ehrlichia organisms to the genus and species levels.

On comparing the efficacy of different techniques in the diagnosis of ehrlichiosis, out of 50 clinical cases suspected, 11(22%) cases were found positive for ehrlichia organisms by ELISA rapid assay test (SNAP-4DX test). None of the cases were found positive for ehrlichia in blood smear and buffy coat smear examination. Forty one (82%) cases were diagnosed positive for ehrlichia based on PCR. Thus, it may be inferred that blood smear examination or buffy coat

smear examination is not a reliable diagnostic test since very few cells are affected and that the organisms / inclusions are not usually detected. Further, the sensitivity of ELISA Rapid Assay kit was not to the extent observed in case of PCR in this study which showed a very high sensitivity and specificity. Thus, the experience of the present study has indicated PCR as a more sensitive and reliable direct diagnostic technique for detection of ehrlichiosis.

CONCLUSION

The study on the efficacy of different diagnostic techniques in the diagnosis of ehrlichiosis indicated that PCR was the most reliable diagnostic test with high sensitivity and specificity followed by ELISA Rapid Assay using kits. Blood and buffy coat smear examination were not reliable diagnostic tests for the diagnosis of ehrlichiosis in dogs



Negative Ehrlichia positive

Plate 1 and 2: ELISA Rapid Assay Test kit results (SNAP-4DX test kit)

Table 1 : Results of ELISA Rapid Assay Test kit

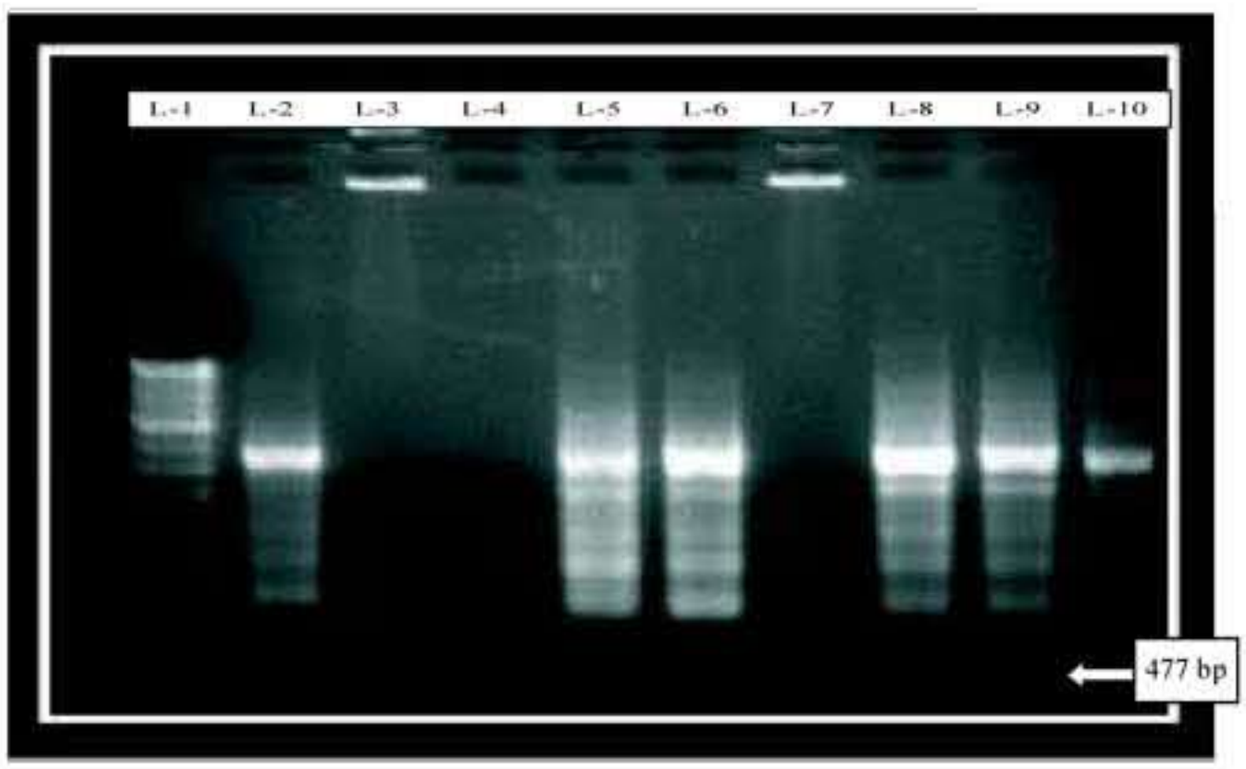
Total numbers tested	Ehrlichia positive (Numbers)	Ehrlichia positive (Per cent)
50	11	22

Table 2 : Results of PCR technique for Ehrlichiosis (n=50)

PCR positive	Ehrlichia genus Numbers (per cent)	Ehrlichia canis (species) Numbers (per cent)
Numbers (%)	41 (82%)	41 (82%)

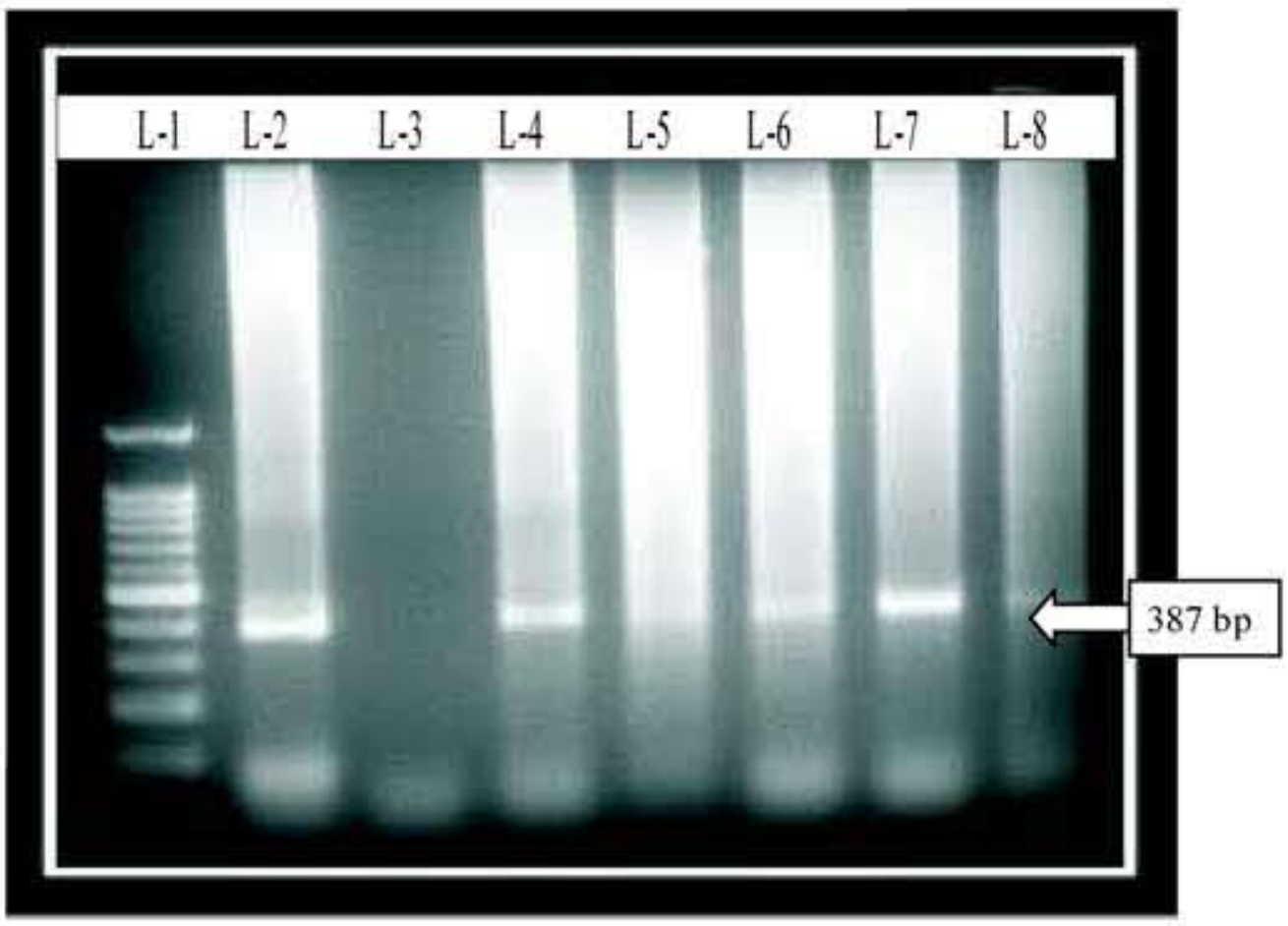
Table 3 : Table showing the comparative efficacy of different diagnostic methods

Sl. No.	Test Method	No. positive (% positive) (Ehrlichiosis-n=50)
1	Blood smear	0
2	Buffy coat smear	0
3	SNAP- 4Dx test	11(22%)
4	PCR	41(82%)



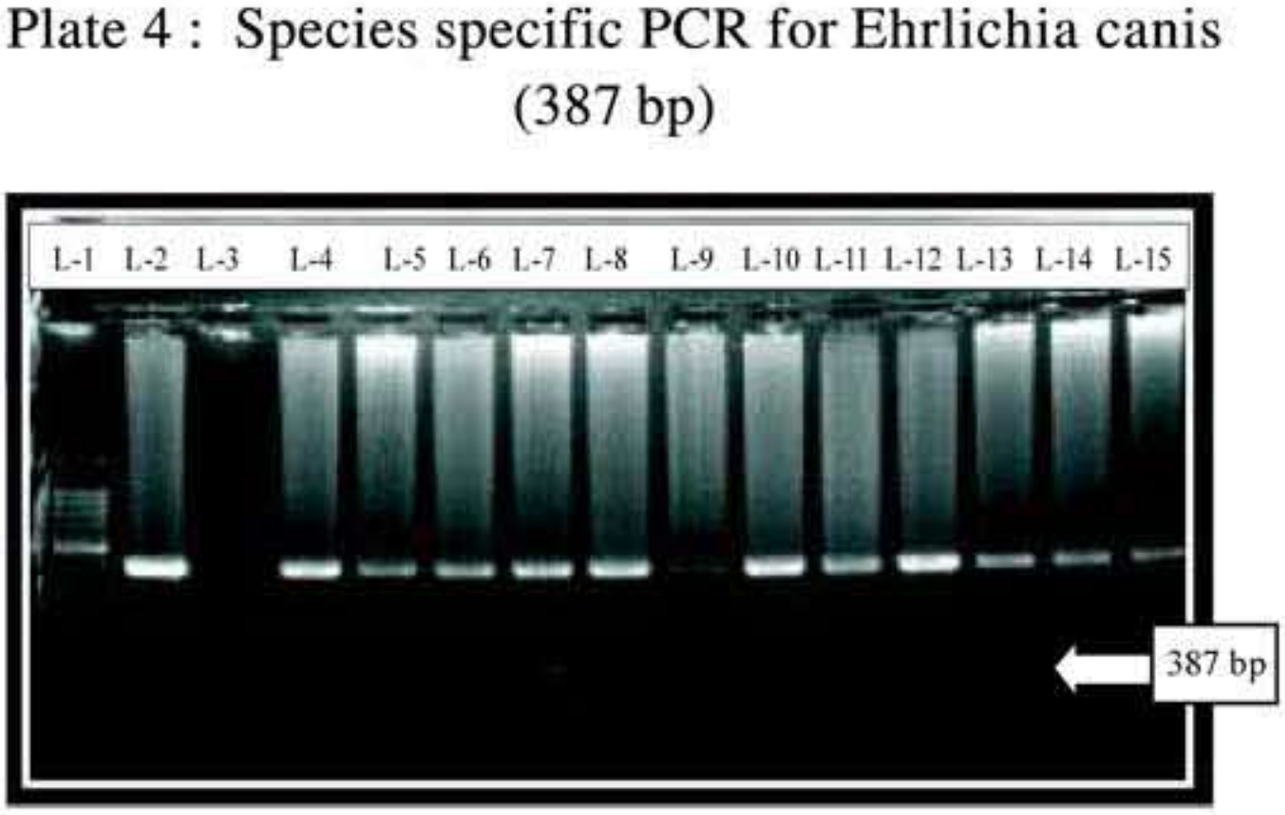
Lane 1	100 bp DNA marker
Lane 2	Known positive sample
Lane 3	Known negative sample
Lane 4	PCR control (no DNA template)
Lane 5, 6, 8 ,9 and 10	Positive test samples
Lane 7	Negative test sample

Plate 3: Genus specific PCR for Ehrlichia (477 bp)



Lane 1	100 bp DNA marker
Lane 2	Known positive sample
Lane 3	Known negative sample
Lane 4, 5, 6, 7, 8	Positive test samples

Plate 4 : Species specific PCR for Ehrlichia canis (387 bp)



Lane 1	100 bp DNA marker
Lane 2	Known positive sample
Lane 3	Known negative sample
Lane 4,5, 6, 7, 8,9, 10, 11, 12, 13, 14 and 15	Positive test samples

Plate 5 : Species specific PCR positive for Ehrlichia canis (387 bp)

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Study of Cardiac Diseases in Dogs with Special Reference to Electrocardiography*

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ABSTRACT

The present study was carried out to evaluate the occurrence of cardiac diseases in dogs. Dogs presented to Department of Veterinary Medicine, Veterinary College Hospital, Bangalore with cardiac insufficiency were selected based on clinical signs, hematology, biochemistry and electrocardiography. In the present study occurrence of cardiac disease was more in male dogs compared to female dogs. The characteristic ECG findings observed in the present study were sagging of ST segment, tall QRS complex, Q dip, P- pulmonale, tall-T wave, deep S wave and ventricular premature complexes.

Key words : Cardiac diseases, electrocardiography.

Canine cardiovascular disease can involve one or more heart structures. These include diseases of the heart valves (mitralvalvular disease), heart muscle (cardiac hypertrophy, dilated and restrictive cardiomyopathy), and those due to abnormal electrical activity (cardiac arrhythmias), ischemic and reperfusion damage and non-fatal infarcts.

The commonly encountered cardiac diseases in dogs which are reported by various authors are dilated Cardiomyopathy (Cobb, 1992; Sisson et al., 2000), degenerative mitral valve disease (Haggstrom, 1996; Pedersen, 2000), hypertrophic cardiomyopathy (Meurs, 2005) and arrhythmogenic right ventricular cardiomyopathy (Bonagura and Lehmkuhl, 2006).

Chronic atrioventricularvalvular insufficiency has been more commonly reported in smaller breeds of dog (Detweiler and Patterson, 1965). Dilated cardiomyopathy (DCM) ismost often associated with large and giant breeds of dogs and Doberman Pinscher, Boxer, English and American Cocker Spaniel breeds (Thomas,1987).

Families of Cavalier King Charles Spaniels and families of Dachshunds have provided evidence that genetic factors play a large role in the occurrence of cardiac diseases (Olsen et al., 1999).

MATERIALS AND METHODS

Dogs presented to the Veterinary College Hospital, Hebbal and those referred from Veterinary Clinical Service Complex, Yelahanka from January 2010 to June 2010 with cardiac insufficiency were considered for the present study.

Total of 18 dogs diagnosed with cardiac insufficiency based on history, clinical signs, hematology, biochemistry and electrocardiography were selected.

Blood samples were collected for hematology in a sterile vacutainer with EDTA as anticoagulant, and subjected for hemoglobin, total erythrocyte count,total leucocyte count and haematocrit value estimation (Schalm et al.,1975).

Serum was subjected for creatinine estimation and the values were expressed in mg/dl. The ECG was recorded using the standard bipolar and augmented

* Part of the MVSc thesis of the first author submitted to KVAFSU, Bidar.

unipolar limb leads at 25mm/s speed and interpreted as described by Tilley (1992).

RESULTS AND DISCUSSION

In the present study, cardiac disease was diagnosed based on history, clinical signs, blood examination and electrocardiographic findings. Blood test was done just to rule out other diseases like renal failure.

In the present study the occurrence of cardiac disease in different breeds ranged from 5.55% to 22.22%. The occurrence was highest in Labrador retriever (22.22%), followed by Golden Retriever and Dachshund at 16.66%. The occurrence in German shepherd, Neapolitan Mastiff and Spitz was 11.11% each and in Non-descript and Cocker spaniel the occurrence was 5.55%. The findings in the present study are in agreement with the results of Martin et al. (2009). However, few reports have indicated common occurrence of cardiac disease in large and medium sized dogs such as Great Danes, Saint Bernard, Dobermann, GSDs, Boxers, Cocker Spaniel (Sisson and Thomas, 2000 and Martin et al., 2009).

The occurrence of cardiac disease is more common in some breeds than in others. This could be due to hereditary mode of transmission of heart disease in dogs as reported by Detweiler et al. (1968).

The gender-wise occurrence of cardiac disease in the present study was 83.33% in male dogs and 16.66% in female dogs, which was in agreement with the findings of Detweiler (1964) and Martin et al. (2009).

The age -wise occurrence of cardiac disease in the present study was 55.55% in more than 8 years to 13 years of dogs, 22.22% in less than 4 years of dogs and rest in 4 to 8 years of dogs. The occurrence was high in dogs between 8 to 13 year old dogs.

The findings of present study was in agreement with the findings of Martin et al. (2009) who reported that dogs aged between 5 to 10 years have high incidence with a peak incidence at 7 years and Haggstrom (1996) has reported that dogs develop decompensated heart failure because of DMVD after 10 years of age.

Age related occurrence of cardiac diseases in dogs could be attributed to decline in the velocity of calcium uptake by the sarcoplasmic reticulum, resulting in impaired myocardial relaxation. In dogs aging is also accompanied by a decrease in the response of the myocardium to beta-adrenergic stimulation (Bright and Mears, 1997).

In the present study, sagging of ST segment was seen in 72.22% of dogs. S-T segment depression in leads II or those with dominant R waves indicates myocardial ischemia, hyperkalemia, hypokalemia and digitalis toxicity. Secondary S-T segment changes from abnormalities of the QRS complex are indicative of hypertrophy, bundle branch block, and Ventricular Premature Complexes (VPC) (Tilley, 1992).

Tall R wave was seen in 66.66% of dogs. Tall R wave is suggestive of left ventricular enlargement. In left ventricular enlargement, amplitude of R wave will be greater than 3mV in lead II (Tilley, 1992).

Q dip was seen in the 33.33% of dogs. Q dip is characterized by Q waves greater than 0.5 mV. Q dip indicates right ventricular enlargement (Tilley, 1992). This correlates with findings of Thomas (1987) who has reported that Q >0.5mV in lead II indicates right ventricular enlargement.

P pulmonale was seen in 22.22% of dogs. P-Pulmonale is characterized by taller P waves with amplitude of more than 0.4 mV. Tall P waves are suggestive of right atrial enlargement (Tilley, 1992).

Tall T wave was seen in the 11.11% of dogs. In general the T wave in dogs should not be more than one – fourth the height of the associated R wave. Large T wave can be seen with myocardial hypoxia, intraventricular conduction disturbance, ventricular enlargement, hypothermia and animals with heart disease and bradycardia (Tilley, 1992).

Deep S wave was seen in 5.55% of dogs. This is commonly observed in right ventricular enlargement (Tilley, 1992). This correlates with findings of Thomas (1987) who has reported deep S waves in precordial chest leads CV_6LU and CV_6LL with right ventricular enlargement.

Ventricular premature complexes were seen in 5.55% of dogs. VPCs are seen in dilated cardiomyopathy, CHF, myocardial infarction, bacterial endomyocarditis (Tilley, 1992).

The mean ± SE of total leukocyte count, total erythrocyte count, hemoglobin and packed cell volume of dogs affected with cardiac disease were 10±0.46 x 10^3 cells/ µL,6.1±0.31 x 10^6 cells/µL, 12±0.58 g% and 41±2.1% respectively. The mean ± SE value of serum creatinine in dogs with cardiac diseases was 1.13±0.10. The mean ± SE value of the total leukocyte count, total erythrocyte count, hemoglobin, packed cell volume were within the normal range.

The mean ± SE of the creatinine levels in dogs with cardiac disease at different intervals of time were within the normal level and there was no significant difference ($P>0.05$) in the creatinine values at different intervals of time.

Table 1 : Breed – wise occurrence of cardiac disease in dogs (n=18)

Breed	No. of dogs	Per cent
Labrador Retriever	4	22.22
Golden Retriever	3	16.66
Dachshund	3	16.66
German Shepherd	2	11.11
Neopolitan Mastiff	2	11.11
Spitz	2	11.11
Non Descript	1	5.55
Cocker Spaniel	1	5.55
Total	18	100

Table 2 : Age– wise occurrence of cardiac disease in dogs (n=18)

Age	No. of animals	Per cent
< 4 years	4	22.22
4- 8 years	4	22.22
>8- 13years	10	55.55
Total	18	100

Table 3 : Electrocardiographic findings in dogs with cardiac disease (n=18)

ECG finding	No. of animals	Per cent
Sagging of ST segment	13	72.22
Tall QRS complex	12	66.66
Q dip	6	33.33
P pulmonale	4	22.22
Tall T wave	2	11.11
Deep S wave	1	5.55
Ventricular Premature Complexes	1	5.55

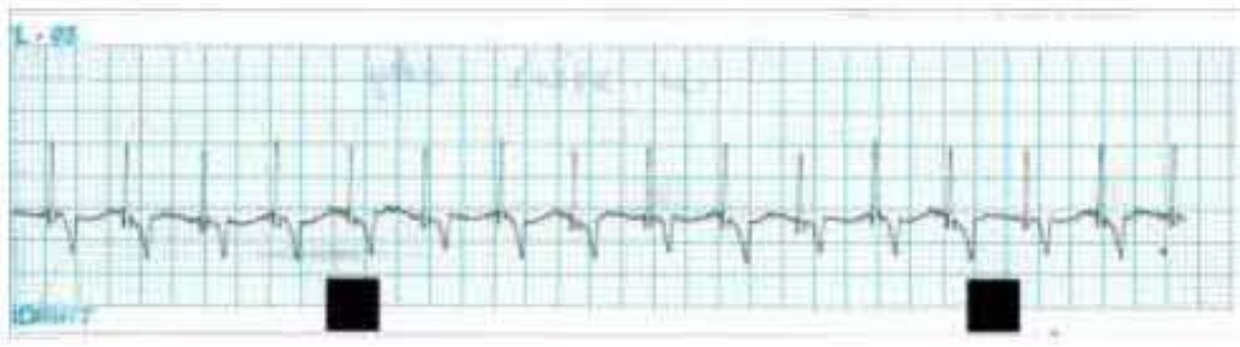

Fig. 1 : Sagging of ST segment

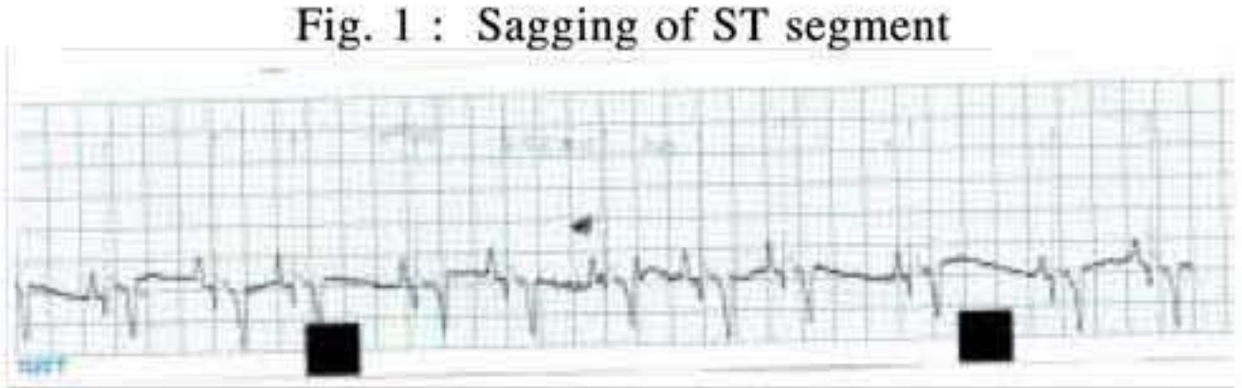

Fig. 2 : P pulmonale - P waves are tall with amplitude more than 0.4 mV

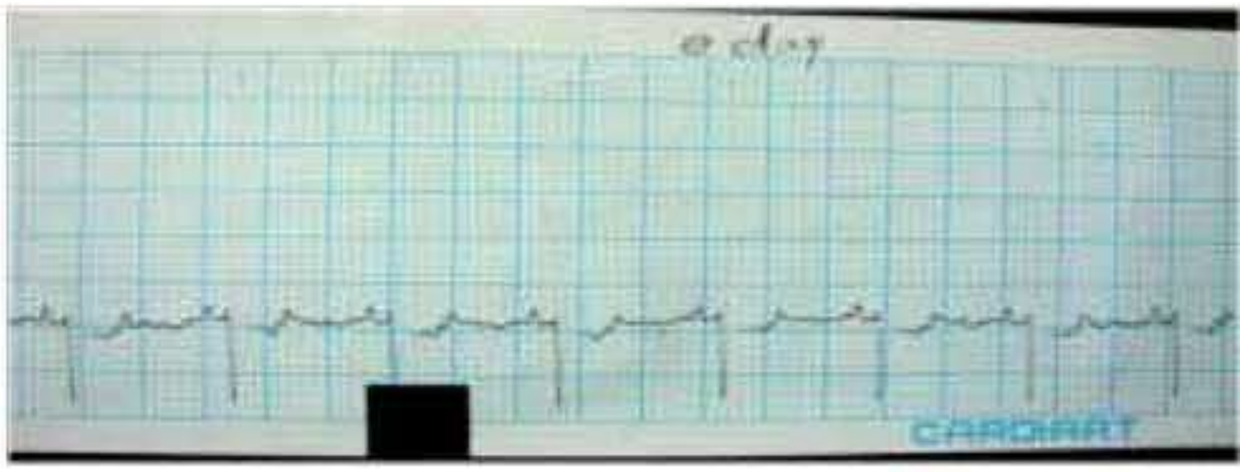

Fig. 3 : Tall QRS complex - Tall QRS complexes. The R wave with amplitude more than 3 mV

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Peritoneal Fluid, Urine and Haemato-biochemical Changes in Clinical Cases of Urethral Obstruction in 48 Bullocks*

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ABSTRACT

Clinical cases of 48 bullocks suffering from urethral obstruction were included in the study. They were randomly divided into two groups, Group A consisted of 28 bullocks with intact bladder and Group B consisted of 20 bullocks with bladder rupture. As a line of treatment urethrotomy, urinary bladder repair and tube cystostomy were performed in all the animals of group A and B. The gross and biochemical changes in peritoneal fluid, urine and blood in bullocks suffering from urethral obstruction pre and post treatment were evaluated. Haematological, uroabdomen and peritoneal fluid changes in bullocks with bladder rupture were of diagnostic but not of prognostic value. Peritoneal urea nitrogen was more of diagnostic value in confirming rupture of bladder.

Key words: Bullocks, peritoneal fluid, obstructive urolithiasis, bladder rupture

Urolithiasis is defined as formation of stones anywhere in the urinary system (Emerick, 1988; Payne, 1989 and Radostits et al., 2000). Abdominocentesis is used as an aid in Veterinary practice to diagnose abdominal disorders (Anderson et al., 1994) and bladder rupture (Parrah et al., 2011). Peritoneal fluid analysis in adult cattle is useful to know the status of disease and assess the severity of lesions in the abdomen. Peritoneal fluid changes have been documented after ascites, abomasal displacement, metritis and peritonitis by Wilson et al. (1985). Abdominocentesis along with peritoneal fluid examination aids in the diagnosis of ruptured urinary bladder. Changes in the peritoneal fluid in calves with urinary obstruction have been described by Parrah et al. (2011). However, there is minimal literature with regard to detailed study of peritoneal fluid changes in the adult cattle suffering from urethral obstruction. Hence, the present study was under taken to record the changes in pH, urea nitrogen and creatinine in peritoneal fluid, urine pH, cells and specific gravity of urine and hemato biochemical changes in bullocks suffering from urethral obstruction.

MATERIALAND METHODS

The study was conducted in clinical cases of 48 adult bullocks suffering from urethral obstruction weighing upto 530 kg and aged between 3 to 16 years, referred to Veterinary College Hospital, Bidar and Hassan for treatment during the year 2012-2016. Clinical, physical, per rectal, urine, peritoneal fluid and hemato-biochemical examination were carried out in all the animals. Group A consisted of 28 bullocks with

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intact bladder and Group B consisted of 20 bullock with ruptured of bladder. The animals were evaluated pre operatively on Days 0 (before operation) and 30 after surgery in both the groups.

The bullocks were restrained in standing position. The site for abdominocentesis was prepared aseptically on the right ventral abdomen or left ventral abdomen 15 cm away and caudal to the umbilicus. Sterile disposable 5 ml syringe was used to aspirate the peritoneal fluid.

The urine sample was collected during intra operative procedure directly from the bladder and from the prepuce in Group A and B respectively. PVC or Foley's catheter was inserted into the urinary bladder for treatment of obstructive urolithiasis. Urine from the bladder was collected after free flow was established intra operatively. After free flow of urine was established through the tube cystostomy catheter 10 ml of urine was collected in sterile vial for urinalysis. Purse string suture was placed to retain the Foley's or PVC catheter inside the lumen of the bladder. Skin was sutured using nylon.

The values were analyzed by Statistical Analysis Software (SAS) (Matange et al., 2011) for calculating descriptive statistics i.e. mean and standard error (SE) of variable. General Linear Model (GLM) of SAS was used for two way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Peritoneal Fluid Analysis :

The quantity of peritoneal fluid collected was 0.8 ml to 5ml. Peritoneal fluid in Group A varied from straw colour to yellow and the quantity also was less, varying from 0.5 ml to 1.6 ml. In Group B, the colour varied from straw colour to pale yellow with blood tinge. Free flow of peritoneal fluid was observed in the bullocks with ruptured urinary bladder. Peritoneal fluid in normal bovines vary from straw colour to yellow (Radostits et al., 2000). Colour of peritoneal fluid collected varied from straw to pale yellow with blood tinge. Normal peritoneal fluid was crystal clear and watery and with urinary bladder rupture the peritoneal fluid was cloudy and clotted when kept open to the environment as also observed by Parrah et al. (2011) and Wilson et al. (1985) because of high content of fibrin.

Cattle have a low volume of peritoneal fluid and failure to obtain a sample is not considered to be abnormal (Wilson et al., 1985 and Radostits et al., 2000). To diagnose rupture of bladder, peritoneal fluid was examined for colour, turbidity, RBC count, Total Nucleated Cell Count, DLC, TP and albumin by Duncan et al. (1994). Peritoneal fluid estimation for urea nitrogen and creatinine was made in case of obstructive urolithiasis to diagnose uroperitoneum by Parrah et al. (2011). In adult normal cattle the quantity of peritoneal fluid was less (0.5 to 1.6 ml). In case of intact bladder where no seepage of urine existed as observed by Anderson et al. (1994). It was more difficult to perform abdominocentesis in ruminants compared to monogastric animals because of the presence of extensive omentum that blocked the needle as reported by Parrah et al. (2011). Free flow is usaully observed in case of excess accumulation of urine (40-60 litres) into the peritoneal cavity by ruptured bladder (Parrah et al., 2011).

Peritoneal fluid estimation for urea nitrogen (mg/dl) in Group A was 22.75±0.19 before treatment and 16.08±0.09 on Day 30 after surgical treatment. Whereas, in Group B, it was 239.25±10.59 mg/dL before treatment and 12.45±0.04 on Day 30 after surgical treatment. There was a significant difference ($P \leq 0.05$) between the groups on Day 0 (before treatment).

The peritoneal fluid urea nitrogen values were significantly higher in ruptured urinary bladder bullocks than normal intact bladder on the day before surgical treatment (Day 0). Similar findings were reported by Parrah et al. (2011).

Peritoneal creatinine (mg/dl) in Group A was 02.39±0.06 before treatment and1.36±0.03 on Day 30 after surgical treatment. Whereas, in Group B, it was 19.22±1.21 mg/dL before treatment and 1.53±0.50 on Day 30 after surgical treatment. There was a significant difference ($P \leq 0.05$) between the groups on Day 0. The peritoneal fluid creatinine values were significantly higher in the bullocks with ruptured urinary bladder belonging to Group B when compared to intact bladder Group A. Similar findings were recorded by Parrah et al. (2011) who mentioned that the ratio of the plasma creatinine to peritoneal fluid was 3.00 : 1

Urea, nitrogen and creatinine were high in urine as waste metablolites in the peritoneal fluid collected in the peritoneum resulting in higher values of urea nitrogen and creatinine. Creatinine has a larger molecule than urea causing slow diffusion into circulation, hence, the peritoneal fluid creatinine to serum creatinine ratio greater than 2:1 indicated uroperitoneum (Bohn and Callan, 2007). Sockett et al. (1986), Larson (1996) and Van Metre (2004) also recorded peritoneal fluid and serum creatinine ratio of greater than 2:1 in bullocks with ruptured bladder and considered it as of diagnostic importance in confirming uroperitoneum.

Urinalysis : Urine pH in Group A was 9.13±0.41 before treatment and 7.10±0.24 on Day 30 after surgical treatment. Whereas, in Group B, it was 9.44±0.20 before treatment and 7.08±0.07 on Day 30 after surgical treatment.

Preoperatively on Day 0 urine was alkaline with a mean urine pH of 9.13±0.41, although the values returned to normal (7.4 to 8.4) after surgical treatment on post operative Day 30. The dissolution of calculi was achieved by altering the pH of the urine as recommended by Dubey (2004). There was no significant difference between the groups. However the values differed significantly (Pd"0.05) within the groups at different intervals.

No marked changes in the colour and specific gravity of urine was observed during the post treatment period in both intact and bladder ruptured bullock Groups on Day 0 and Day 30.

Specific gravity of urine in Group A was 1.035±0.03 before treatment and 1.015±0.03 on Day 30 after surgical treatment. Whereas, in Group B, it was 1.048±0.08 before treatment and 1.018±0.04 on Day 30 after surgical treatment.

Mild numbers of RBCs were found in most of the bullocks in both the groups; however, the RBCs were recorded in the initial days after surgery. The appearance of the RBC may be due to damaged to the mucosa of the bladder by PVC catheter during movement of bullock.

Epithelial cells were present in the urine in most of the animals in the initial days of treatment and reduced significantly during the end of observation period. The presence of cells might be due to pathological changes developed due to accumulation of urine in the bladder (Dubey, 2004) and due to acidic nature of ammonium chloride (Kane et al., 1989).

Hematological Analysis :

In all the animals, haemoglobin concentration (Hb) and packed cell volume (PCV) were found slightly less within the normal physiological range before treatment on Day 0. Hemoglobin level improved significantly ($P \leq 0.05$) on Day 30 after treatment. There was no significant difference between the group. The mean values of total leukocyte count (TLC) were increased in all the animals in both the groups A and B before treatment on Day 0 which reduced significantly on Day 3 after treatment. The values returned to normal physiological range by 7- 30 days. There was no significant difference between the groups.

The changes in the Hb and packed cell volume indicated the extent of dehydration by way of haemoconcentration (Sockett et al., 1986).

The total leukocytic count (normal physiological range 4-12*10^3/µL) were higher than the normal range before treatment on 0 day which reduced significantly

to normal range after the treatment in both the groups. This decrease is attributed to the elimination of stress, pain and infection. Similar decrease in the values of TLC was reported by Pandey et al., (1986) in experimental buffalo calves after relieving obstruction. However, the total leukocytic count was within normal range on Day 1 after treatment in both the groups which gradually and significantly reduced over different intervals of the study period.

The base value of DLC indicated marked neutrophilia and lympocytopenia in all animals on the day of presentation. Similar findings were reported by Gera and Nigam (1981), who opined that these changes might be due to infection and stress inflicted on the affected animals. The improvement indicated recovery from the stress, pain, and infection as result of diversion of urine, administration of antibiotics and anti-inflammatory drugs.

Biochemical parameters

Serum urea nitrogen (mg/dl) in Group A was 17.17±1.42 before treatment and 14.08±0.49 on Day 30 after surgical treatment. Whereas, in Group B, it was 115.35±14.66 before treatment and 11.15±0.56 on Day 30 after surgical treatment. There was a significant difference ($P \leq 0.05$) between the groups on Day 0.

Serum creatinine (mg/dl) in Group A was 2.20±1.29 before treatment and 0.95±0.22 on Day 30 after surgical treatment. In Group B, it was 6.16±2.97 before treatment and1.03±0.21 on Day 30 after surgical treatment. There was a significant difference ($P \leq 0.05$) between the groups on day 0.

The serum urea nitrogen and creatinine levels were significantly higher than normal range on 0 day of observation in bullocks with ruptured bladder when compared to the intact bladder bullocks. Singh and Singh (1990) reported that the measurement of these parameters may not be of diagnostic or prognostic value since the alterations in the parameters may indicate pre renal azatomia without involvement of kidney. However, mentions that both lesion and the cause along with the duration of disease might indicate the prognosis more accurately (Divers et al., 1982). Massive fluid accumulation in the abdomen, following bladder rupture, acts as a reservoir. Therefore, much more increase in the urea nitrogen and creatinine concentration of abdominal fluid occurs than in plasma (Sockett et al., 1986). Thus, serves as a way of diagnosing rupture of bladder. Further, Sockett et al. (1986) considers the measurement of creatinine more reliable for diagnosis of uroperitoneum owing to the larger size of creatinine molecules leading to decrease in equilibration across the peritoneum.

Alanine amino tansferase and aspertate aminotransferase were not altered in the study period except higher levels of aspertate aminotransferase on the day after surgery. All the values returned to normal (Radostits et al., 2000) and were used to assess the effect of different treatment groups.

CONCLUSION

The gross and biochemical changes in peritoneal fluid, blood and urine in bullocks suffering from urethral obstruction before and after treatment was studied. Peritoneal fluid analysis along with per rectal examination was confirmative of bladder rupture. Lesion and the cause along with the duration of disease might indicate the prognosis more accurately. Haematological, uroabdomen and peritoneal fluid changes in bullocks with bladder rupture were of diagnostic but not of prognostic value. Peritoneal urea nitrogen was more of diagnostic value in confirming rupture of bladder.

Table 1 : Showing Mean±SE values of various parameters of peritoneal fluid , serum and urine in bullocks suffering from urethral obstruction before and after treatment.

	Peritoneal fluid			Serum		Urine	
	P- Urea Nitrogen (mg/dl)	P- CRT (mg/dl)	pH	Urea Nitrogen (mg/dl)	CRT (mg/dl)	pH	Sp. gravity
Intact Bladder- Group A							
Day 0	22.75±0.19^{a} A	02.39±0.06^{a} A	8.42±0.31^{a} A	17.17±1.42^{a}	2.20±1.29^{a} A	9.13±0.41^{a} A	1.035±0.03^{a} aA
Day 30	16.08±0.09^{aA} A	1.36±0.03^{aA}	7.24±0.11^{aA} A	14.08±0.49^{abA}	0.95±0.22^{aA} A	7.10±0.24^{aB} A	1.015±0.03^{aB} aB
Ruptured bladder- Group B							
Day 0	239.25±10.59^{bA}	19.22±1.21 bA	9.38±0.26^{b} A	115.35±14.66 bA	6.16±2.97^{b} A	9.44±0.20^{a} A	1.048±0.08 aA
Day 30	12.45±0.04^{a} B	1.53±0.50^{aB}	7.46±0.18^{a} B	11.15±0.56^{cbB}	1.03±0.21^{a} B	7.08±0.07^{a} B	1.018±0.04 aB

Superscript A,B differ significantly ($P \leq 0.05$) from 0 day within the group. Superscript a,b,c differ significantly ($P \leq 0.05$) between groups at corresponding intervals.

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Traumatic Pneumothorax in a Labrador Retriever: A Case Report

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ABSTRACT

A 2 years old, female, Labrador retriever was presented to Veterinary College Hospital, Bengaluru with a history of difficulty in breathing, unable to sleep and bark after it met with an automobile accident. On clinical examination animal was dull and depressed with rectal temperature of 103.2°F, dyspnoea and tachypnea. Electrocardiography revealed tachycardia (150 beats/min) and thoracic radiography revealed elevation of the heart off the sternum, collapse of lung lobes and retraction from the chest wall. Presence of radiolucent area of free air in which no pulmonary vascular structures were visible, suggestive of pneumothorax and the casewas managed successfully by thoracocentesis.

Key words : Traumatic pneumothorax and thoracocentesis

Pneumothorax is defined as an accumulation of air in the pleural space. Traumatic pneumothorax is the most common cause of pneumothorax in dogs. It can be open or closed (Ettinger and Feldman, 2005).

In open traumatic pneumothorax there will be a direct communication between the pleural space and the atmosphere via a thoracic wall wound, which usually occurs as a result of penetrating trauma of the thoracic wall, such as a bite or in case of rib fracture with perforation of the thoracic wall (Puerto et al., 2002) Closed traumatic pneumothorax occurs as a result of blunt trauma (e.g., automobile accident) When the chest is compressed against a closed glottis, the bronchial tree or lung parenchyma can rupture with resultant air leakage into the pleural space (Fossum, 2002). Any pneumothorax is usually bilateral in dogs because they have a delicate and usually fenestrated mediastinum.

CASE HISTORY AND OBSERVATIONS

A 2 years old, female, Labrador retriever, weighing 16Kg was presented to Veterinary College Hospital, Hebbal, with a history of difficulty in breathing, unable to sleep and bark after it met with an automobile accident.

On physical examination animal was dull with open mouth breathing (Fig. 1) (abdominal respiration) and tachypnea. Conjunctival mucous membrane was congested and rectal temperature was 103.2°F. Electrocardiography revealed tachycardia (Fig. 2) and hematobiochemical findings were within normal range except there was a mild leucocytosis (22000 cells/cumm).

Radiography revealed elevation of the heart off the sternum, collapse of lung lobes and retraction from the chest wall. Presence of radiolucent area of free air in which no pulmonary vascular structures were visible in the caudal thorax (Fig. 3). Based on history of trauma, clinical signs, electrocardiography and radiography it was diagnosed as traumatic pneumothorax

TREATMENT

Dog was stabilised by removing air (400 ml) by bilateral thoracocentesis in lateral recumbancy at 7th to 9th intercostal space in the dorsal one third of thoracic cavity using 21 gauze two way canula. Treated with inj. meloxicam at 0.2 mg subcutaneously and antibiotics. (Tab. Cephalexin 250mg) b.i.d. for 3 days and animal completely recovered after one week.

The automobile accident had resulted in pneumothorax and acute onset of dyspnoea. The signs were acute in traumatic pneumothorax and the cause was obvious from the history. Similar clinical signs were reported by Deepa (2016).

In animals with closed traumatic pneumothorax, thoracocentesis can be curative, and recurrence is uncommon (Ludwig, 2000; Crisp, 2000). Thoracocentesis should restore negative pressure within the thoracic cavity. Traumatic pneumothorax is most commonly managed conservatively and surgical intervention is rarely necessary. Thoracocentesis can be used to manage dyspnoea while pulmonary lesions heal usually by 3-5 days (Ettiger and Feldman, 2005). In the present case, animal recovered with medical management without surgical intervention.

CONCLUSION

Surgery is rarely needed to correct traumatic pneumothorax. Thoracocentesis is usually sufficient to allow pulmonary healing in 3 to 5 days. The prognosis for animals with traumatic pneumothorax is considered excellent, if there are no other life threatening injuries.

Fig. 1 : Dog suffering from traumatic pneumothorax with open mouth breathing

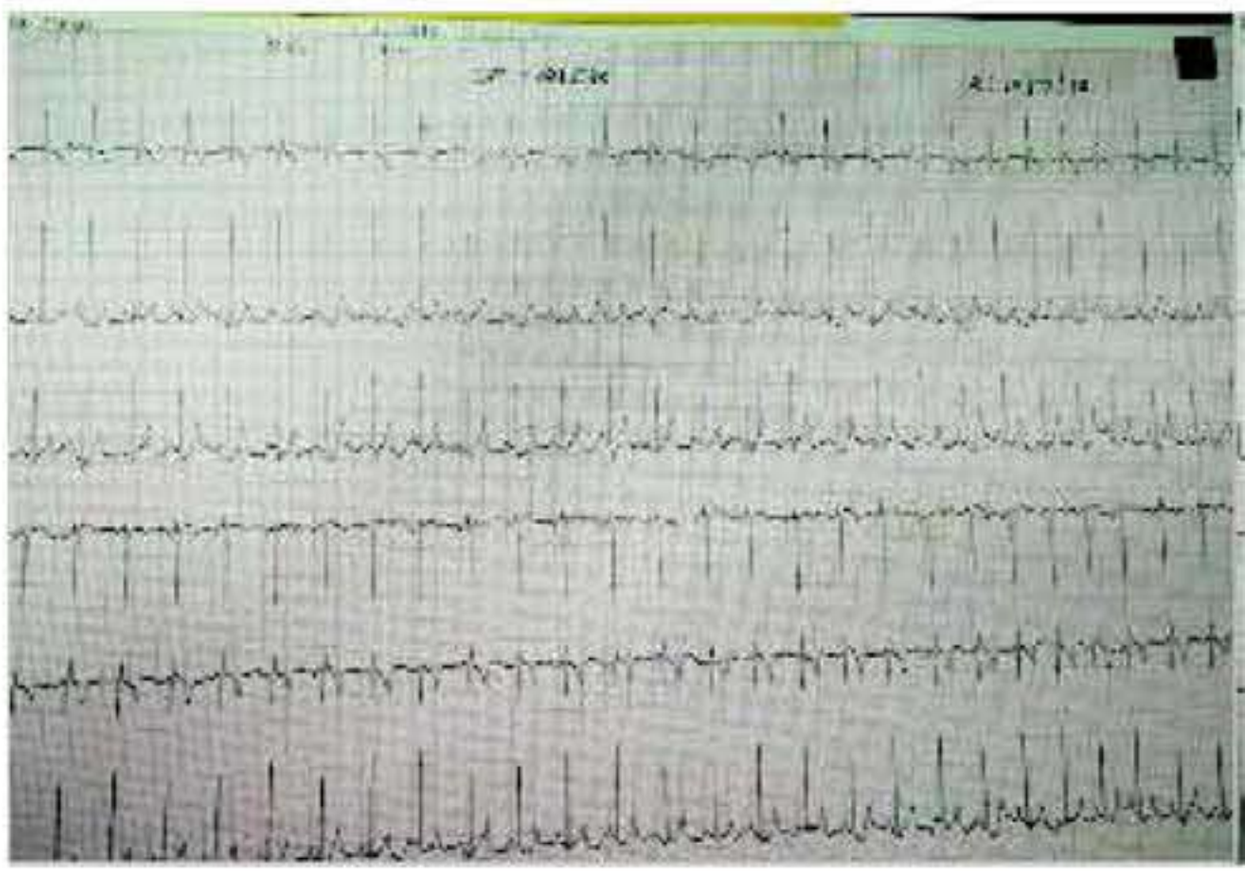

Fig. 2 : Electrocardiogram showing tachycardia (Lead II)

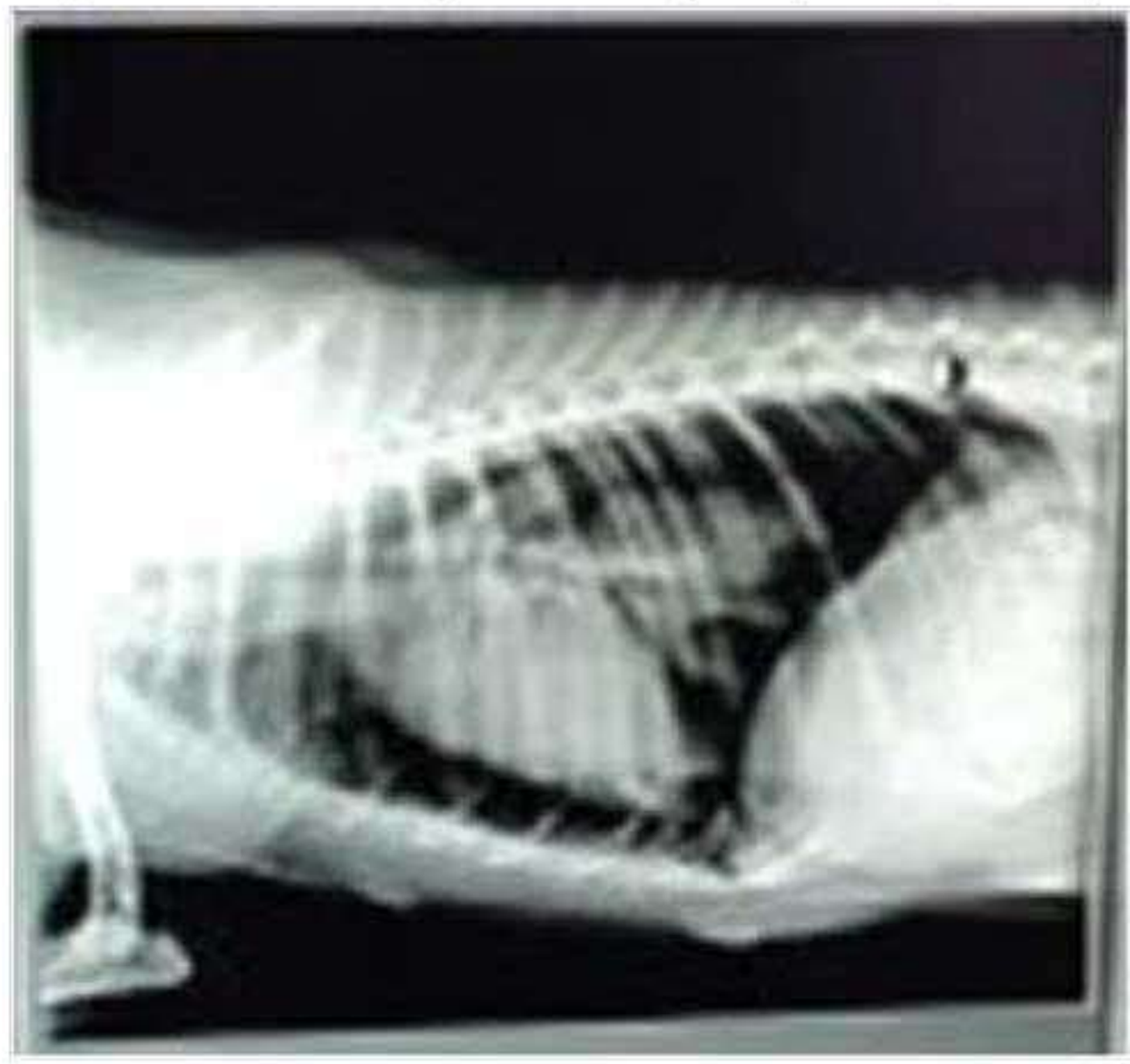

Fig. 3 : Lateral thoracic radiography showing pneumothorax

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Prevalence and Pathological Features of Whitespot Disease of Fish in and around Bangalore*

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ABSTRACT

Ichthyophthirius multifiliis is one of the most important ciliated protozoan parasites of freshwater fish and has been frequently reported from the different fish species from India and other parts of the world. The parasite is regularly seen on the skin and gills. The lesions are more seen in the gills and may result in death due to disturbance in the osmoregulation. The current study was done during January to August of 2015 as a part of study on the occurrence and pathomorphological changes in fish diseases in and around Bengaluru. A total of 311 fish were examined in the Department of Veterinary Pathology, Veterinary College Bengaluru of which 74 (23.93%) were positive. Affected fish showed pin head white spots scattered all over the body and gills. Histological sections showed that the trophonts were found encysted in gills and skin covering the fins. Microscopic features of the lesions are also explained.

Key words: Ich, Ichthyophthirius multifiliis and Trophonts.

INTRODUCTION

Ich, also known as white spot disease is one of the most common protozoan parasitic diseases of freshwater fish caused by protozoa Ichthyophthirius multifiliis (Lom and Dykova, 1992, Noga, 2010, Donna, 2011 and Roberts, 2012). In 1876, Forquet reported for the first time, the fresh water ciliate Ichthyophthirius multifiliis in France which caused problems due to rapid multiplication in trout ponds during the warmer season.

All fresh water fishes are susceptible to the infection, however the scale less fishes such as catfish and loaches are highly vulnerable with mortality up to 100 per cent (Meyer, 1974 and Dickerson, 2006). Itch is commonly observed in fish that live in warm water and outbreak of Ich commonly occurs at 15-25°C (Noga, 2010). The life cycle of I. multifiliis is divided into three distinct stages. The trophont resides and feeds on the epidermis of the host, where it can grow to a diameter of up to 1 mm and the mature trophont escapes from the epidermis to the surroundings, where some of the parasites settle and develop into encysted tomonts. In the tomont cystic stage numerous daughter cells known as theronts are produced. The number of theronts resulting from one tomont varies between 50 and few thousands (Li and Buchmann, 2001). Theronts get access to epidermis by invading mucosal cells and invade and exert pathogenic effect and elicit the condition Ichthyophthiriosis or white spot disease (Lom and Cerkosova, 1974; Hass et al., 1999; Buchmann and Neilson, 1999 and Buchmann et al., 2001).

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In this background, the present work was undertaken to study the occurrence and pathomorphological changes of white spot disease in and around Bengaluru.

MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru during 2014-15 to record the occurrence white spot disease / Ich in fresh water edible and ornamental fish in and around Bengaluru. A total of eight lakes located at Hebbala, Madivala, Nagavara, Hulimavu, Hessaraghatta, Yelahanka, Rachenahalli and Sadaramangala and three aquaculture farms including Main Research Station (MRS) farm, Fisheries Regional Investigation Centre (FRIC), Hebbala and FRIC, Hesaraghatta and two wholesale and five local aquarium shops in and around Bengaluru were visited regularly to collect ailing and freshly dead fish for examination. A total of 101 fresh water edible and 210 aquarium fish formed the source of material for the current study. Ailing fish were collected in aerated polythene bags with water and brought to the laboratory for further examination. The skin and fins of fish were examined for the ulcers and lesions and presence of white spots and symptoms of the disease. Gills were removed and placed in petri dish containing distilled water and examined for the presence of I. multifiliis. Parasite quantification was performed directly on wet mount of fins and gills under microscope. The identification of parasites was made as per Elsayed et al. (2006) and Osman et al. (2009). Methods used for collection and fixing the parasitic specimens were as per standard protocols (Lom and Dykova, 1992). In addition, the water quality parameters were also analysed in specific association with Ich occurrence, as per specified guidelines.

RESULTS AND DISCUSSION

A total of 311 fish samples were examined to record occurence of Ich. White spot disease caused by I. multifiliis, was found to be the most common parasitism, affecting 74 fish (Table 1). Earlier reports have suggested that white spot disease is one of the most commonly occurring protozoan diseases accounting for significant economic losses to the aquaculture industry (Mathews, 1994; Noga, 2010 and Roberts, 2012). This condition was more commonly observed among ornamental fish, especially in Gold fish; followed by fresh water shark and common carp (Table 1). Higher occurrence of Ich among Gold fish was suggested to be due to poor water quality, overcrowding in aquarium, improper diet and sudden temperature changes that predispose them to Ich (Noga, 2000; Ostrow, 2003 and Raissy and Ansari, 2011), In the present study, the water quality examination showed higher alkalinity, ammonia and carbon dioxide mean concentrations than normal prescribed values. Although earlier studies indicated Ich infestation among fresh water fish, it was found to be not significant in the present study.

Clinically, affected fish showed numerous white, slightly raised spots throughout the body, especially on the tail fin (Fig. 1and 2). The skin surface was slimy with loss of bright skin colour. In fresh water shark, there was darkening of skin with increased mucus production. These could be attributed to the extensive changes that occur in the integument tissue and gills when the trophonts feed and encyst viz. destruction of adjacent epithelial cells, dilatation of blood vessels, haemorrhages and infiltration of inflammatory cells with proliferative changes (Noga, 2010; Roberts, 2012). Frayed out fins, surface swimming, upside down positioning and rapid mouth water pumping were also observed. These findings were in agreement with Kabata (1985) who stated that, the proliferative and degenerative processes that occur in the skin and gills disrupt the normal ionic exchange, affecting normal excretion and osmoregulation.

The wet mount preparations from the affected skin and gills revealed numerous trophonts, which are the feeding and growing stages of the I. multifiliis. They were seen actively moving, by rolling and rotating

on the scales, epidermis and gills. The trophonts varied in their size and measured 0.16 mm to 0.27 mm in diameter. The trophonts were spherical to oval in shape and were covered by short cilia throughout the surface. They contained within, a single horse shoe shaped macronucleus and a single round or oval micronucleus. The cytoplasm was dark and granular (Figs. 3 and 4).

Microscopically, the trophonts were found encysted in gills and skin covering the fins. Within the fins, the trophonts were either single or multiple with more than one trophont encysted together. In gills, the trophonts occurred singly, however occasionally, more than one trophont were also observed. The encysted trophonts appeared large and elongated with clearly appreciable macronucleus and micronucleus. In gills, most of the trophonts were found encysted at the tip of the primary lamellae (Fig. 5). The cytoplasm of the trophonts appeared eosinophilic and granular. Degenerated trophonts were also observed in both gills and skin which appeared as a mass of eosinophilic granular debris with absence of outer most layers of cilia and infiltration of inflammatory cells around (Fig. 8). The encysted trophonts were covered completely by varying thickness of hyperplastic lamellar epithelial cell layers which appeared flattened due to pressure atrophy. Goblet cell hyperplasia was observed at the base and tips of secondary lamellae and also in the areas adjacent to encystment of trophonts. Lamellar fusion was also observed. The lamellae at the site of localization of trophonts were distended and appeared bulged out. Majority of the primary lamellae revealed shortening, clubbing, thickening and loss of secondary lamellae. In addition, some of the lamellae showed congestion of central vein, secondary lamellar capillaries, desquamation of lining cells and presence of clumps of granular melanin pigments.

In the fins, the trophonts were surrounded by many layers of malphigian cells. The trophonts were located at different levels in the spinous fin. However, they were more concentrated at the free end where more space was available. Small sized newly formed trophonts were also observed encysted in the wall. The interspinous epithelium was widened and hyperplastic and showed numerous fright sense cells. The number of mucus producing cells also increased. Around the degenerating trophonts infiltration of granular cells was observed. Also, within the trophonts erythrocytes and other cell debris were appreciable (Fig. 6). In Ich affected fish, the microscopical changes observed in the liver included congestion and diffuse vacuolar degeneration, congestion, degeneration and necrosis of tubular epithelial cells in kidney and congestion in heart (Fig. 10). These microscopic findings were in accordance with earlier observations (Tumbol et al., 2001, Yu et al., 2012 and Roberts, 2012). The tissue damage caused by the parasite can also lead to altered osmoregulatory process and ion regulation and can serve as portal of entry for secondary bacterial or fungal infection, eventually leading to mass mortality (Raissy and Ansari, 2011; Yu et al., 2012).

The values of various water quality parameters observed in white spot disease/Ich condition were shown in the Table 3. These included temperature range of 24.3 to 25.8 ^{o}C, pH of 6.90± 0.29, hardness of 126 ±3.67, carbon dioxide of 15.2 ± 1.47 mg/L, alkalinity of 200 ± 19.36 mg/L, nitrate of 0.36 ±0.12 mg/L phosphate 0.33± 0.12 of mg/L, ammonia of 0.16± 0.02 mg/L, dissolved oxygen of 4.8 ± 0.35 mg/L and hydrogen sulphide 0 mg/L. While the mean values of alkalinity, ammonia and carbon dioxide concentrations were higher than the normal, the temperature, hardness, nitrate, phosphate and dissolved oxygen of water were found within the normal range. Since, various physicochemical factors such as temperature, pH, alkalinity etc. have strong influence on fish health and their resistance against the disease causing agents (Plumb et al., 1988 and Shresta, 1994), such an altered aquatic environment might have predisposed the fish to infection. Nearly, same observations have also been made by Hossain et al. (2007) with respect to white spot diseases occurrence. Temperature ranging between 23-26 ^{o}C, might have favoured the I. Multifiliis infection, as the outbreaks usually occurs at 15-25 ^{o}C (59-77 ^{o}F) (Noga, 2010).

CONCLUSION

White spot disease/Ich caused by I. multifiliis, was found to be the most common parasitism among fresh water ornamental fish. Water quality parameters such as alkalinity, ammonia and carbon dioxide play important role in the occurrence of the disease.

Table 1 : Total number of freshwater edible and ornamental fish examined

Freshwater edible fish	Numberof samples	Ornamental fish	Number of samples
Rohu	37	Gold fish	67
Silver Carp	9	Red kodango	1
Common Carp	17	Molly	14
Mrigal	1	Angel fish	3
Murrel	4	Eel	3
Tilapia	33	Flower horn	1
Total	101	Fresh water shark	14
		Pot belly	1
		Koi carp	2
		Red sword tail	1
		Black molly	1
		Guppy	50
		Silver dollar	2
		Gourami	20
		combined fish	30
		Total	210

Table 2 : Prevalence of Ich in freshwater fish in and around Bengaluru

Disease	Type of fish	Number of fish affected	Total number of fish examined	Per cent
Ich	Gold fish	60	311	19.29
	Freshwater shark	12		4
	Common carp	2		0.64
Total		72		23.93

Table 3 : Water quality and occurrence of Ich / White spot disease in Freshwater fish

Water Sampling/ Water Quality	Temperature °C	Ph	Hardness	Co_2 (Mg/ L)	Alkalinity (Mg/L)	Nitrate (Mg/L)	Phosphate (Mg/L)	Ammonia (Mg/L)	Do (Mg/ L)	H_2s (Mg/L)
Normal Values	24-30	6.5-9	50-150	<10	50-150	0.005-0.5	0.005-0.5	0.1	>4	0.002
S 1	25 - 26.5	6.5	135	12	225	>1	0	0.1	4	Nil
S 2	25-26	7.5	120	16	225	0.5	0.5	o.1	5.6	Nil
S 3	24.5-26	7	135	16	125	>1	0	0.2	4	Nil
S 4	23-24.5	6	120	20	200	0.1	0	0.2	5.6	Nil
S 5	24-26	7.5	120	12	225	0.5	0.5	o.1	4.8	Nil
MEAN	24.3- 25.8	6.9	126	15.2	200	0.36	0.33	0.16	4.8	Nil

Fig. 1. Gross picture showing numerous white spots on the tail fin of gold fish in white spot disease.

Fig. 2. Gross picture showing tiny raised white spots scattered on the body in fresh water shark.

Fig. 3. Picture showing trophonts of Ichthyophtheriusmulti feliis in wet mounts preparation. X 40

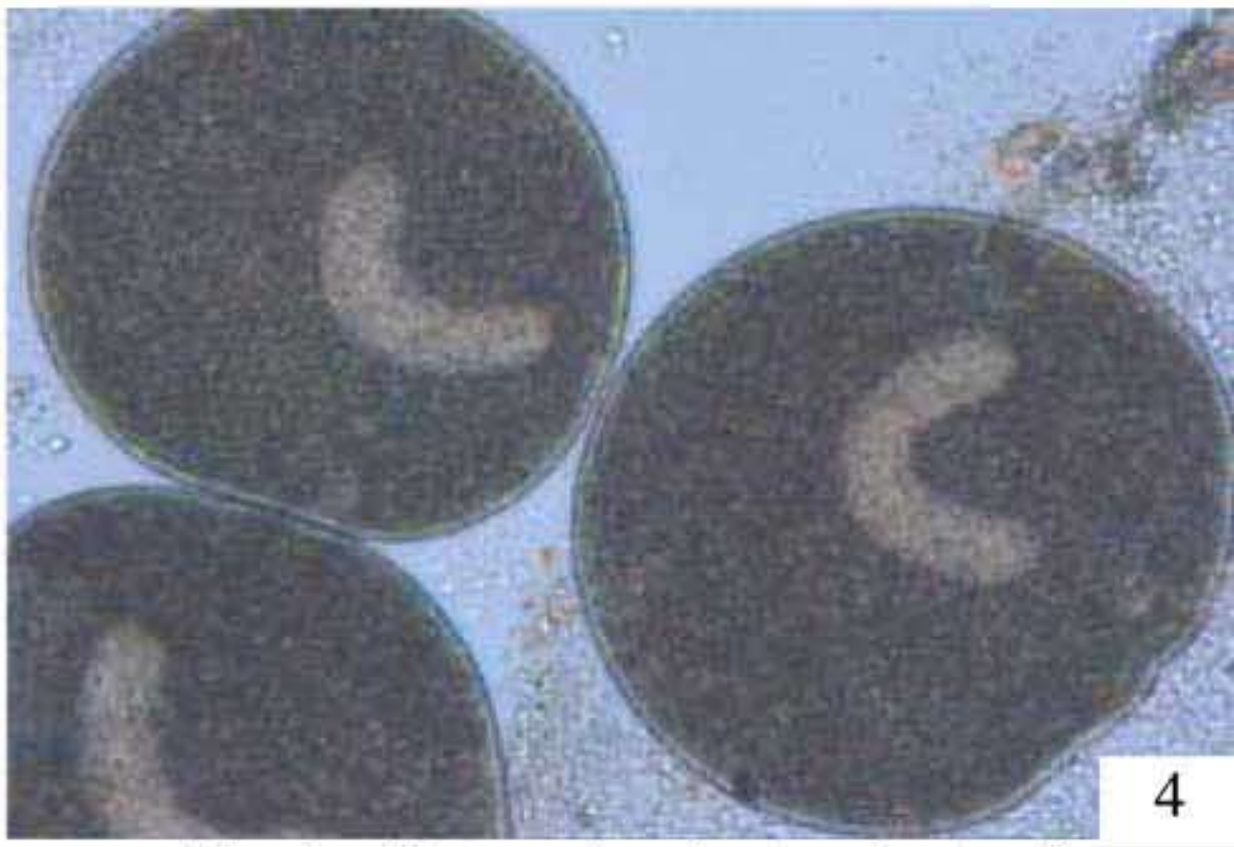

Fig. 4. Picture showing trophonts of Ichthyophtheriusmulti feliis in wet mounts preparation. Note, horse shaped macronucleus. X 40

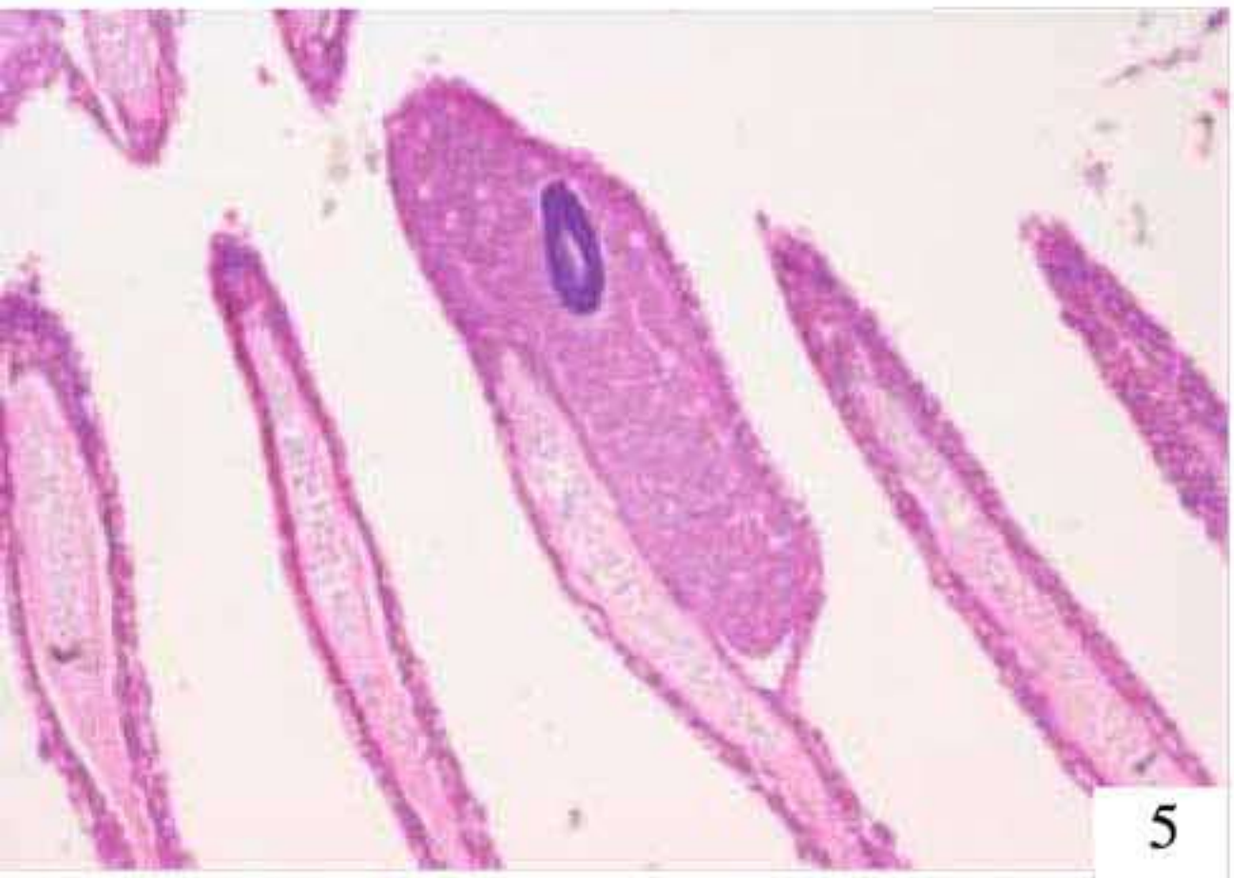

Fig. 5. Section of gills showing encysted trophont at the tip a filament in Ich diseases H&E X 100

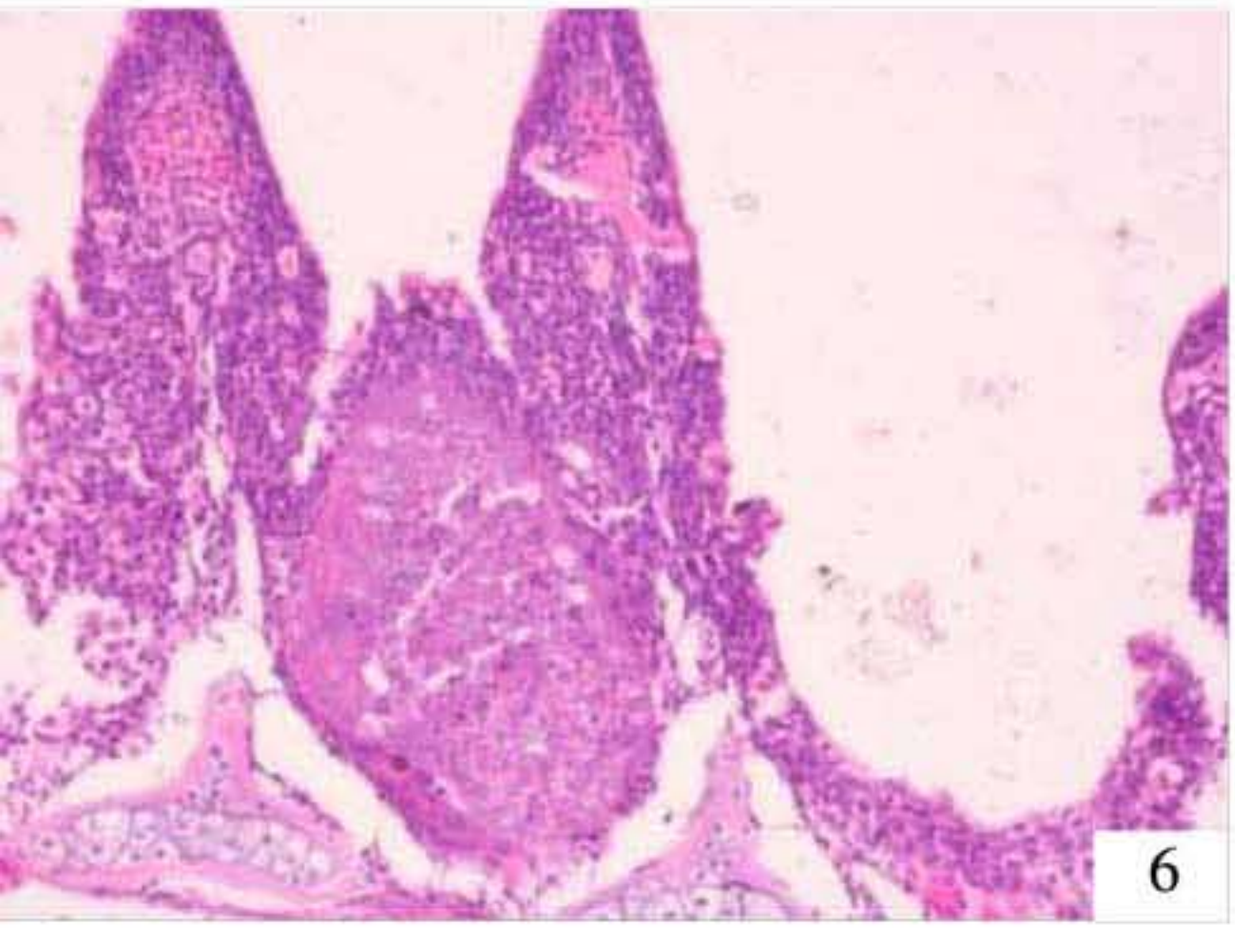

Fig. 6. Section of gills showing a degenerating trophont at the base of the filament in Ich disease. H&E X 100

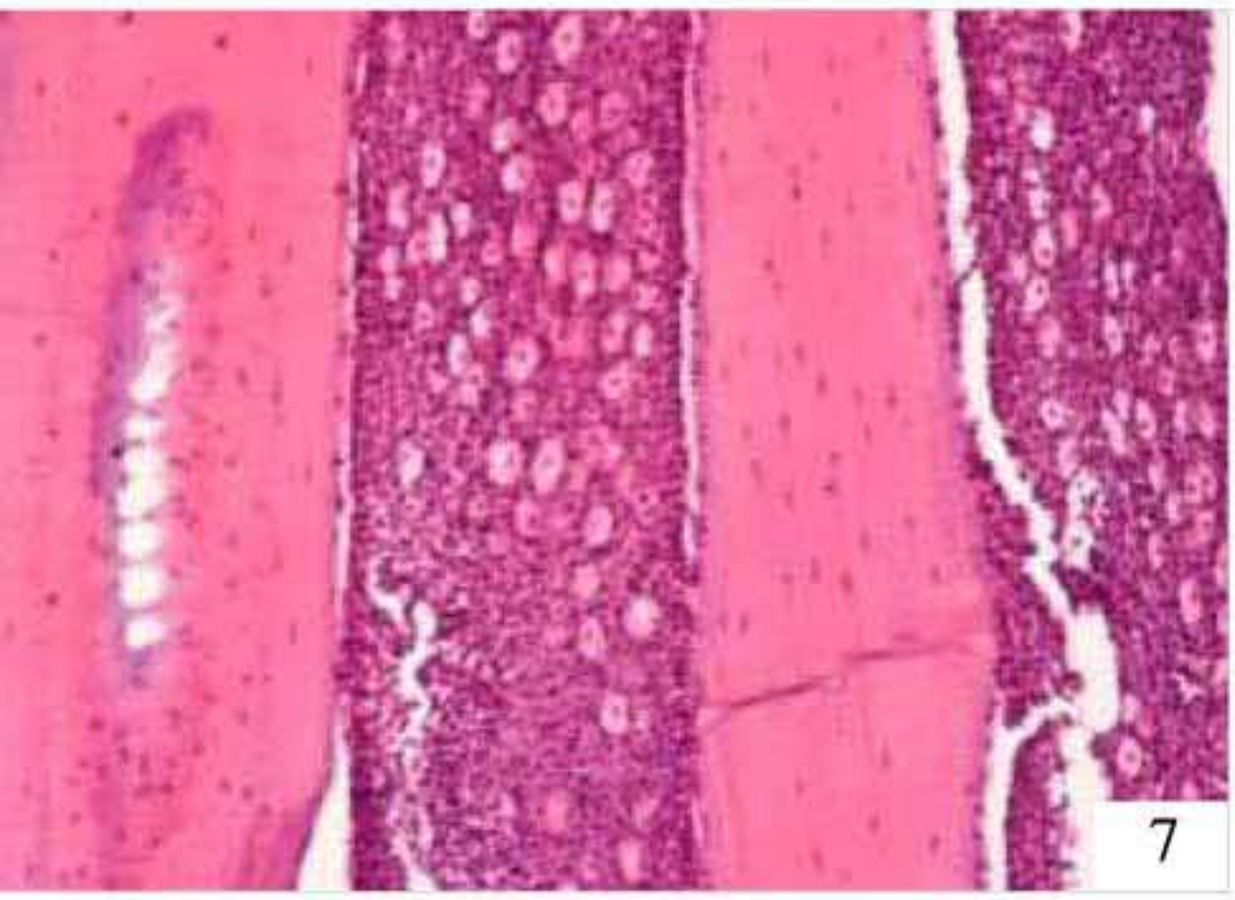

Fig. 7. Section of fin showing interspinous epithelial hyperplasia in Ich disease. H&E X 1000

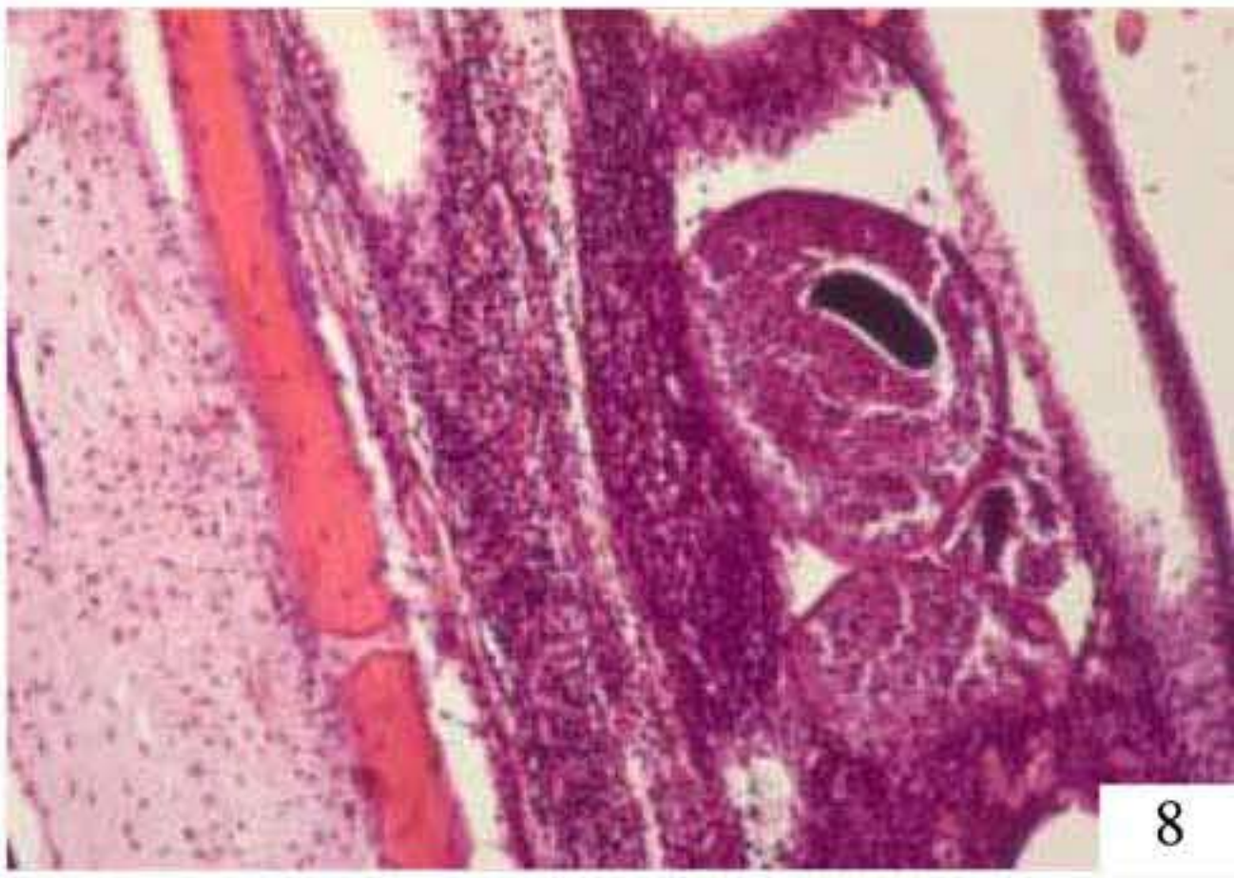

Fig. 8. Section of the fin showing encysted trophonts with epithelial hyperplasia around in white spot disease. H&E X 100

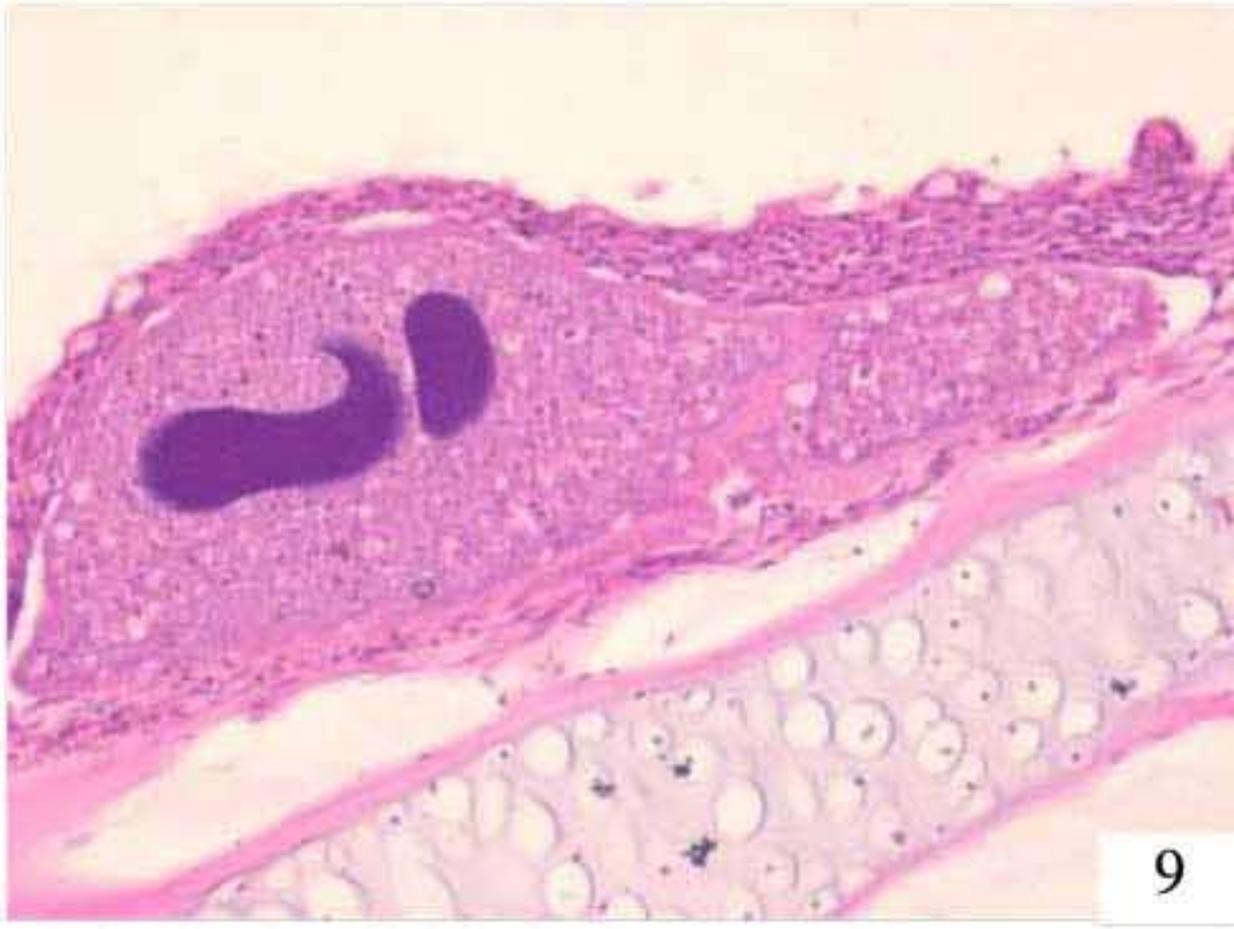

Fig. 9. Section of gills showing encysted trophont covered with layers of hyperplastic epithelial cells in Ich disease. Note, the macro and micronucleus of the same.H&E X 100

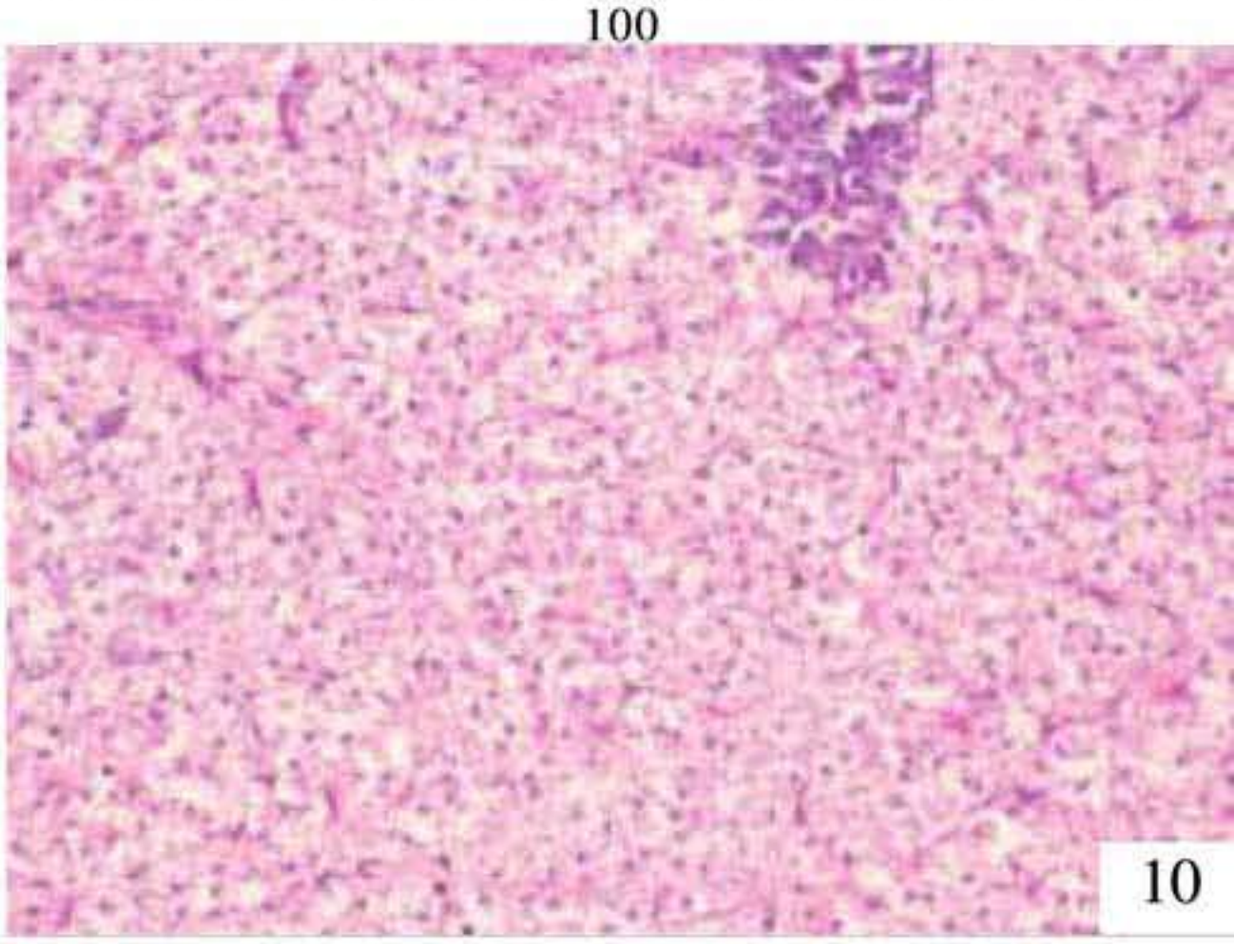

Fig. 10. Section of the liver in white spot disease showing diffuse vacuolar degeneration H&E X 100

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