Introduction
Rotaviruses are major causes of acute diarrhoeal diseases in newborn animals and humans (Saif et al., 1994; Dhama et al., 2009). Results of virus infection range from subclinical infection to death. Rotaviral diarrheas are common in calves, and affected young calves may die as a result of severe dehydration or secondary bacterial infections (Holland, 1990; Saif and Theil, 1990; Chauhan and Singh, 1996). Rotavirus belongs to the family Reoviridae, genus Rotavirus and is non-enveloped, double stranded RNA virus, with a diameter of 65-70 nm and have icosahedral core from which the 32 capsomers radiate, giving them a wheel like appearance. There are 11 segments of double stranded RNA (16-21 kbp) surrounded by an inner and outer capsid layer (Murphy et al., 1999; Desselberger et al., 2005). Rotaviruses are classified into groups, subgroups and serotypes (Mathews, 1979; Dhama et al., 2009). The immune response to rotaviruses is serotype specific. The group and subgroup specificity was confirmed by VP6 protein, which differentiated the rotavirus into 7 groups (A-G) and into two subgroups (I-II) (Steele et al., 2004; Desselberger et al., 2005). Group A Rotaviruses comprised of important pathogens of human beings, cattle, and other animals (Mathews, 1979). Group B rarely affected calves, lambs, piglets and human beings. Group C might affect swine and occasionally humans. Group D, F and G could affect poultry and Group E might affect swine (Steele et al., 2004; Villarreal et al., 2006). However, Group A rotaviruses are the major cause of rotaviral infections in domestic animals, even though atypical rotaviruses (belonging to other groups) had also been isolated in some instances (Steele et al., 2004; Ghosh et al., 2007).

Several methods used for rotavirus diagnosis, plaque reduction neutralization test, ELISA, nucleic acid hybridization, electron microscopy (EM), and immune electron microscopy (IEM), possess limited sensitivity (Hoshino et al., 1984; Xu et al., 1990; Parwani et al., 1993; Gouvea 1994; Lucchelli et al., 1994; Fedorova et al., 2005). The reverse transcriptase-polymerase chain reaction (RT-PCR) offers many advantages besides high sensitivity and specificity in detection of rotavirus in faecal samples (Kang et al., 2004; Fedorova et al., 2005). It helps in the detection of viral nucleic acid during initial stages of infection without waiting for higher virus titer and development of immune response in the affected host species. Detection of rotavirus infection in

DETECTION OF GROUP A BOVINE ROTAVIRUS IN DIARRHOEIC CALVES BY REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) AND ELECTROPHEROTYPING

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ABSTRACT

In the present study, reverse transcription polymerase chain reaction (RT-PCR) was standardized and applied for detection of bovine rotavirus A (BRA) in diarrhoeic clinical samples (n=112) collected from dairy farms of Bareilly (Uttar Pradesh) and Nammakkal (Tamil Nadu) regions, India. The presence of virus was also confirmed by electropherotyping analysis. For RT-PCR, two pairs of published primers were used for amplification of VP6 and VP7 gene of group A rotavirus. BRA RNA extracted from faecal samples and infected MDBK cells by TRIzol LS method was used for RT-PCR detection of the virus and electropherotyping. RT-PCR amplification yielded virus specific amplicons of 309 bp and 304 bp sizes for VP6 and VP7 gene, respectively. Out a total of 112 clinical samples rotavirus RNA was detected in 27 (24.11%) samples. Electrophoretic analysis of rotavirus dsRNA segments yielded 4-2-3-2 long electrophoretic segmental pattern for bovine rotavirus group A. The molecular tool of RT-PCR and electropherotyping were found suitable for detection of bovine rotavirus group A and could be well applied for rapid detection of rotaviruses in clinical/field samples.

Key words: Bovine rotavirus, reverse transcription-PCR, electropherotyping

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reservoir animals and symptomless carriers is another advantage of RT-PCR. The aim of present study was the standardization and application of RT-PCR using VP6 and VP7 genes based detection of group A bovine rotavirus in diarrhoeic calves and electrophoretotyping analysis.

Materials and Methods

Collection of faecal samples from diarrhoeic calves

Faecal samples were collected from 112 calves (103-clinical case and 9-dead cases) evincing symptoms of diarrhoea from organized dairy farms of Namakkal (Tamil Nadu) and Military dairy farm, Bareilly and post-mortem room of Indian Veterinary Research Institute (IVRI), Izatnagar (Uttar Pradesh). All the clinical samples under test were suspended in 10% (W/V) phosphate buffered saline (PBS), solution (pH 7.2), and used as inoculums for adaptation on MDBK cell line, giving five passages in cell cultures. On observation of characteristic cytopathic effect (CPE), the cells were harvested by freezing and thawing thrice and centrifuged at 12,000 rpm for 20 min at 4°C for the removal of cell debris. The cell culture supernatants were collected and stored at -20°C till tested. All the 112 faecal samples and respective infected cell cultures were subjected to RNA extraction and detection of rotavirus by RT-PCR. Also electrophoretotyping analysis was performed to confirm the presence of bovine rotavirus A.

RT-PCR

For the standardization of RT-PCR, two published primers specific for VP6 and VP7 genes of group A bovine rotavirus were used viz., Rot3 F- 5‘AAAGATGCTAGGGACAAAAATTG3’ (nt 57-78) and Rot5 R-5’ TTGACATTGAGCTTATCC3’ (nt 344-365) for the VP6 gene (Song et al., 2006); and F-5’GATCGAATGTTGTAATCC A3’ (nt 531-550) and R-5’ AAT TCG CTA CGT TTT CTCTTG G3’ (nt 824-808) for the VP7 gene of group A rotavirus (Husain et al., 1995). Viral RNA was extracted with TRIzol LS reagent (Invitrogen, USA) as per the manufacturer’s instructions. All the 112 diarrhoeic clinical samples as well as infected cell culture fluid were used for RNA extraction. The extracted RNA pellets were air dried for 15 min and mixed with 30 µl of nuclease free water and stored at -70°C until further use. The purity of RNA was checked by measuring the OD value at 260 nm wavelengths. The extracted RNA was used to synthesize cDNA using random hexamer primers. Reverse transcription (RT) for first strand synthesis was carried out using Revert aid H minus - Moloney Murine Leukemia Virus Reverse Transcriptase (MMuLV-RT) (MBI Fermentas, USA) in a standard 20 µl reaction mixture. First, 5.0 µl of RNA template, 1.0 µl of Random Hexamer Primer (0.2 µg/µl) and 5.0 µl of nuclease free water, were added in sterile nuclease free 0.2 ml PCR tube. The mixture was incubated at 70°C for 5 min and immediately snap chilled on ice. After that, 4.0 µl of 5X RT buffer, 2.0µl of dNTP mix (10 mM each), 0.5 µl of RNAse inhibitor (Recombinant-Ribonuclease-inhibitor, 40 U/µl) and 0.1 µl of MMuLV-RT enzyme (200 U/µl) were added. The mixture was incubated at 25°C for 5 min; snap chilled on ice and RT enzyme was added. The RNA was subsequently reverse-transcribed at 25°C at 10 min, 42°C for 60 min, followed by 10 min at 70°C to denature the enzyme. The cDNA samples were then cooled at 4°C and stored at -20°C.

PCR reaction was carried out in 25 µl volume containing 2 µl cDNA, 25 pmol of each primer, 5 µl of PCR mixture consisting of 2.5 µl 10x PCR buffer, 0.5 µl 10 mM dNTPs, 1.5 µl 25 mM MgCl2 and 0.5 µl Taq polymerase (5 U/µl, MBI Fermentas). The amplification of gene segment 9 (VP7) was done with an initial denaturation at 95°C for 5 min; followed by 30 cycles of denaturation, annealing and extension at 94°C for 1 min; 55°C for 1 min; 72°C for 1 min, respectively. The final extension was done at 72°C for 10 min. The amplification was carried out using the thermocycler (PTC 200, MJ Research, USA). Keeping reaction mixture same as mentioned for VP7 gene, the amplification of gene segment 6 (VP6) was done with an initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation, annealing and extension at 94°C for 1 min; 52°C for 1 min; 72°C for 1 min, respectively. The final extension was done at 72°C for 10 min, and the amplification was carried out using the thermocycler. The electrophoresis was carried out in 1x Tris borate buffer (TBE) and current was applied at the rate of 12 V/cm of gels. PCR products were visualized on an ethidium bromide-stained 1.5% agarose gel along with a molecular weight marker (100 BP DNA Ladder plus).

Electrophoretotyping

The presence of rotaviral RNA was also checked by agar gel electrophoresis. A slight modification of horizontal electrophoresis in a 5 mm thickness of 1% agarose was used as described by Psikal et al. (1991). The agarose gel was prepared by boiling agarose in 0.5x Tris Boric Acid EDTA (TBE) buffer, pH 8.2 and allowed to cool for 5 min to about 50°C, and ethidium bromide added (0.5 µg/ml). Approximately 30 ml of the liquid gel was poured into gel cast/mold (8.5 × 8 cm), starts/wells formed by combing, and placed in a horizontal gel electrophoresis apparatus containing 300 ml of 0.5 X TBE buffer. Ten µl of sample mixture (5 µl of RNA+ 2 µl of loading buffer (6x) + 3 µl of nuclease free water) was added to the wells, and electrophoresis carried out at 80 V for 3 hr. Then the gel was observed under ultra violet (UV) transilluminator to visualize
the amplified PCR products, and photographed.

**Results and Discussion**

RT-PCR was standardized and applied for the detection of bovine rotavirus genomic RNA in the 112 field/clinical samples collected from diarrheic calves from Tamil Nadu and Bareilly regions of India. Of these, a total of 27 (24.11%) diarrheic samples were found to be positive for rotavirus RNA. RT-PCR amplification yielded rotavirus specific products of 304 bp and 309 bp for VP7 and VP6 viral genes, respectively (Fig. 1, 2, 3 and 4). However, no amplification was observed in negative controls using the same set of amplification conditions indicating the specificity of the primers. Simultaneously, the standardized RT-PCR techniques when applied for detection of the presence of rotavirus in infected MDBK cell lines revealed the adaptation of rotaviruses in the cell cultures and along with CPE observed at 5th passage level. All the faecal samples (27) found positive by RT-PCR were also tested positive during adaptation in cell cultures.

All the RT-PCR positive clinical samples along with positive cell culture supernatant when subjected to electropherotyping analysis, exhibited 4-2-3-2 (eight bands observed visibly) migration/electrophoretic pattern (electropherotype) on agarose gel electrophoresis which is characteristic of RNA segments to Group A rotavirus (Fig 5 and 6). First 4 bands were closely migrating of which second and third bands were fused (class 1- segments 1, 2, 3, 4), bands 5 and 6 known as class II (segments 5 and 6) and class IV showed tightly migrated triplet band 7, 8 and 9 and long migration pattern of 10 and 11 segments.

In the present study VP6 and VP7 gene based RT-PCR and horizontal electrophoresis (electropherotyping) of the bovine rotavirus genome in agarose were used for the identification of bovine rotavirus group A (BRA) in 112 faecal samples collected from scouring calves in Bareilly (Uttar Pradesh) and Namakkal (Tamil Nadu) regions. RT-PCR, using primers from highly conserved regions of rotavirus A genes encoding VP7 and VP6, is more sensitive than other tests like ELISA, Electropherotyping and electron microscopy (EM) for detection of rotaviruses (Husain al., 1995; Buesa al., 1996; Song al., 2006; Eilschner al., 2002). Group A rotavirus genome segment six encodes for VP6 protein, which is the major inner capsid protein and there is a high degree of nucleotide sequence conservation among different viral strains (Tarlow et al., 1990). VP6 contains common epitopes shared by group A rotavirus and is the target for antibodies used in ELISA (Holland al., 1990; Saif and Theil, 1990). The VP7 protein expresses the major neutralization antigen and is distinguishable by means of both serological and genomic techniques in different G types, with good correlation between the serological and genomic classifications (Kapikian et al., 2001).

RT-PCR assay was standardized for the detection of BRA by amplifying VP6 and VP7 genes. Standardization and optimization of RT-PCR included the reverse transcription of the RNA extracted, various annealing temperature from 52-55°C, various concentration of primers 5-20 pmol and template volume of 2-4 µl and cycling conditions (30 for VP7 and 35 for VP6). After standardization of cycling conditions, expected amplicon size of 309 bp and 304 bp were obtained for VP6 and VP7 gene, respectively. These results were agreed with the reports of Husain et al. (1995) and Song et al. (2006).

Kalica et al. (1978) describes 11 segments resulting from polyacrylamide agarose gel electrophoresis (PAGE) of the rotavirus, however during RNA electrophoresis in the present study only 8 bands were discernible. This was up to three bands less than described for the rotavirus RNA analysis in PAGE. This reduction in number of bands due to the positions of the segments 2 and 3, and 7, 8 and 9 which were so close in agarose electrophoresis that the segments became indiscernible. This observation in present study is in agreement with earlier report of Chudzio et al. (1989) and Psikal et al. (1991). All rotavirus group A positive samples showed long electropherotypes. These results are in agreement with the predominance of long electropherotype showed in previous studies (Kasule et al., 2003; Kusumakar et al., 2007). Recently, Manuja et al. (2010) showed that sensitivity and specificity of ELISA, RNA-PAGE and RT-PCR for Group A rotavirus detection was 100%, 66.67% and 71.43%; and 97%, 100% and 100%, respectively.

All 27 (24.11%) samples found positive for rotavirus A by RT-PCR also showed electropherotyping positivity and similar migration pattern. This indicates that the rotavirus RT-PCR can detect all bovine group A rotaviruses, even in the presence of other enteric pathogens, and also indicates specificity and sensitivity of the RT-PCR standardized for detection of BRA. RT-PCR equally detected the presence of BRA in clinical diarrheic samples as well as adaptation of virus isolates in cell cultures. The study also revealed that bovine rotavirus group A, an economically important viral pathogen, known to possess public health significance, was found to be prevalent in organized dairy farms of Bareilly and Namakkal, India. The study supports the established fact that rotavirus infections are widespread in nature and inflicts diarrhoea (scour) in young ones of bovines (Chauhan and Singh 1996; Malik et al., 2005; Steele et al., 2004; Dhama et al., 2009). Rotavirus shows genetic and antigenic diversity in terms of subgroup,
electropherotypes and G and P serotypes/ genotypes. The diversity of rotavirus strains and the high prevalence of mixed infections among human and animals India are unique features of rotavirus epidemiology (Broor et al., 2003; Dhama et al., 2009). Many a few laboratories are working on rotaviruses in India, under such conditions RT-PCR assays which are highly sensitive, specific, and rapid for detection of bovine rotaviruses, may facilitate diagnosis of rotaviral diarrheas in animals, assist in molecular studies of the virus, and help plan appropriate prevention and control strategies for this important pathogen.

References
Electrophoresis of rotavirus RNA in Agarose gel electrophoresis, extracted from faecal samples showing 4-2-3-2 migration typical for Group A rotavirus (Lane 1,2,3,6,7,8,9,10,11,12 -positive faecal samples; Lane 4,5 -Negative faecal samples). Number 1-11 indicating 11 dsRNA genomic segments of bovine group A rotaviruses. The close migration 7, 8 and 9 as a triplet is characteristic of group A rotaviruses.

INFLUENCE OF EXOGENOUS FIBROLYTIC ENZYMES ON IN VITRO FERMENTATION OF BAJRA STRAW IN GOATS

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ABSTRACT

An experiment was carried out to evaluate the effects of exogenous fibrolytic enzymes supplemented to bajra straw @ 0, 1, 2, 3 and 4 g/kg substrate dry matter on degradability of DM and NDF as well as total gas production (TGP) using goat rumen liquor. There was significant (P<0.01) improvement in nutrient degradability of DM and NDF in bajra straw and TGP production showing maximum values at enzyme level of 2 g/kg DM. Second in vitro trial was performed to evaluate the effects of exogenous fibrolytic enzymes (2 g/kg DM) on rumen fermentation characteristics in complete feed containing bajra straw 60 parts and concentrate mixture 40 parts. There was significant (P<0.01) improvement in degradability of DM, NDF, TGP and total volatile fatty acids compared to control. Enzyme application did not alter rumen pH, total nitrogen as well as ammonia nitrogen in rumen fluid.

Key words: Bajra straw, complete feed, exogenous fibrolytic enzymes, in vitro degradability, rumen fermentation

Introduction

Crop residues are indispensable part of ruminant feeding through out world particularly in developing countries like India, but its utilization in ruminants is limited due to lack of lignolytic activity of ruminal microbes (Singh and Oosting, 1993), since the fibre fraction recovered from faeces is fermentable (Krause et al., 2003). Hatfield et al. (1999) pointed out that increasing cell wall degradation by 10% would significantly increase global milk and meat production (TGP) using goat rumen liquor. There was significant (P<0.01) improvement in nutrient degradability of DM and NDF in bajra straw and TGP production showing maximum values at enzyme level of 2 g/kg DM. Because responses to enzyme addition can be non linear and optimum dose level to improve fibre utilization by ruminants is not yet well established. It is important to establish the optimum level of exogenous fibrolytic enzymes for crop residues before enzyme technology can be used cost effectively in ruminant rations for bringing out consistent improvement in roughage utilization. The present investigation was under taken to study the effect of exogenous fibrolytic enzymes at different levels on in vitro degradability characteristics (DM and NDF) and total gas production (TGP) of bajra straw and fermentation pattern in bajra straw based complete feed using rumen liquor of goats.

Materials and Methods

Experiment I

The proximate constituents of bajra straw and complete feed was done as per AOAC (1999) and fibre fractions as per Van Soest et al. (1991). Exogenous fibrolytic enzyme mix (ECONASE RDE D) in powder form was procured form AB enzymes, Rajmaki, Finland, Containing cellulase (14140 E u/g) and xylanase (39300 B x u/g). The in vitro procedure as per modified method of Tilley and Terry (1963) was used to determine the IVMD and IVNDFD at 48 and 72 h post incubation and total gas production at 24 h post incubation. The experiment was conducted in completely randomized block design. Three adult bucks were adopted to experimental complete feed for 20 days containing bajra straw at 60% level to meet the nutrient requirement as per ICAR (1998). Rumen fluid was collected from these bucks before morning feeding (Solvia and Hess, 2007) by perforated tubing device under negative pressure and squeezed through four layers of muslin cloth to get inoculums (SRL), then transferred to pre-warmed flask (39°C) and flushed with CO2. The 10 ml of SRL was dispensed into pre-warmed (39°C) fermentation vessels (Jackmola et al., 2010) containing 500 mg finely ground bajra straw (< 1mm) without (To) or with 1 (T1), 2 (T2), 3 (T3) and 4 g enzyme (T4) + 40 ml buffer and flushed with oxygen free CO2. Each treatment was run in triplicate with negative controls (SRL plus buffer alone). These controls were used to correct for fermentation residues resulting directly form SRL. Each vessel cork fitted with control value was kept in incubator at 39°C for 48 h. In vitro gas production was measured periodically up to 24 h incubation by Doctor’s syringe. At the end of 48 h incubation two drops of saturated HgCl2 was added in each vessel to stop microbial activity. Contents of each vessel were transferred into a spout less beakers of 1000 ml capacity. The vessels were thoroughly washed with neutral detergent solution and final volume in beaker was made to 150 ml. The contents were refluxed for 1 h at 100°C, filtered and washed though pre weighed.
Gooch crucible (Grade 1, 50 ml capacity). This undigested residue (NDF) was oven dried at 100°C for 24 h and cooled in desiccators and weighed. Loss in DM and NDF with regard to per cent of sample (500 mg) was the digested dry matter and NDF and degradability was calculated accordingly. For in vitro studies at 72 h, incubation of samples was run same way as mentioned above for 48 h incubation. After 48 h of incubation the reaction was stopped by adding 2 ml of 6 N hydrochloric acid and 0.1 g pepsin powder (1:3000) to each vessel and vessels were incubated for another 24 h and procedure repeated as for 48 h incubation except addition of HgCl₂ at 48 h.

Experiment II

Optimum level of enzyme (2 g) selected from experiment I, was mixed with finely ground (<1 mm) complete feed containing bajra straw 60 parts and concentrate mixture 40 parts on DM basis. The parts of concentrate mixture were barley 04, deoiled rice bran 04, groundnut cake 15, gaur korma 14, mineral mixture 02 and salt 01. The experimental procedures for degradability of nutrients in complete feed were similar to those as described for experiment I. The pH in rumen liquor was determined immediately after incubation was terminated at 48 h using portable digital pH meter (pen type). Total volatile fatty acids were determined as per method of Barnett and Reid (1957) using Markham still distillation apparatus, total nitrogen as per AOAC (1999) and ammonia nitrogen by spectrophotometer method (Chaney and Marbach, 1962).

The data were compiled and analyzed statistically (Snedecor and Cochran, 1994) to draw the inference.

Results and Discussion

The chemical composition of bajra straw (Table 1) was comparable to those of the earlier reports by Venkata Ramana et al. (1989) and Dhuria et al. (2007). The complete feed contained 16.42% crude protein, 2.41% ether extract, 22.53% crude fibre, 47.94% nitrogen free extract, 10.70% total ash, 58.11% neutral detergent fibre, 37.41% acid detergent fibre, 20.70% hemicelluloses and 24.35% cellulose. The crude detergent fibre, 37.41% acid detergent fibre, 20.70% hemicelluloses and 24.35% cellulose. The crude protein and ether extract in enzyme mix were 16.17 and 1.63 per cent, respectively.

The in vitro dry matter degradability (IVDMD) of bajra straw supplemented with enzyme mix was significantly (P<0.01) higher as compared to control irrespective of level of enzyme and time of incubation (Table 2). Enzyme supplementation improved DM degradability up to 7.52 and 10.64, 21.29 and 22.74, 16.95 and 22.26 and 15.24 and 18.56 per cent in T₁, T₂, T₃ and T₄, treatments at 48 h and 72 h incubation, respectively, over the control. A similar trend was observed in NDF degradability as that observed for IVDMD. The respective improvement in NDF degradability for 48 h and 72 h incubation was 4.89 and 9.06, 19.14 and 23.41, 16.47 and 16.46 and 12.01 and 10.75 per cent at 1, 2, 3 and 4 g/kg DM, respectively, over that of control. A linear increase in IVDMD and IVNDFD at 48 h and 72 h incubation with the increase in the level of supplementation of enzyme up to 2 g/kg level (T₂) was observed and thereafter, inclusion of enzyme did not have any additional improvement on IVDMD and IVNDFD even at 4 g/kg a non significant improvement on mean IVDMD and IVNDFD was recorded. Chopra et al. (2007) reported increased DM, NDF, ADF and cellulose degradability of wheat straw, maize stovers, bajra stalks and berseem hay on account of addition of fibrolytic enzymes. Other workers (Eun et al., 2007; Srinivas et al., 2008; Moharrery et al., 2009; Gallardo et al., 2010) also reported improvement in degradability pattern of nutrients in different roughages either in vitro or in sacco due to supplementation of enzymes. Addition of enzymes in the substrate might have caused fibre hydrolysis and/ or solubilization of ADF which may have increased digestion rate and/or extent of digestion (Feng et al., 1996; Gado and Salem, 2008; Krueger et al., 2008). Enzyme application to the substrate enhances the attachment of rumen microorganisms to the feed particles thereby increasing hydrolytic capacity of the rumen (Wang et al., 2001). Increased NDF degradability enhances the energy density of diets and stimulates the microbial production (Oba and Allen, 2000). Another possibility is that exogenous enzymes can access greater surface area compared with cell bound microbial enzymes (Thakur et al., 2008). The decline in DM and NDF degradability at higher levels might be due to the fact that beneficial disruption of the feed area may get diminished because the excess exogenous enzyme attached to feed may have restricted microbial attachment and limited digestion of feed.

The per cent improvement in IVTGP in bajra straw supplemented with 1, 2, 3, and 4 g/kg DM of bajra straw was 23.38, 35.44, 32.56 and 27.75 over that of control at 24 h incubation. Srinivas et al. (2008) and Tang et al. (2008) indicated that application of fibrolytic enzyme tended to increase in vitro gas production parameters, IVDMD, IVOMD and IVNDFD of grass and cereal straws respectively. The increase in total gas production appeared to be associated with higher DM and NDF degradability in substrate due to increase in bacterial number and activity rate of fermentation.

Supplementation of fibrolytic enzymes was equally effective in improving IVDMD, IVNDFD and TGP (Table 3) in complete feed as compared with unsupplemented feed. IVDMD, IVNDFD and TGP increased by 16.43, 12.20 and 11.61 per cent, respectively, over control. The results of the study fall in line with those of Thakur et al. (2008), reported increased digestibility when enzymes were added in total mixed rations. Similar findings were also...
Enzyme supplementation did not alter pH, total nitrogen and ammonia nitrogen concentration in rumen fluid of goats. The total volatile fatty acid concentration was significantly higher (84.43 mEq/l) in enzyme treated feed as compared to control (81.22 mEq/l). Higher concentration of TVFA supports the theory that enzyme application in the diet resulted in higher ruminal digestion which is confirmed by improvement in DM and NDF degradability. Almaraz et al. (2010) and Chopra et al. (2007) also reported an increase in TVFA concentration in in vitro experiments. The results of in vitro study indicated significant improvement in IVDMD, IVNDF and IVTGP as well as TVFA concentration in rumen fluid of goats as an effect of supplementation of enzymes at 2 g/kg dry matter which needs further testing in in vivo studies.

Table 1: Chemical composition of feeds (% DM basis)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Bajra straw</th>
<th>Complete feed</th>
<th>Enzyme mix</th>
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<tbody>
<tr>
<td>DM</td>
<td>93.05</td>
<td>94.03</td>
<td>98.06</td>
</tr>
<tr>
<td>OM</td>
<td>91.02</td>
<td>89.30</td>
<td>100.00</td>
</tr>
<tr>
<td>CP</td>
<td>4.60</td>
<td>16.42</td>
<td>16.17</td>
</tr>
<tr>
<td>EE</td>
<td>1.24</td>
<td>2.41</td>
<td>-</td>
</tr>
<tr>
<td>CF</td>
<td>34.21</td>
<td>22.53</td>
<td>-</td>
</tr>
<tr>
<td>NFE</td>
<td>50.97</td>
<td>47.94</td>
<td>-</td>
</tr>
<tr>
<td>NDF</td>
<td>78.51</td>
<td>58.11</td>
<td>-</td>
</tr>
<tr>
<td>ADF</td>
<td>54.41</td>
<td>37.41</td>
<td>-</td>
</tr>
<tr>
<td>HC</td>
<td>24.10</td>
<td>20.70</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>28.51</td>
<td>24.35</td>
<td>-</td>
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</table>

Table 2: in vitro DM, NDF degradability and total gas production in bajra straw (%DM basis)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Incubation</th>
<th>T₀</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVDMD</td>
<td>48</td>
<td>41.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48.55&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>72</td>
<td>47.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.48&lt;sup&gt;d&lt;/sup&gt;</td>
<td>56.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.976</td>
</tr>
<tr>
<td>IVNDFD</td>
<td>48</td>
<td>29.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>35.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.50&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>72</td>
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<td>36.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.552</td>
</tr>
<tr>
<td>IVTGP ml/500mg</td>
<td>24</td>
<td>28.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>35.90&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.842</td>
</tr>
</tbody>
</table>

Note: Means superscripted with different letters in a row differ significantly (P<0.01)

Table 3: Effect of enzymes on fermentation characteristics and rumen metabolites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Un-supplemented complete feed</th>
<th>Enzyme supplemented complete feed</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVDMD</td>
<td>51.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.927</td>
</tr>
<tr>
<td>IVNDFD</td>
<td>45.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.527</td>
</tr>
<tr>
<td>IVTGP ml/500mg</td>
<td>53.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.961</td>
</tr>
<tr>
<td>pH</td>
<td>6.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.011</td>
</tr>
<tr>
<td>TVFA mEq/l</td>
<td>81.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.112</td>
</tr>
<tr>
<td>Total N mg/dl</td>
<td>92.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.241</td>
</tr>
<tr>
<td>Ammonia N mg/dl</td>
<td>32.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.067</td>
</tr>
</tbody>
</table>

Note: Means superscripted with different letters in a row differ significantly (P<0.01)

References
CLINICO-HEMATOBIOCHEMICAL STUDIES IN EQUINES INFECTED WITH T. EVANSI

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Faculty of Veterinary Sciences and Animal Husbandry, S.K.U.A.S.T.-J,
R.S. Pura-181102 (J&K), India

ABSTRACT

Trypanosomiasis, an arthropod borne blood protozoan disease commonly known as Surra is caused by Trypanosoma evansi and transmitted by Tabanus spp. In the present study a total of twelve (12) equines tested positive for presence of T. evansi were evaluated for clinical and hematobiochemical alterations. The main clinical signs observed in clinically infected equines (n=12) include intermittent high fever (103-104° F), dullness, lachrymation, persistent watery nasal discharge, depression, severe anaemia, loss of body weight, weakness and emaciation. Petechiae were observed on the conjunctival mucous membrane. There was oedema of dependant parts particularly neck. Apart from these clinical observations one mare showed nervous signs like incoordination of gait, circling movement, paddling movement, head tilt and falling down. The infected equines showed marked anaemic tendency as revealed by significant decrease in Hb, PCV and TEC level as compare to their healthy counterpart (n=12). The diseased equines showed significant leukopenia due to related neutropoenia along with agranulocytosis as compare to the healthy one. Basophils and eosinophils remain unaffected. Marked hypoglycaemia, hypoproteinaemia along with significant decrease in serum iron concentration and uraemia was recorded in infected equines. No significant alteration has been recorded in Na, K and creatinine level as compare to the healthy counterpart of the study.

Key words: Trypanosomiasis, clinical signs, haemato-biochemical, T. evansi.

Introduction

Equines are considered as beasts of burden. Equines hold special position in livestock both for civil and military purposes in view of its multifaceted utility. In northern India particularly in the States of Jammu and Kashmir, Haryana, Punjab, Uttar Pradesh, Rajasthan and Gujarat donkeys and horses are extensively used as a mode of transportation for men and material. Trypanosomiasis, an arthropod borne blood protozoan disease commonly known as Surra (Hoare, 1964) is caused by Trypanosoma evansi and transmitted by Tabanus spp. (Radostits et al., 2000). This was the first trypanosome identified in mammals by Sir Griffith Evans in Dera Ismail Khan (Now in Pakistan) It is widely prevalent in livestock of Africa, Asia and South America (Hunter, 1990) and is endemic in China, the Indian sub continent, Northern America, The Philippines, Bulgaria, parts of the former U.S.S.R. and parts of Indonesia (O.I.E., 2008). The "Office international des epizootics" categorized the disease Surra under 'B' disease of significance (O.I.E., 2004). Zoonotic potential of the disease has been documented through a report of Trypanosoma evansi infection in a person from Maharashtra, India (Powar et al., 2006).

The disease is clinically characterized by intermittent fever directly associated with parasitaemia, progressive anaemia, oedema of dependent parts, loss of condition, listlessness, dullness despite good appetite, nasal and ocular discharges, paddling movement, falling down and head tilt. Urticarial plaques and petechial haemorrhages of the serous membrane are often observed. A wide-based stance, loss of balance and hind limb proprioceptive defects may also occur particularly in Equides. There are indications that the disease also causes immunodeficiency (Onah et al., 1998).

Materials and Methods

The present study was conducted in three different agro climatic zones of Jammu division. The equines were screened randomly for presence of T. evansi by three parasitiological methods i.e., peripheral wet blood film examination (PWBF), Giemsa’s staining and microhaematocrit centrifugation technique (MHCT). A total of twelve (12) equines tested positive for presence of T. evansi. The positive equines were further evaluated for clinical and haematobiochemical alterations. The data generated from the present study was subjected to statistical analysis.

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to the test of significance (one way ANOVA) as per the method described by Snedecor and Cochran (1994).

**Clinical observations**

Each animal was observed critically and clinically by noting respiration, pulse, and body temperature, observing serous membrane of eyelids for presence/absence of petechial haemorrhages, oedema of dependant parts, presence/absence of nasal and ocular discharges. Animals were also observed for any neurological signs like paddling movements, circling movements, standing with legs placed wide apart, falling down and head tilt. Overall body condition was taken into account including dullness, depression and loss of condition.

**Haematological studies**

Whole blood was processed for routine haematological parameters like Hb, PCV, TLC, TEC, DLC as per the standard techniques described by Jain et al. (1986).

**Biochemical studies**

Serum was separated from blood by centrifugation at 3000 rpm for 15 min and was used for estimation of total protein (Biuret method), albumin (BCG Dye method) blood urea nitrogen (GLDH-Urease method) using commercially available kits of Transasia Biomedicals, Ltd. H.P., India, creatinine by Jaff's method (Erba Mann Heim Pvt. Ltd.) sodium and potassium (Thiocyanate method) using commercially available kits of Crest Biosystems and iron by bichromatic method using commercial kit (Recon Diagnostics Pvt. Ltd. Gujarat., India). Estimation of glucose was done on the spot of sample collection by using glucometer (Accu-Chek Active).

**Results and Discussion**

**Clinical signs**

The main clinical signs observed in clinically infected equines (n=12) include intermittent high fever (103-104°F), dullness, lachrymation, persistent watery nasal discharge, depression, severe anaemia, loss of body weight, weakness and emaciation. Petechiae were observed on the conjunctival mucous membrane. There was oedema of dependent parts particularly neck. Apart from these clinical observations one mare showed nervous signs like in-ordination of gait, circling movement, paddling movement, head tilt and falling down.

Presence of fever was due to the toxins liberated by the parasites which were present in the blood, irrespective of concentration of the parasite (Manohar, 1984). This leads to the change in the body temperature set point in the hypothalamus under the influence of pyrogenic stimuli released during infection (Baracos et al., 1987). All other clinical signs were also observed by Levine (1961), Richardson and Kendall (1963), Manohar (1984), Gill (1991) and Soulsby (2005).

Beside this in Jammu similar clinical signs were observed by Soodan et al. (2007) with high rise of temperature (103-105°F) with decreased appetite, dullness and weakness, congestion in conjunctival mucous membrane with petechial haemorrhages.

Nervous signs like incordination of gait, circling movements, paddling movement, head tilt and falling down, legs spread apart were seen in one mare which may be due to long standing chronic infection resulting in invasion of brain by Trypanosoma evansi. Similar findings were reported by Yadav and Kumar (2010) in a horse at NRCE farm, which was heavily infested with Trypanosoma evansi and died during 2009.

**Hematological studies**

The results of haematological investigation in healthy and diseased equines are given in (Table 2). The infected equines shows marked anaemic tendency as revealed by significant decrease in Hb, PCV and TEC level as compare to their healthy

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Clinical Signs</th>
<th>No. of cases (12)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weakness, dullness, emaciation, listless and depression</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Normal thirst and appetite</td>
<td>02</td>
<td>16.66</td>
</tr>
<tr>
<td>3</td>
<td>Varying body temperature</td>
<td>10</td>
<td>83.33</td>
</tr>
<tr>
<td>4</td>
<td>Increase in body temperature after administration of dextrose</td>
<td>02</td>
<td>16.66</td>
</tr>
<tr>
<td>5</td>
<td>Pale mucous membrane</td>
<td>10</td>
<td>83.33</td>
</tr>
<tr>
<td>6</td>
<td>Nervous signs like in-ordination of gait, head tilt, circling movement, standing with legs held wide apart, falling down</td>
<td>01</td>
<td>8.33</td>
</tr>
<tr>
<td>7</td>
<td>Generalized urticarial eruptions</td>
<td>03</td>
<td>25.00</td>
</tr>
<tr>
<td>8</td>
<td>Small and rapid pulse</td>
<td>08</td>
<td>66.66</td>
</tr>
<tr>
<td>9</td>
<td>Rapid and laboured respiration</td>
<td>06</td>
<td>50.00</td>
</tr>
<tr>
<td>10</td>
<td>Serous nasal discharge</td>
<td>01</td>
<td>8.33</td>
</tr>
<tr>
<td>11</td>
<td>Oedema of hind limb and neck</td>
<td>01</td>
<td>8.33</td>
</tr>
<tr>
<td>12</td>
<td>Petechiae on nictitating membrane</td>
<td>04</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Figure in parenthesis indicates total number of clinical cases observed.
counterpart. The diseased equines showed significant leucopenia due to related neutropenia along with agranulocytosis as compare to the healthy one. Basophils and eosinophils remain unaffected.

**Biochemical studies**

The biochemical observations (mean±SE) in control and diseased animals are given in Table 3. Marked hypoglycaemia, hypoproteinaemia (decreased albumin and total protein) along with significant decrease in serum iron concentration and uraemia was recorded in infected equines. No significant alteration has been recorded in Na, K and creatinine level as compare to the healthy counterpart of the study.

In the present study it was observed that there was marked fall in the mean values of Hb, PCV and TEC. Similar findings were reported by Killick and Kendrick (1964), Wust (1980), Manohar et al. (1983) and Soodan et al. (2007). Richardson and Kendall (1963) reported that the most noticeable pathologic change was the progressive anaemia in Trypanosomiasis in general. The number of red blood cells and haemoglobin being reduced to 25 per cent of the normal values. The anaemia may be caused due to inhibition on the haemopoietic system by the toxins liberated by the parasites resulting in failure in production of the red blood cells. Jennings (1976) speculates on the mechanisms by which RBC are made more susceptible to erythrophagocytosis. These include the attachment of Trypanosome antigen to RBC, reported by Herbert and Inglis (1973), which may increase the cell susceptibility to erythrophagocytosis, which may be further increased by the union of surface absorbed antigen with antibody. This could decrease RBC, PCV and Hb values.

A decrease in TLC observed in the present study is in conformity with the findings of Samaddar (1969), Manohar et al. (1983) and Suryanarayana (1984). All of them reported leucopenia to be the consistent feature in Trypanosomiasis. The leucopenia could be due to toxins liberated by the parasites. There was also a marked decrease in neutrophils and increase in lymphocytes leading to neutropenia and lymphocytosis, respectively. Monocytes also showed a significant increase leading to monocytosis. No significant change was observed in other cell types like eosinophils and basophils. This confirms the work of Seegert (1930), Gill and Sen (1971) and Suryanarayana (1984) who reported that differential leucocyte count registered a decrease in the proportion of neutrophils and increase in the relative number of lymphocytes in *Trypanosoma evansi*.
Fig. 1: General weakness and emaciation in equine severely infected with *T. evansi*

Fig. 2: Petechial hemorrhages on the inner surface of eye lid in equine severely infected with *T. evansi*

Fig. 3: Equine standing with wide stance due to acute *T. evansi* infection

Infected ponies. Neutropoenia may be due to the predominant effect of toxins liberated by parasites on bone marrow rather than lymphoid organs. Increase in lymphocytes may be relative to decrease in neutrophils rather than an absolute increase in the number of lymphocytes.

References


UNUSUAL CASE OF CYCLOPIA WITH COENUROUS CEREBRALIS CYST IN A JERSEY CROSS BRED COW- A CASE REPORT

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Introduction
Cyclopia has been reported in many species and is a teratological defect, mostly reported in pigs and sheep (Roberts, 1971) but rarely observed in bovine (Gupta and Anand, 2002; Ozkan et al., 2005 and Khasatiya, 2010) and caprine (Ashokan et al., 1990). It is characterized by single eye orbit with doubling of eye balls, rudimentary eyelids; tubular appendage like nose placed above the centrally located eye and has longer lower jaw than upper one. Usually this condition is incompatible with foetal life and may cause dystocia at the time of parturition. The present case report deals with dystocia due to monster and its successful per vaginal delivery by forced traction.

Case history and observations
A cross bred jersey cow five years of age in its second parity at full term was brought to teaching veterinary clinical complex Hisar, having dystocia due to foetal cause since one day. Per vaginal manipulations were already done by field doctor to solve the problem earlier but in vain. On vaginal examination, it was observed that both the water bags were ruptured and a dead foetus in anterior longitudinal presentation, dorso-sacral position with lateral deviation of head was present in the birth canal.

Treatment and Discussion
The birth canal was lubricated with paraffin and traction was applied on forelimbs to deliver dead foetus. The placenta was shed along with foetus. The delivered foetus had two eye balls in single eye socket. Eyelids were absent and head was covered with Coenurous cerebralis cyst. The lower jaw contained large teeth and larger than upper jaw. The tongue was oedematous and large. The ears were long. The whole body of foetus was covered with long black hairs. All these physical characteristics of the deformed foetus were suggestive of a condition known as cyclopia and classified under teratological defects of embryonic development (Roberts, loc. cit). Probably, this was due to anomalies of foetus. The animal was treated with antibiotics, liver extract, anti-inflammatory, intrauterine bolus along with supportive therapy for 5 days. Follow up of case revealed successful recovery of dam. In sheep the condition has been reported due to ingestion of Veratrum californicum (Binns et al., 1963). In cross bred heifer, Honparkhe et al. (2009) reported similar congenital defective fetus.

Reference
INCIDENCE OF HAEMOPROTOZOAN INFECTION IN CANINES IN AND AROUND MATHURA

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ABSTRACT

A total of 103 blood smears were collected between January and August 2010 from dogs exhibiting clinical signs of haemoprotozoan infection. On blood smear examination, 9 blood smears were found to be positive for various haemoprotozoan which were identified as Babesia canis (4.9%) and Trypanosoma evansi (3.8%). Highest prevalence was observed to be in the monsoon months and no significant difference was found between breeds and sexes. Lower haemoglobin level, total erythrocyte count and packed cell volume values were recorded along with marked neutrophilia and lymphopenia.

Key words: Canine, incidence, haemoprotozoa, trypanosomiasis, babesiosis.

Introduction

Haemoprotozoan infections are common in canines in tropical countries. Most of the haemoprotozoan parasites are tick borne and are of great socio-economic and public health importance. Amongst them, canine babesiosis is a disease of worldwide significance, where members of genus Babesia readily parasite RBCs causing progressive anaemia. Canine Babesia is morphologically classified into large and small forms. Babesia canis (large) and Babesia gibsoni (small) have been documented to infect dogs (Schoeman and Leisewitz, 2006). Trypanosomiasis caused by T. evansi is also an important infection although there are few reports of natural infection with Trypanosoma evansi in dogs (Losos, 1986). The present study was conducted to document the prevalence of haemoprotozoan in dogs in and around Mathura city as well as to project the haematological status for the ailing animals for effective therapeutic management.

Materials and Methods

To ascertain the pattern of occurrence of the haemoprotozoan, cases attended in the Teaching Veterinary Clinical Complex of College of Veterinary Sciences and Animal Husbandry, DUVASU (Mathura) from January 2010 to August 2010 were included in the study. Blood samples were collected from 103 dogs with clinical signs of fever, inappetance, dullness, depression, anaemia, conjunctivitis, bilateral corneal opacity, jaundice, swollen lymph nodes etc.

Leishman stained blood smears were examined microscopically and parasites were identified based on the description by Soulsby (1982). To get the haematological picture of the disease, total erythrocyte count, total leukocyte count, differential leukocyte count, PCV and haemoglobin level were estimated following standard techniques (Jain, 1993).

Results and Discussion

The examination of 103 canine blood samples for haemoprotozoan infections revealed 9 (8.7%) positive cases. The main protozoa identified in those blood smears were Babesia canis with a maximum of 5 (4.9%) cases followed by 4 cases of Trypanosoma evansi (3.9%). Singh et al. (1993) found 4.68% prevalence of canine Trypanosomiasis in Ludhiana and Senthilkumar et al. (2009) reported the incidence of Babesia canis to be 3.9% and that of Trypanosoma evansi to be 0.4% in Chennai city. In another study, Chowdhary and coworkers reported prevalence of Trypanosoma evansi in dogs to be 1.72% in and around Kolkata. (Chowdhary et al., 2005). The overall prevalence of blood-parasites was recorded as 11.66% by Gadahi et al. (2008) in a survey for blood parasites in Hyderabad city of which the incidence of Babesia canis was found to be 5%.

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3Assistant Professor, Deptt. of Animal Genetics & Breeding, DUVASU, Mathura.
4Incharge, Clinics, DUVASU, Mathura.
A considerable seasonal variation was found with the occurrence of haemoprotezoan disease in dogs. Most of the animals suffered during monsoon months, which might be due to more number of ticks in monsoon that had developed during summer months. This is in accordance with the observations made by Singh et al. (1993) and Senthilkumar et al. (2009) who found highest prevalence of canine haemoprotezoan diseases in monsoon months. Gadahi et al. (2008) also recorded the highest percentage of haemoprotezoan infection in the month of July (13.3%).

No significant difference was found to be present between breeds and sexes. Similar findings were reported by Chowdhary et al. (2005); Senthilkumar et al. (2009) and Dash and Dutta (2001). Although Gadahi et al. (2008) found that the percentage of infection was greater in females (18.6%) than males (9.33%) and the prevalence in stray and pet dogs 13% and 9%, respectively. This discrepancy in the observation might be due to smaller sample size, locality and health status of dogs.

In the present study, the anaemia caused was reflected by low haemoglobin content (9 g/dl), lower TEC (4.2 x 10^6/cumm) and PCV (30%) values. Differential leukocyte counts indicated that the percentage of neutrophils increased and lymphocytes decreased significantly in cases of Trypanosomiasis as compared with un-infected animals. This finding is in agreement with the results obtained by Chowdhary et al. (2005) who have also reported a decrease in the above haematological values. Changes in DLC profile may lead to immune suppression and render the animals prone to opportunistic pathogenic infection. More long-term observation must be made to conclude the epidemiological nature of haemoprotezoan diseases in canines in this geographical location.

References
EFFECT OF DROUGHT ON WHITE BLOOD CELL INDICES IN SHEEP

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ABSTRACT

The experiment was designed to assess the effect of drought on white blood cell indices in 100 sheep of both sex in western part of Rajasthan. All animals belonged to farmers’ stock. The parameters included total leucocyte count (TLC) (10³/µl), differential leucocyte count (DLC) viz. neutrophils (%), lymphocytes (%), eosinophils (%), basophils (%) and monocytes (%) and the over all mean values were 7.746±0.113, 27.370±0.252, 63.139±0.233, 2.440±0.099, 0.560±0.049 and 2.790±0.111, respectively. The effect of sex and age was also observed. It was found that the sex and age effect was significant (P<0.05) only on the mean values of monocyte in sheep.

Key words: Sheep, sex and age

Introduction

Role of sheep has their role in economy of arid and semi arid tract is paramount since they are the backbone of landless and small farmers. Low rainfall and scanty vegetation are common to this area. Low water intake tends to create dehydration (Kataria et al., 2001) which is reflected in various haematological parameters. The knowledge of blood norms is important in diagnosing many diseases in animals (Vaidya et al., 1970). Although blood biochemical analyses have been carried out extensively in sheep but reports are not available on this aspect during drought conditions. It would be of great importance to estimate the different blood values during drought conditions particularly regarding management of the animals in such emergency conditions. In this way these values become a real aid to the diagnosis of normal vs. pathological conditions of the animals.

Materials and Methods

The experiment was designed to evaluate the effect of stress due to drought on white blood cell indices in sheep (up to 5 years of age). The effects of sex and age were also determined on various parameters during drought. The animals belonged to farmers’ stock of drought stricken area of western part of Rajasthan particularly area in and around Jaisalmer district. The animals belonged to similar environmental habitat and managemental practices. Field grazing pattern was adopted for the animals. Animals browsed Ziziphus nummularia leaves and also received small amounts of loppings of Prosopis cineria trees. Blood was collected from jugular vein under complete aseptic condition into the test tubes with anti coagulant. All the samples were collected in duplicate and processed on the same day of collection. White blood cell indices i.e. total leucocyte count (TLC) (10³/µl) and DLC i.e. neutrophils (%), lymphocytes (%), eosinophils (%), basophils (%) and monocytes (%) were determined by standard technique as described by Jain (1986) To determine the effect of age and sex the mean values were compared statistically by using ‘t’ test (Snedecor and Cochran, 1994) with the respective mean values.

Results and Discussion

The overall mean values of various white blood indices i.e. total WBC (TLC) (10³/µl) and DLC and their mean values according to sex and age are presented in Table 1. The overall mean value of total leucocyte obtained in the present investigation was higher than those reported by Dutta et al. (1996) in sheep. The overall mean value of total leucocyte obtained in the present investigation were lower than those reported by Lewis (1976), Bhargava (1980), Pyne et al. (1982), Upadhayay and Rao (1985), Rastogi and Singh (1990) and Kalleswarappa and Jayprakash (1999), in sheep. The overall mean value of total leucocyte obtained in the present investigation was higher than those reported by Dutta et al. (1996) in sheep. The overall mean value of total lymphocyte in the
present investigation were higher than those reported by Reda and Hathout (1956), Bhargava (1980), Pyne et al. (1982), Hawkey et al. (1983) and Alonso et al. (1997) but lower than those reported by Rastogi and Singh (1990) in sheep.

The overall mean value of neutrophil in the present investigation was lower than those reported by Reda and Hathout (1956), Sharma et al. (1973), Hawkey et al. (1983), Dutta et al. (1996) in sheep.

The overall mean value of eosinophil, basophil and monocyte in the present investigation were lower than those values reported by Reda and Hathout (1956) but higher than those reported by Pyne et al. (1982), Bhargava (1980), Hawkey et al. (1983), Dutta et al. (1996) in sheep.

Effect of sex and age was found non significant (P>0.05) on the different white blood cell indices in the present study. However, the age effect was significant (P<0.05) on monocyte in sheep the values being higher in Ist group.

Reda and Hathout (1956) recorded an increase in eosinophils with advancement of age in sheep while in present investigation no effect was noticed. Study of age related variations in haematological parameters is very important as they can change the interpretation in sick animals (Hawkey et al., 1983).

Neutrophils secrete a broad spectrum of cytokines. Most of these are involved in the innate immune response, however, neutrophils also secrete cytokines that affect adaptive immunity. Several studies indicate that neutrophils may modulate the adaptive immune response to infectious agents (Kaneko, et al., 2008). During drought conditions due to scanty vegetation long transportation is common so the effect of critical environmental, transportation and exercise on neutrophil is noticed. Neutrophils become activated during transportation despite the potentially suppressive effects of cortisol on neutrophil function.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Effects</th>
<th>TLC (10³/µl)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Basophils (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Overall</td>
<td>7.746±0.113 (6.20 - 10.20)</td>
<td>27.370±0.252 (24 - 31)</td>
<td>63.139±0.233 (59 - 69)</td>
<td>2.440±0.099 (1 - 4)</td>
<td>0.560±0.049 (0-1)</td>
<td>2.790±0.111 (1-5)</td>
</tr>
<tr>
<td>2.</td>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Male</td>
<td>7.479±0.147 (6.20-9.64)</td>
<td>27.700±0.496 (25-31)</td>
<td>63.000±0.502 (59-69)</td>
<td>2.368±0.176 (1-4)</td>
<td>0.566±0.092 (0-1)</td>
<td>2.500±0.218 (1-5)</td>
</tr>
<tr>
<td>b.</td>
<td>Female</td>
<td>7.860±0.135 (6.25-10.20)</td>
<td>27.228±0.292 (24-31)</td>
<td>63.200±0.256 (60-66)</td>
<td>2.471±0.121 (1-4)</td>
<td>0.577±0.059 (0-1)</td>
<td>2.941±0.126 (1-5)</td>
</tr>
<tr>
<td>3.</td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>0 yrs</td>
<td>7.879 ± 0.197 (6.20-10.20)</td>
<td>27.549±0.0377 (24-31)</td>
<td>62.745±0.342 (59-69)</td>
<td>2.529±0.143 (1-4)</td>
<td>0.549±0.070 (0-1)</td>
<td>3.058±0.159 (2-5)</td>
</tr>
<tr>
<td>b.</td>
<td>Above 2 yrs</td>
<td>7.606±0.160 (6.25-9.26)</td>
<td>27.183±0.335 (24-31)</td>
<td>63.651±0.307 (60-66)</td>
<td>2.346±0.138 (1-4)</td>
<td>0.571±0.071 (0-1)</td>
<td>2.510±0.146 (1-5)</td>
</tr>
</tbody>
</table>

(i) Figures in the parentheses indicate numbers of animals for the effects and range for the different parameters.

(ii) In sex effect mean values of all parameters of female animals have been compared with respective mean values of animals.

(iii) In age effect mean values of all parameters of the animals of above 2 years of age group have been compared with respective mean values of animals of below 2 years of age group.

(iv) Mean superscripted by letter “a” do not differ significantly (P>0.05) and letter “b” differ significantly (P<0.05) from the respective male and female age group.

References
Introduction

India is full of biodiversity and blessed with rich fauna and flora but many endangered or critical species of wild animals and birds are being killed for food or aesthetic purposes. Administrative authorities of forest department in most of the states often face the problem of species identification. Deer are believed to be vulnerable to poaching for the purpose of meat, skin and antlers. A hoof is the tip of a toe of an ungulate mammal, strengthened by a thick horny (keratin) covering. The poaching of wild ungulates is very common in India and after getting the demandable body parts, other material are discarded or sometimes the poacher are caught with the hooves of species. As the habits of wild and domestic ungulates are quite different, hence the hooves get adopted as per their habitat and this may help in the identification of the species.

Materials and Methods

The present investigation has been performed on the hooves of forelimb and hind limb of four adult female chital and goat. The gross morphometric parameters were length and width of different surfaces and borders of hooves and the angle between anterior border and solar surface was measured with measuring tape and thread.

Results and Discussion

The hooves of forelimb and hind limb of chital and goats were resembled almost same in the morphology as found in ungulates. But morphometrically, both were found very dissimilar which may be due to difference in their habitat and running habit.

In the fore hooves solar surface was of 6.56±0.024 cm long laterally in chital (Fig. 1a) where as it was 4.04±0.024 cm in goat (Fig. 3a) and 5.46±0.014 cm medially in chital while 4.04±0.024 cm in goat. The interdigital border was 3.48 ±0.014 cm long in chital and 3.68 ±0.014 cm in goat. The posterior surface was 1.39 ±0.013 cm long in chital and 2.04±0.024 cm in goat. The maximum width of solar surface, lateral surface and coronet were 2.09±0.013 cm, 3.28±0.014 cm and 0.58±0.014 cm in chital, respectively (Fig. 1b), and those were 2.20±0.020 cm, 3.01±0.013 cm and 0.60±0.020 cm in goat, respectively (Fig. 3b). The angle of hoof was 45.0 ±0.41° in chital where as it was 59.9±0.43° in goat (Table 1 and Table 2).

In the hind hooves solar surface was of 5.79±0.013 cm long laterally in chital (Fig. 2a) where as it was 3.49±0.013 cm in goat (Fig. 4a) and 4.78 ± 0.014 cm medially in chital while 3.98±0.014 cm in goat. The maximum width of solar surface, lateral surface and coronet were 2.09±0.013 cm, 3.28±0.014 cm and 0.58±0.014 cm in chital, respectively (Fig. 1b), and those were 2.20±0.020 cm, 3.01±0.013 cm and 0.60±0.020 cm in goat, respectively (Fig. 3b). The angle of hoof was 45.0 ±0.41° in chital where as it was 59.9±0.43° in goat (Table 1 and Table 2).

In the hind hooves solar surface was of 5.79±0.013 cm long laterally in chital (Fig. 2a) where as it was 3.49±0.013 cm in goat (Fig. 4a) and 4.78 ± 0.014 cm medially in chital while 3.98±0.014 cm in goat. The interdigital border was 3.41 ±0.013 cm long in chital

Key words: Chital, goat, hooves, morphometry.
and 3.79±0.013 cm in goat. The posterior surface was 1.89±0.013 cm long in chital and 1.99±0.013 cm in goat. The maximum width of solar surface, lateral surface and coronet were 1.73±0.014 cm, 3.40±0.020 cm and 1.23±0.014 cm in chital, respectively (Fig. 2b), and those were 2.23 ±0.014 cm, 2.80±0.020 cm and 0.43±0.014 cm in goat, respectively (Fig. 4b). The angle of hoof was 48.3 ±0.25° in chital where as it was 60.8±0.15° in goat (Table 3 and Table 4).

Table 1: Different measurements and mean with standard error for fore hooves of chital

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C₁ (cm)</th>
<th>C₂ (cm)</th>
<th>C₃ (cm)</th>
<th>C₄ (cm)</th>
<th>AM±SE (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of lateral border of solar surface</td>
<td>6.60</td>
<td>6.50</td>
<td>6.55</td>
<td>6.60</td>
<td>6.56±0.024</td>
</tr>
<tr>
<td>Length of medial border of solar surface</td>
<td>5.50</td>
<td>5.50</td>
<td>5.45</td>
<td>5.45</td>
<td>5.46±0.014</td>
</tr>
<tr>
<td>Length of inter-digital border</td>
<td>3.50</td>
<td>3.45</td>
<td>3.45</td>
<td>3.50</td>
<td>3.48±0.014</td>
</tr>
<tr>
<td>Length of posterior surface</td>
<td>1.40</td>
<td>1.35</td>
<td>1.40</td>
<td>1.40</td>
<td>1.39±0.013</td>
</tr>
<tr>
<td>Maximum width of lateral surface</td>
<td>3.30</td>
<td>3.25</td>
<td>3.25</td>
<td>3.30</td>
<td>3.28±0.014</td>
</tr>
<tr>
<td>Maximum width of solar surface</td>
<td>2.10</td>
<td>2.05</td>
<td>2.10</td>
<td>2.10</td>
<td>2.09±0.013</td>
</tr>
<tr>
<td>Maximum width of coronet</td>
<td>0.60</td>
<td>0.55</td>
<td>0.55</td>
<td>0.60</td>
<td>0.58±0.014</td>
</tr>
<tr>
<td>Angle of hoof</td>
<td>45°</td>
<td>46°</td>
<td>45°</td>
<td>44°</td>
<td>45±0.41</td>
</tr>
</tbody>
</table>

Table 2: Different measurements and mean with standard error for fore hooves of goat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G₁ (cm)</th>
<th>G₂ (cm)</th>
<th>G₃ (cm)</th>
<th>G₄ (cm)</th>
<th>AM±SE (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of lateral border of solar surface</td>
<td>4.00</td>
<td>4.10</td>
<td>4.05</td>
<td>4.00</td>
<td>4.04±0.024</td>
</tr>
<tr>
<td>Length of medial border of solar surface</td>
<td>4.00</td>
<td>4.10</td>
<td>4.05</td>
<td>4.00</td>
<td>4.04±0.024</td>
</tr>
<tr>
<td>Length of inter-digital border</td>
<td>3.70</td>
<td>3.65</td>
<td>3.70</td>
<td>3.65</td>
<td>3.68±0.014</td>
</tr>
<tr>
<td>Length of posterior surface</td>
<td>2.00</td>
<td>2.05</td>
<td>2.00</td>
<td>2.10</td>
<td>2.04±0.024</td>
</tr>
<tr>
<td>Maximum width of lateral surface</td>
<td>3.00</td>
<td>3.05</td>
<td>3.00</td>
<td>3.00</td>
<td>3.01±0.013</td>
</tr>
<tr>
<td>Maximum width of solar surface</td>
<td>2.20</td>
<td>2.15</td>
<td>2.25</td>
<td>2.20</td>
<td>2.20±0.020</td>
</tr>
<tr>
<td>Maximum width of coronet</td>
<td>0.60</td>
<td>0.55</td>
<td>0.60</td>
<td>0.65</td>
<td>0.6±0.020</td>
</tr>
<tr>
<td>Angle of hoof</td>
<td>60°</td>
<td>59°</td>
<td>59.5°</td>
<td>61°</td>
<td>59.9±0.43</td>
</tr>
</tbody>
</table>

Table 3: Different measurements and mean with standard error for hind hooves of chital

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C₁ (cm)</th>
<th>C₂ (cm)</th>
<th>C₃ (cm)</th>
<th>C₄ (cm)</th>
<th>AM±SE (cm)</th>
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</thead>
<tbody>
<tr>
<td>Length of lateral border of solar surface</td>
<td>5.80</td>
<td>5.75</td>
<td>5.80</td>
<td>5.80</td>
<td>5.79±0.013</td>
</tr>
<tr>
<td>Length of medial border of solar surface</td>
<td>4.80</td>
<td>4.75</td>
<td>4.75</td>
<td>4.80</td>
<td>4.78±0.014</td>
</tr>
<tr>
<td>Length of interdigital border</td>
<td>3.40</td>
<td>3.40</td>
<td>3.45</td>
<td>3.40</td>
<td>3.41±0.013</td>
</tr>
<tr>
<td>Length of posterior surface</td>
<td>1.90</td>
<td>1.85</td>
<td>1.90</td>
<td>1.90</td>
<td>1.89±0.013</td>
</tr>
<tr>
<td>Maximum width of lateral surface</td>
<td>3.40</td>
<td>3.35</td>
<td>3.40</td>
<td>3.45</td>
<td>3.40±0.020</td>
</tr>
<tr>
<td>Maximum width of solar surface</td>
<td>1.70</td>
<td>1.70</td>
<td>1.75</td>
<td>1.75</td>
<td>1.73±0.014</td>
</tr>
<tr>
<td>Maximum width of coronet</td>
<td>1.20</td>
<td>1.20</td>
<td>1.25</td>
<td>1.25</td>
<td>1.23±0.014</td>
</tr>
<tr>
<td>Angle of hoof</td>
<td>48°</td>
<td>49°</td>
<td>48°</td>
<td>48°</td>
<td>48.3±0.25</td>
</tr>
</tbody>
</table>

Table 4: Different measurements and mean with standard error for hind hooves of goat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G₁ (cm)</th>
<th>G₂ (cm)</th>
<th>G₃ (cm)</th>
<th>G₄ (cm)</th>
<th>AM±SE (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of lateral border of solar surface</td>
<td>3.50</td>
<td>3.45</td>
<td>3.50</td>
<td>3.50</td>
<td>3.49±0.013</td>
</tr>
<tr>
<td>Length of medial border of solar surface</td>
<td>4.00</td>
<td>3.95</td>
<td>3.95</td>
<td>4.00</td>
<td>3.98±0.014</td>
</tr>
<tr>
<td>Length of interdigital border</td>
<td>3.80</td>
<td>3.80</td>
<td>3.75</td>
<td>3.80</td>
<td>3.79±0.013</td>
</tr>
<tr>
<td>Length of posterior surface</td>
<td>2.00</td>
<td>1.95</td>
<td>2.00</td>
<td>2.00</td>
<td>1.98±0.013</td>
</tr>
<tr>
<td>Maximum width of lateral surface</td>
<td>2.80</td>
<td>2.75</td>
<td>2.80</td>
<td>2.85</td>
<td>2.8±0.020</td>
</tr>
<tr>
<td>Maximum width of solar surface</td>
<td>2.20</td>
<td>2.20</td>
<td>2.25</td>
<td>2.25</td>
<td>2.23±0.014</td>
</tr>
<tr>
<td>Maximum width of coronet</td>
<td>0.40</td>
<td>0.40</td>
<td>0.45</td>
<td>0.45</td>
<td>0.43±0.014</td>
</tr>
<tr>
<td>Angle of hoof</td>
<td>60.4°</td>
<td>61°</td>
<td>61°</td>
<td>60.6°</td>
<td>60.75±0.15</td>
</tr>
</tbody>
</table>
Conclusion

On the basis of present findings the chital and the goat can be differentiated on the basis of hooves by taking different parameters in considerations especially the length of solar surface and the angle of hoof which can play an important role in Wildlife forensic.

References


BIOCHEMICAL ALTERATIONS AND THERAPEUTIC EFFICACY OF ASCORBIC ACID IN RECURRENT CASES OF BOVINE MASTITIS IN INDIA

Seema Tiwari\textsuperscript{1} and M.P. Gupta

Department of Epidemiology and Preventive Veterinary Medicine
Punjab Agricultural University, Ludhiana

ABSTRACT

A herd consisting of 80 animals was screened for clinical and subclinical mastitis on the basis of pH, SLST, SCC and CST. Six animals which were free from mastitis served as healthy control group, six animals which are affected with mastitis but didn’t get any treatment served as infected control group and other six animals which were affected with mastitis and given treatment with Inj. Redoxin 20 ml i/m s.i.d for 5 days served as treatment group. The treatment started on 7th day of observation. pH of milk ranged from 6.65 0.199 to 6.80 0.289, 7.39 0.376 to 8.18 0.203, 6.76 0.0745 to 7.75 0.359 in healthy control, infected control and treatment group, respectively. SCC ranged from 0.166 0.235 to 0.166 0.235, 11.1 14.20 to 12 13.14 and 33 15.45 to 1.67 1.67 lac cells/ml in healthy control, infected control and treatment group, respectively. The mean value of vitamin C level was 1.096 0.011 mg/dl to 1.0988 0.134 mg/dl, 1.006 0.0177 to 1.019 0.0193 mg/dl and 1.0081 0.0307 to 1.3888 0.0148 mg/dl, respectively, in healthy control group, infected control group and treatment group, respectively. Compared to the healthy control animals, there was 8% decrease in vitamin C level in infected control group. Four animals out of six were completely cured. This shows that vitamin C has got therapeutic efficacy in mastitis.

Key words: Mastitis, ascorbic acid, CST, SCC, pH

Introduction

Mastitis is a global problem of dairy farmers and is a serious hindrance in the development of dairy industry (Dhillon \textit{et al.}, 2000). In dairy animals it results in heavy economic losses mainly due to subclinical mastitis (Sena and Sahni, 2001) or economically mastitis is the most disastrous disease of dairy animals (Dhillon \textit{et al.}, 1995). The subclinical mastitis causes three times more loss than the clinical mastitis (Shukla and Supekar, 1982). Bovine mastitis is the single most common cause for antibiotic use in lactating dairy cattle (Moore and Heider, 1984; and Kaneene and Miller, 1992) and thereby one of the most common causes of illegal antibacterial residues in marketed milk (Erskine, 1996). Antibacterial therapy of bacterial induced disease in cattle has been incriminated as a catalyst for resistance in bacteria isolated form treated animals, other animals within the herd and food derived from cattle for human consumption (Berghash \textit{et al.}, 1983 and Griggs \textit{et al.}, 1994). Additionally, antibacterial use has been suggested as a selective force in determining the bacterial ecology of bovine mastitis (Myllys \textit{et al.}, 1994). Treatment of mastitis without antibiotic is preferred because of resistant effect of antibiotic leading to a threat to safe milk supply, high cost of antibiotic therapy per clinical case and development of resistance and cross resistance to various antibiotics among pathogens of both animals and human origin (Mellenberger, 1996). Also in view of WTO regulation, milk with antibiotic is not considered fit for processing in milk plant.

Free radicals can be extremely damaging to biological systems (Padh, 1991). Also, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy intracellular pathogens. However, these oxidative products can in turn, damage healthy cells if they are not eliminated. Antioxidants serve to stabilize these highly reactive free radicals thereby maintaining the structural and functional integrity of cells (Chew, 1995). Therefore, antioxidants are very important to immune defense and health of animals. Therefore, this study has been carried out to find out the efficacy of ascorbic acid in treatment of mastitis.

Materials and Methods

Eighty animals were screened for mastitis from a private dairy farm in Ludhiana region. Tests used for screening were pH, sodium lauryl sulphate test (SLST), somatic cell count (SCC) and culture sensitivity testing (CST). Thirty three animals were found positive for clinical mastitis, out of which, 12

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animals were randomly selected and divided into 2 groups of six animals each i.e. infected control group and treatment group. Six apparently healthy animals from the same herd were taken as healthy control group i.e., free from mastitis. Treatment has been done with inj Redoxin* (20 ml i/m s.i.d for 5 days). Pre-treatment sampling (blood and milk) has been done on 0th and 7th day and post-treatment sampling (blood and milk) has been done on 14th and 21st day of observation. Milk sample has been processed for estimation of pH (standardized pH meter), SLST (with freshly prepared sodium lauryl sulphate reagent), SCC, standard microscopic counting method of Indian standard institute (1960) and CST (Cruickshank et al., 1970) and blood sample has been processed for estimation of Vitamin C (Baker and Frank, 1968). Statistical analysis was done by using completely randomized design (CRD) and randomized block design (RBD) as per method of Snedecor and Cochran (1968).

Results and Discussion

History and symptoms

The animals were in 3rd to 5th lactation and recently calved. Hind quarters were infected more readily than fore quarters (60% vs. 40%). It might be due to more exposure of hind quarter to floor urine and dung and high milk content in the hind quarter than in pain response (purposeful lifting and kicking of the adjacent hind limb) during palpation of mammary gland was noted (Maunsell et al., 1998). There was presence of flakes in milk of most animals. Out of 6 animals, blood was present in milk of two animals. The consistency of milk was watery in three animals out of six.

Treatment group

The animals taken in this group were in first to third lactation, four to sixth month of calving and recently calved. All animals were previously treated with Lemox (ampicillin and cloxacillin) but there was reoccurrence of mastitis. The affected quarters were swollen, hot, oedematous and painful to touch in starting. After treatment, four animals out of six were cured and came in normal condition. The inflammation subsided and the animals become clinically normal. One animal out of six did not get cured. In one animal, the mammary gland got fibrosed.

pH

Healthy group

The mean value of pH in healthy group ranged from 6.65 0.2 to 6.80 0.3 (Table 1). There was non-significant variation in pH of milk during whole observation period. The observed pH value is in accordance to normal pH of milk which is 6.5 (Dhillon et al., 1989).

Infected control group

The mean pH of milk in infected control group animals ranged from 7.39 0.4 to 8.18 0.2 (Table 1) which is similar to finding of Dhillon et al. (1995); Pal et al. (1993) and Kamal et al. (1998). Higher is the pH of milk, the more severely affected quarter. Similar finding was observed by Dhillon et al. (1995). In mastitis, increased permeability of the gland to blood components brings about elevated pH, due to increase movement of bicarbonate ions into the milk (Schalm et al., 1971).

pH may be used as a reliable index of inflammation in case of mastitis (Kamal et al.,1998). There was no significant variation in pH during whole observation period. While comparing pH of milk of animals of this group to pH of milk of animals of healthy group at 5% Confidence Interval, there was significant difference (Fig. 1).

Treatment group

pH may be used as a reliable index of inflammation in case of mastitis (Kamal et al.,1998). There was no significant variation in pH during whole observation period. While comparing pH of milk of animals of this group to pH of milk of animals of healthy group at 5% confidence Interval, there was significant difference (Fig. 1).

Treatment group

The mean value of pH in treatment group ranged from 6.76 0.1 to 7.75 0.2 (Table 1). Treatment with Redoxin injection was given at 7th day of observation. The latter value indicates pretreatment value and former indicates post treatment value. There was significant difference in pH of milk during pre-treatment period and post treatment period at 5% confidence interval (Table 1). The change in pH value from 0 day to 7 day and 7 day to 14 day was significant but there was no significant change in pH value from 14-21 days (Table 1). This indicates that treatment had cured the animals.

While comparing pH value of infected control animals which are vitamin C deficient to animal of this group, there was non-significant difference during pre-treatment period but significant difference occurred during post treatment period (Fig. 1). There was significant difference between pH values of milk during whole observation period (Table 1) and while comparing with healthy group animals, there was significant difference. This finding is similar to finding of Dhillon et al. (1995) who found that there is reciprocal decrease in milk pH from 7.33 to 6.5 following treatment with tri sodium citrate.

Sodium lauryl sulphate test (SLST)

Healthy group
The animals gave negative test with SLS reagent. There was no gel formation when milk from the quarters was mixed with SLS reagent (Table 1). The milk sample gave 1+ which was categorized normal (Mulkalwar et al., 1999).

Infected control group
The milk of animals showed severe SLS reaction. Four out of six animals showed thick gel formation in affected quarter. Two out of six animals, showed moderate reaction. The SLS reaction categorized as subclinical ++, clinical +++ (Kuralkar et al., 2000). These animals belong to clinical group as they showed ++++ reaction. There was further increase in reaction from 0 day to 7 day and 7 day to 14 day (Table 2). But from 14 day onwards there was decrease in reaction (Table 2) or gland became fibrosed and no milk secretion occurred in two animals.

Treatment group
The milk of animals on 0 day showed severe reaction i.e. ++++. There was increase in reaction from 0 day to 7 day i.e. +++. The animals were treated with injection Redoxin afterward there was decrease in SLS reaction and it came to + in four animals out of six on 14 day post treatment (Table 3). This showed that treatment has got curing effect in animals of this group.

The milk of animals on 0 day showed severe reaction i.e. ++++. There was increase in reaction from 0 day to 7 day i.e. +++. The animals were treated with injection Redoxin on 7th day of observation afterward there was decrease in SLS reaction and it came to + in four animals out of six on 14 day post treatment.

Somatic cell count (SCC)

Healthy group
The mean value of SCC ranged from 0.16 0.2 lac cells/ml to 0.16 0.2 lac cells/ml of milk (Fig. 1). There was no significant variation in SCC of milk from quarter to quarter of these animals (Table 1). This finding is similar to findings of Munoz et al. (2002) and Maunsell et al. (1998). Four animals out of six were having nil SCC. Absence of SCC corresponds to absence of bacterial growth i.e. there is association between bacteriological growth and SCC.

Infected control group
The mean value of SCC varied from 11.1 14.20 to 12 13.14 lac cells/ml of milk (Table 1). There was no significant variation in SCC during whole observation period. There was slight decrease in SCC value from 0 day to 7 day. While comparing SCC value of milk of animals of this group with that of healthy group, there was significant difference at 5% confidence interval. Bakrema et al. (1998) categorized SCC as low (<1.5 lac cells/ml), medium (1.5-2.5 lac cells/ml) and high (2.5-4 lac cell/ml) bulk milk SCC.

Different mastitis pathogens elicit different SCC responses and in particular that associated with environmental organisms such as E. coli may result in high SCC for a short period of time.

Major pathogens (e.g. Streptococci, Coliforms) cause SCC over 600 x 10^3 cells/ml in cows (Dohoo and Meek, 1982). In this group similar results were found as causative organisms were mostly Gram negative bacilli.

The SCC value increases in mastitis and is directly related to bacterial load. The increase in SCC occurred because during inflammatory response, the animal's immune system is mobilized to protect the host and eliminate the pathogens (Mukherjee et al., 2004). Polymorphonuclear cells are major cell types found in mammary secretion during mastitis and play a major role in immune response in the udder (Paape et al., 1972).

Treatment group
The mean value ranged from 33.33 15.45 to 1.67 1.67 where former indicates SCC value during pre-treatment period and latter during post treatment period. There was significant difference between SCC value of 7th and 14th day at 5% confidence interval i.e. it has decreased from 33.33 15.45 to 4.75 3.73 lac cells/ml (Table 1). It indicates that treatment has got effect on mastitis. While comparing with SCC values of animals of infected control group, there was non-significant difference but there was significant difference between SCC values on different days of sampling within this group.

While comparing SCC value with healthy group there was significant difference.

Culture sensitivity test (CST)

Healthy group
There was no bacterial growth when milk samples were streaked on trypticase soya agar media and blood agar media. Clinically negative glands were bacteriologically negative at four sampling times. This finding is similar to finding of Maunsell et al. (1998).

Infected control group
The organisms Escherichia coli were isolated from milk sample of two animals and no growth found in milk sample of three animals. Waage et al. (1999) isolated E. coli in 6.4% of the milk samples in cows.

The organisms were mostly sensitive to Pefloxacin and Gentamicin. Intermediate sensitivity was found for polymyxin B and tetracycline. They were resistant to neomycin, ampicillin, streptomycin and amikacin (Table 1). Similar finding were observed by Mallikeswaran and Padmanabhan (1990) where E. coli were sensitive to oxytetracycline, neomycin and trimethoprim.
Two isolates of E. coli species and one isolate of Kleebsiella species were identified in milk samples of animals but in one animal there was no growth. This finding was similar to finding of Dhillon et al. (1995) who isolated E. coli, Kleebsiella and Staphylococcus spp. from mastitic milk samples. Isolation of Gram -ve bacteria as major pathogen occurred due to previous antibiotic treatment with lemox which causes inhibition of growth of Gram +ve bacteria and so Gram -ve bacteria predominates. The isolates were susceptible to pefloxacin and gentamicin, intermediately sensitive to neomycin and polymixin B and tetracycline and resistant to ampicillin, streptomycin and amikacin (Table 1).

Vitamin C
Healthy group
Mean vitamin C level in plasma ranged from 1.09 0.1 mg/dl to 1.09 0.1 mg/dl (Table 1). There was no significant variation in vitamin C level during whole observation period. This finding is similar to finding of Chaiyotwittayakun et al. (2002) who states that mean ascorbic acid concentration rapidly increased after the second infusion of vitamin C injection.

Infected control group
The mean value of vitamin C ranged from 1.02 0.1 to 1.02 0.1 mg/dl (Table 1). This finding is in accordance with Sharma (2001). While comparing with healthy group, there was significant difference. It showed the animals of this group were vitamin C deficient. There was significant difference between vitamin C level of animals within the group during whole observation period (Fig. 3).

Ascorbate functions as antioxidants by neutralizing the peroxidase and other free radicals responsible for tissue injury thus protecting cells of body. Beside its anti-oxidant properties, ascorbate also has a pre-oxidant role to activate...
Fig. 1: Comparison of pH of milk of animals of different groups.

Fig. 2: Comparison of SCC of milk of animals of different groups.

Fig. 3: Comparison of vitamin C level of animals of different groups.
myeloperoxidase inside the neutrophils and thus enhancing the immune response.

**Treatment group**

The mean value of vitamin C ranged from 1.01 to 1.39 mg/dl (Table 3). The former indicates vitamin C level during pre-treatment period and latter indicates vitamin C level during post treatment period. There was significant difference between pre-treatment and post treatment value i.e. which indicates positive effect of treatment. Antioxidant treatment may have cured the epithelium of teat canal and mammary gland of mastitis affected animals. Integrity of epithelium has been maintained as no cases of mastitis were recorded after treatment.

While comparing with infected control group, there was significant difference. There was also significant difference between vitamin C level of animals within the group during whole observation period. While comparing with healthy group, there was significant difference (Fig. 3).

Further ascorbate, a cofactor in the hydroxylation reaction of proline and lysine, is also involved in the maintenance of natural barrier. Thus a depression in udder defence due to high production and lower ascorbic acid concentration may initiate multiplication of mastitogens (Singh and Pachauri, 2003).

**Conclusion**

pH, SLC and SCC can act as good barometer for detection of subclinical mastitis. Keeping in view the isolation of E.coli in the most of the milk samples indicates emergence of environmental mastitis in place of contagious mastitis. So, the cleanliness of farm is must for decreasing the incidence of mastitis. Sterile cases of galactorrhagic mastitis can be effectively treated with ascorbic acid. Ascorbic acid treated animals become clinically normal but bacteriologically they remain positive. We can forecast the incidence of mastitis in a particular herd by estimating vitamin C level in plasma of animals.

**References**


ANTIOXIDANT POTENTIAL OF **ALLIUM SATIVUM** (GARLIC) IN IRON-INDUCED OXIDATIVE STRESS#

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Division of Medicine  
Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, India

ABSTRACT

The present investigation was carried out to prophylactic efficacy of *Allium sativum* in mitigating oxidative stress in iron-overloaded rabbits. A total of 15 New Zealand white female rabbits were divided into three equal groups. Animals of group I received no treatment and served as healthy control throughout the experimental period of 28 days. The animals of group II received iron intramuscularly on alternate days starting from day 1 to day 14. The animals of group III received juice of *A. sativum* orally for a period of 28 days in addition to iron injection as in group II. The blood samples were collected at weekly intervals to assess blood iron concentration, oxidative stress indices viz. lipid peroxide, superoxide dismutase and catalase and change in haematocrit values. Oral feeding of *Allium sativum* alleviated oxidative stress by inhibition of lipid peroxide levels and decreased in antioxidant enzymes activity.

**Key words**: Antioxidant, oxidative stress, *Allium sativum*, iron

Introduction

Oxidative stress, antioxidants, free radicals and certain dietary supplements have received considerable interest during the recent years for their role in the process of ageing and pathogenesis of certain diseases like cancer, cardiovascular problems, arthritis and diabetes etc. (Bulkley, 1993). In many disease conditions, free radical production is secondary to the initial disease process and oxidative damage exacerbates the primary lesions (Halliwell *et al*., 1992). Iron is an essential constituent of the body and is believed to catalyze free radical mediated oxidation of cellular proteins, DNA and membrane lipids (Aust *et al*., 1985, Britton *et al*., 1987). Several dietary substances present in plants have been reported to act as superoxide scavenger and inhibit lipid peroxidation (Singh, 1997; Khajuria *et al*., 1997). *Allium sativum* has been used against various disease conditions both in humans and animals, since antiquity (Aviello *et al*., 2009) and reported to scavenge free radical, hydroxyl radicals and reactive oxygen species (Imai *et al*., 1994; Yamasaki *et al*., 1994 and Reitz *et al*., 1995).

Geng *et al*., (1997) has reported antioxidant effect of aged garlic through modulation of the GSH redox cycle and SOD activity in vascular endothelium. Limited information is available on use of herbal antioxidants in mitigating iron-induced oxidative stress. The present study was, therefore, undertaken to evaluate the antioxidant potential of *A. sativum* in iron overloaded rabbits.

Materials and Methods

The experiment was conducted on fifteen (15) New Zealand white adult female rabbits procured from IVRI Mukteshwar campus and Division of Laboratory Animal Resource of IVRI, Izatnagar. The animals were maintained in individual rabbit cages and were acclimatized for a month before starting the experimentation. They were also given coocidiostats and were maintained on standard ration and *ad lib* water.

The animals were divided into three groups viz., I, II and III, each consisting of 5 animals and received different treatments over a period of 28 days. Group I received no treatment and served as negative healthy control during the study period. Group II received prooxidant only and served as positive (iron overloaded stress) control. Iron sorbitol citric acid complex (*Jectofer®*) was given @ 120 mg/kg b. wt./ day i/m on alternate days starting from day 1 to 14 of the experimental period to induce oxidative stress.

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³Professor and Head, Clinical Medicine, Orissa University of Agriculture and Technology, Orissa

*Jectofer—Marketed by CFL laboratories containing 50 mg of elemental iron per ml as iron sorbitol citric acid complex.
Group III received prooxidant along with juice of *A. sativum* bulbs root @ 100 mg/kg b.wt. orally for a period of 28 days (day 1 to 28) once daily. The fresh bulbs of garlic were procured from the local market and were crushed with a grinder after proper cleaning. The extract was collected through a four layered muslin cloth after squeezing the crushed material over the cloth layer. The gram equivalent of garlic was obtained after dividing the weight of fresh garlic bulbs by the amount of juice obtained. The juice was reconstituted in triple glass distilled water in such a way that each ml of solution was meant for one kg body weight and was kept in air tight containers under refrigeration (4°C).

Blood samples were collected directly by cardiac puncture from all the experimental animals on day 0 and thereafter at weekly intervals (day 7, 14, 21 and 28). Approximately, 2 ml blood was collected in heparinized vials, 1 ml of which was used immediately for haematobiochemical analysis, and 1 ml was stored at -20°C in nitric acid washed heparinized vials for digestion and estimation of iron concentration.

The blood samples were centrifuged at 200 rpm for 10 minutes and the supernatant was discarded. The sediment cells were washed and centrifuged three times with 0.9% NaCl solution. The freshly washed erythrocytes were haemolysed with distilled water (1:10). Lipid peroxide level (LPO) in the haemolysate was determined following the method of Placer *et al.* (1966). Briefly, 0.2 ml of RBC haemolysate was added to 1.3 ml of 0.2 M Tris 0.16 M KCl buffer of pH 7.4 after which 1.5 ml of TBA reagent was added and the mixture was heated in boiling water bath for 10 min using glass beads as reagent. After cooling, 3 ml of pyridine/nbutanol (3:1 v/v) and 1 ml of 1N NaOH were added to it and mixed by shaking. Blank was prepared by taking 0.2 ml of distilled water instead of RBC haemolysate. The absorbance was read at 548 nm and the nmol of absorbance by 0.05 units.

The activity of antioxidant enzymes was estimated in 10% RBC haemolysate after proper dilution by the method of Cohen *et al.* (1970). The reaction was started by addition of 50 µl of diluted sample to 3 ml of phosphate buffer- H₂O₂ solution. Initial absorbance of 240 nm was read after 20 seconds against reference cuvette in which same amount of distilled water was added in place of H₂O₂. Time required for the initial absorbance to decrease by 0.05 unit was noted and catalase activity in the assay mixture was calculated using following formula:

\[
\text{Catalase (units/assay mixture)} = \log \frac{E_1}{E_2} \times \frac{2300/6.93 \times 1}{\Delta t}
\]

\[
E_1 = \text{Initial absorbance of assay mixture}
\]

\[
E_2 = \text{Absorbance after decrease}
\]

\[
\Delta t = \text{Time required in seconds for decreased of absorbance by 0.05 units}
\]

Catalase activity in erythrocytes was expressed in units/mg of Hb.

Haemoglobin in 10% RBC haemolysate was estimated colorimetrically by cyano-met haemoglobin method (VanKampen and Ziglstra, 1961). The values were estimated in mg/ml of haemolysate.

Packed Cell Volume (PCV) was estimated in blood samples by microhaematocrit method (Coles, 1980). Blood samples were digested as per the procedure described by Kolmer *et al.* (1951). Briefly, 1 ml of sample was mixed with 5 ml of conc. HNO₃ in 50 ml digestion tube. The samples were kept overnight at room temperature for cold digestion. Next day, the tubes were heated (below 90°C) using a microdigestion bench. The samples were allowed to digest slowly. This procedure was repeated till the solution became transparent like water and the volume reduced to 1 ml, approximately. Final volume was made with distilled water after warming the digested material. Iron content in the digested blood samples was measured colorimetrically (Sandell, 1959).

The data were analysed statistically using two way analysis of variance and paired t-test to find out significance of difference in mean values (Snedecor and Cochran, 1975).

**Results and Discussion**

The iron injected rabbits of group II showed a significant (P<0.05) increase in their blood iron levels with the peak reaching on day 14. However, the increase in blood iron concentration was comparatively lower in group III rabbits co-treated with garlic juice. The animals of group II exhibited...
### Table 1: Blood iron, haematocrit and oxidative stress indices in different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Iron (mg/dl)</td>
<td>Group I</td>
<td>36.13 ±1.88</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>35.70 ±1.47</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>34.73 ±2.72</td>
</tr>
<tr>
<td>LPO (nmol of MDA/ mg of Hb)</td>
<td>Group I</td>
<td>5.30 ±0.19</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>5.02 ±0.47</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>4.09 ±0.21</td>
</tr>
<tr>
<td>SOD (U/mg of Hb)</td>
<td>Group I</td>
<td>9.94 ±1.07</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>10.64 ±0.91</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>11.31 ±1.18</td>
</tr>
<tr>
<td>CAT (U/mg of Hb)</td>
<td>Group I</td>
<td>34.20 ±2.44</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>32.41 ±2.54</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>30.46 ±1.75</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>Group I</td>
<td>38.80 ±1.02</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>39.60 ±1.47</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>39.20 ±0.80</td>
</tr>
</tbody>
</table>

LPO—Lipid peroxide; SOD—Superoxide dismutase; CAT—Catalase; PCV—Packed cell volume
Group I—Negative control; Group II—Positive control; Group III—A. sativum treated
The values (Mean±SD) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly (P<0.05) for a parameter.

### Table 2: Per cent change in blood iron, haematocrit and oxidative stress indices in different groups between day 0 and 28 of the study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>% changes in values (between day 0 and 28) in different groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>Iron</td>
<td>†2.23%</td>
</tr>
<tr>
<td>LPO</td>
<td>†0.4%</td>
</tr>
<tr>
<td>SOD</td>
<td>†0.76%</td>
</tr>
<tr>
<td>CAT</td>
<td>†0.19%</td>
</tr>
</tbody>
</table>

LPO—Lipid peroxide; SOD—Superoxide dismutase; CAT—Catalase
Group I—Negative control; Group II—Positive control; Group III—A. sativum treated
† - increase; † - decrease
26.55% increase in the lipid peroxide levels between day 0 and 28 as compared to 23.28% in garlic treated group. The antioxidant enzymes viz. superoxide dismutase and catalase showed an increase up to day 14 followed by a decline in the garlic treated group. No significant difference was observed in haematocrit values of all the groups at different observation period. A slight increase in the haematocrit values was observed in the groups II and III after iron administration (Table 1 and 2).

Results of the experiment indicated that the juice of *A. sativum* successfully reduced oxidative stress and reduced blood iron concentration. The antioxidant potential of garlic has been suggested by many workers (Ide *et al.*, 1996; Geng *et al.*, 1997; Lee *et al.*, 2009). The five diallyl polysulphide components obtained from garlic were reported to inhibit lipid peroxidation whereas the aged garlic extract has been found to inhibit early formation of thiobarbituric acid reactive substances in vitro (Horie *et al.*, 1992; Imai *et al.*, 1994; Vidyashankar *et al.*, 2010). The protective role of garlic in the present study in iron-induced oxidative stress might be due to either inhibition of membrane lipid peroxidation or due to free radical scavenging effect.

References
PATHOLOGICAL AND HEMATO-BIOCHEMICAL OBSERVATIONS OF DERMATOMYCOSES IN CAMEL (CAMELUS DROMEDARIUS)

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ABSTRACT
In the present study, a total of one hundred eighty seven skin biopsies showing frank macroscopic lesions were collected from camel. Histopathological aspects of these skin problems were studied along with haematobiochemical parameters and isolation of organisms. These samples were processed mechanically for paraffin embedding by acetone and benzene technique. The dermatomycoses was found in 4.28 per cent cases. Haematobiochemical observations revealed increase in haemoglobin, TLC, MCHC, neutrophil count, eosinophil count and decrease in lymphocyte count and serum glucose levels. Microsporum gypseum, Microsporum distortum and Aspergillus fumigatus were isolated from the positive cases. Grossly, hard and fibrous crusts and papules alongwith alopecia were found on the skin. Microscopically, there was thickening of epidermis with rete pegs extending downwards. The lesions consisted of tissue debris, inflammatory infiltration predominantly of mononuclears alongwith fungal hyphae confirmed with Grocotts methanamine silver stain gives characteristic black colour to them.

Key words: Camel, dermatomycosis, histopathology, haematology

Introduction
The camel has been a necessity of mankind in desert and semi-desert areas. It plays a very important role in transportation and production of milk, meat, wool, hair and hide in these areas. In recent recurring droughts in state where there had been huge loss of livestock especially the cattle and to a lesser extent of sheep and goats, camel remained marginally affected. The skin is the largest organ of the body and the anatomic and physiological barrier between the animal and environment. The skin infections are caused by bacteria, viruses, parasites and fungi. Mycotic dermatitis caused by fungi of two genera- Microsporum and Trichophyton. According to Bucek et al. (1992) it has zoonotic importance. Dermatophilosis is transmitted to man by contact with the infected animals. In this study, incidence of dermatomycoses was confirmed by histopathological examination along with haematobiochemical parameters and with the identification of specific etiological factors.

Materials and Methods
For the present study, 187 camels showing frank skin lesions were examined to identify the various disease conditions, which are commonly prevailing in camels. Histopathological aspects of these skin problems were studied along with the haematobiochemical parameters and isolation of fungal organisms by standard techniques. The areas of skin showing lesions were preserved in 10 per cent formal saline and processed mechanically for paraffin embedding by acetone and benzene technique (Lillie, 1965). The sections of 4-6 micron thickness were cut and stained with haematoxylin and eosin method of staining. Grocotts methanamine-silver (GMS) staining used to highlight the fungal hyphae in the tissue sections. The blood samples were also collected for haematobiochemical studies of these cases by standard techniques.

Results and Discussion
In the present study, dermatomycoses was found in 8 cases (4.28 per cent) out of 187 cases whereas, observations of other scientists such as Khamive, 1982 and Agab, 1988 were higher than the present observations.

Haematobiochemical studies
Increase in haemoglobin, TLC, MCHC, neutrophil count, eosinophil count and decrease in lymphocyte count and serum glucose were recorded. The decrease in serum glucose recorded in positive cases of fungal infection in camels included in study could possibly be due to utilization of glucose by the

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³Contractual Teacher, Department of Clinical Veterinary Medicine, Ethics and Jurisprudence, CVAS, Bikaner-334001
fungus and this finding agrees well with the report of Ibrahim et al. (1984) suggested a decrease in blood glucose, total protein and A:G ratio. A non-significant decrease in A:G ratio have also been recorded in present study in comparison to normal healthy animals but instead of decreased serum total protein, it was found to be unaffected of fungal infection in this study in contrast to the work of Ibrahim et al. (1984). Except for the TLC and neutrophil count, though the other haematological parameters revealed significant variation in comparison to normal healthy animals but were well within the normal physiological range suggested by Khanna (2002) in dermatomycoses in dogs.

Fungal isolation
In the present study Microsporum gypseum, Microsporum distortum and Aspergillus fumigatus were isolated. These findings were in line with Das (2001) and Khanna (2002).

Pathological studies
Grossly, hard and fibrous crusts and papules alongwith alopecia were found on the skin, these findings were similar to those recorded by Chatterjee et al. (1977). Arora et al. (1973) biopsied tissue of two camels, the tissue was hard and fibrous and revealed yellowish white purulent foci which were granulomatus. Whereas, Boever and Rush (1975) observed circumscribed, crusty, hairless lesions on the shoulder and foreleg in Microsporum gypseum infection in a dromedary camel which are similar to the findings of present study. Microscopically, there was thickening of epidermis with rete pegs extending downwards. The lesions consisted of tissue debris, inflammatory infiltration predominantly of mononuclears alongwith fungal hyphae (Fig.1). There were marked hyperkeratosis, parakeratosis and acanthosis in the epidermis. Duplicate sections stained with Grocott's methanamine silver stain gives characteristic black colour to the fungal hyphae (Fig.2). These findings were similar to those recorded by Khamiev and Kubentaeva (1984).

Acknowledgements
The authors are thankful to the Dean, College of Veterinary and Animal Science, Rajasthan Agricultural University, Bikaner for providing necessary facilities.

References
HAEMATO-BIOCHEMICAL EFFECT OF LOW LEVEL LASER IRRADIATION THERAPY IN DOGS

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Madhya Pradesh, INDIA

ABSTRACT

The study was conducted on 18 clinical cases of dogs, divided into three groups comprising of 6 animals in each group. The Group-I animals were treated with antiseptic dressing + parenteral antibiotic, Group II-animals were treated with He-Ne laser (10Hz + 5min, 3 Joules) + dressing + parenteral antibiotic and Group III - animals were treated with He-Ne laser (20Hz + 10min, 6 Joules) + dressing + parenteral antibiotic. The haematobiocchemical studies were done on days 0th, 3rd, 7th, 10th, and 14th. The TEC, Hb, and PCV value did not have any significant differences between the groups and with in groups. A highly significant (P< 0.01) increased was observed in the TLC in laser treated group, but value were with in the normal range. A significant decrease in the TLC was observed as the wound healing progressed. No significant differences were observed in the DLC but values of lymphocytes increased within normal range. The value of total protein, blood glucose and ALT did not show any significant differences between the groups and with in groups. The haematological and biochemical parameters remained unaltered by LLLT, suggestive of no adverse effect of laser therapy on vital functions of the body, making it a safer therapeutic modality free from any side effects.

Key words: Haemato-biochemical parameters, laser therapy, dogs.

Introduction

There is always a need of therapy which can accelerate the rate of wound healing, without affecting the normal physiological process involved in wound healing to evolve a safer and effective with minimal side effects. The low energy laser radiation was found to have a stimulating effect on cells and high energy radiation had an inhibitory effect (Mester et al., 1985).

Materials and Methods

The present study was undertaken in 18 dogs which were grouped into three groups irrespective of age, sex, breed, and location of wound. Thus, each group was consisting of 6 animals. Group I-Dressing of contaminated wound with antiseptic liquid and ointment (povidine iodine) + parenteral antibiotic (Ceftriaxone) dose 7 mg/kg b. wt., was given i/m at the 12 hrs., interval for 5 consecutive days. Group II-Dressing of contaminated wound + LLLT (10Hz + 5min, 3 Joules) + dressing + parenteral antibiotic (as group I). Group III- Dressing of contaminated wound + LLLT (20 Hz + 10 min, 6 Joules)+ parenteral antibiotic (as group I).

Instruments

EC Laser- 306 Machine having Laser tube of He-Ne + Infrared with a wavelength of 632.8 nm. Power output of 10 mW and Laser diode of 830 nm was used for the treatment. Frequency and amplitude on X and Y axis was set according to wound. The energy (joules) was calculated using following formula.

\[ \text{Energy (Joules)} = \text{power (Watt)} \times \text{time (seconds)} \]

Haemato-biochemical parameters

Five blood samples from each dogs was collected at 0th day, 3rd day, 7th day, 10th day and 14th day for the estimation of haematological parameters, total erythrocytes count (TEC), haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count (DLC). For haematology blood was collected in vials containing anticoagulant EDTA @ 2 mg/ml of blood. All parameters were studied as per the procedure Described by Jain (1986). Blood glucose was estimated by Glucose Oxidase (GOD/POD) method using Merckotest reagent kit as described by Trinder (1969). Alanine aminotransferase (ALT) were determined by Reitman and Frankel (1957) method of kit (Glaxo).

The data was analyzed by using simple analysis of variance to test the different among the interval under each group, the standard procedure as outlined by Snedecor and Cochran (1994).

Results and Discussion

There were no significant differences in the TEC, Hb, and PCV value between the groups and within groups. A highly significant (P< 0.01) increased was observed in the TLC in laser treated group, but value were with in the normal range. A significant decrease in the TLC was observed as the wound healing progressed. No significant differences were observed in the DLC but values of lymphocytes increased within normal range. The value of total protein, blood glucose and ALT did not show any significant differences between the groups and with in groups. The haematological and biochemical parameters remained unaltered by LLLT, suggestive of no adverse effect of laser therapy on vital functions of the body, making it a safer therapeutic modality free from any side effects.
haemoglobin concentration and PCV between the groups or within group. All the values were the normal range. Inspite of this, a definite increase in its value was observed in the all groups, as the wound healing progressed due to generalized activation of sympatho-adrenal mechanism and haematocrit values were on normal range in all groups this indicate normal hydration status of animals.

There were statistically significant difference TLC between the groups and with in groups. Increased of total leukocytes count due to inflammatory response of wound but all the values fell within the normal limits. This indicated that all the cases under study have been selected, when they were in the repair phase of healing. In this stage the inflammatory reaction had subside which correlated with the total leukocyte counts on day 0th. The higher values in the laser treated groups could not be indicated as a ‘laser induced leukocytosis’ since these values were also within the normal limits. The pattern of reduction in these values during the course of study indicated that there was a direct correlation between the local inflammatory response

Table 1: Mean Values (±SE) of haematological parameters at different time intervals, in three groups of dogs.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Periods (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEC (million/ cu mm)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>6.68±0.12</td>
<td>6.74±0.10</td>
</tr>
<tr>
<td>II</td>
<td>6.68±0.09</td>
<td>6.71±0.07</td>
</tr>
<tr>
<td>III</td>
<td>6.71±0.07</td>
<td>6.86±0.06</td>
</tr>
<tr>
<td>Hb(g/dl)</td>
<td></td>
<td>13.38±0.25</td>
</tr>
<tr>
<td>I</td>
<td>13.40±0.19</td>
<td>13.46±0.14</td>
</tr>
<tr>
<td>II</td>
<td>13.46±0.14</td>
<td>13.68±0.11</td>
</tr>
<tr>
<td>III</td>
<td>40.26±0.77</td>
<td>40.58±0.63</td>
</tr>
<tr>
<td>II</td>
<td>40.33±0.55</td>
<td>40.50±0.42</td>
</tr>
<tr>
<td>III</td>
<td>40.50±0.42</td>
<td>41.16±0.30</td>
</tr>
<tr>
<td>PCV (%)</td>
<td></td>
<td>12.31±0.07</td>
</tr>
<tr>
<td>I</td>
<td>13.33±0.15</td>
<td>13.41±0.15</td>
</tr>
<tr>
<td>II</td>
<td>13.32±0.14</td>
<td>13.33±0.15</td>
</tr>
<tr>
<td>TLC (Thousand/ cumm)</td>
<td></td>
<td>71.33±0.33</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>I</td>
<td>71.16±0.40</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>I</td>
<td>21.16±0.30</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>I</td>
<td>3.66±0.21</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>I</td>
<td>3.33±0.33</td>
</tr>
</tbody>
</table>
| Group I (n=6) - Dressing of wound + Parenteral Antibiotic.
Group II (n=6) - Dressing of wound + LLLT (10Hz + 5min, 3 Joules) + Parenteral Antibiotic.
Group III (n=6) - Dressing of wound + LLLT (20Hz + 10min, 6 Joules) + Parenteral Antibiotic.
Mean under same superscript in rows did not differ significantly (p < 0.01).
and the white blood cells in systemic circulation. All the values of neutrophils, monocytes and eosinophils were within the normal range and there were no significant differences between the groups or within the groups but lymphocytes values of laser treated group marginal increase but in normal range and they decreased during course of study. This increase might be suggestive of their participation in some immune reactions towards the later stages of wound healing.

Mester et al. (1978) reported that laser rays caused suppression of some undesirable immune reaction. Stadler et al. (2000) they reported lymphocyte proliferation significantly higher with an increase in free radical and lipid peroxide production.

There were no significant differences in the total protein between the groups or within groups.

Fowler (1993) opined that hypoproteinaemia delayed wound healing. But Probst (1993) reported that rate of wound healing was not well correlated with plasma protein levels, unless the serum protein concentration was less than 2 g/dl.

The values in the laser treated group were lower than the first group, but none of them came down to 2 g/dl. Thus, not only were the levels inadequate to cause delayed healing, the laser treated group had a faster rate of wound healing compared to the first group.

Thus, it seemed appropriate to agree with the statement made by Probst (1993), that the rate of wound healing was not well correlated with plasma protein levels. Above studies are indicated that low level laser therapy has no influence on protein concentration, neither it releases exudates nor the organ synthesizing proteins were affected. There was no statistically significant difference in the blood glucose values between the groups or within the groups. There was concluding all animal take during course of study are healthy.

There was no statistically significant difference in the ALT values between the groups or within the groups. Inspite of this, a definite reduction in its value was observed in the all groups, as the wound healing progressed. It was conclude that other enzymes need to be studied that can be used in conjunction with ALT to correlated with wound healing. Other enzymes specific to liver damage and soft tissue damage need to be compared to come to conclusion as to which enzymes could be a specific indicator for wound healing.

References


STUDIES ON CLINICO-PHYSIOLOGICAL AND HAEMATO-BIOCHEMICAL CHANGES FOLLOWING EPIDURAL ANALGESIA BY ROPIVACAINE AND ROPIVACAINE-XYLAZINE COMBINATION IN GOATS

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ABSTRACT

Anaesthetic effect of ropivacaine (0.6 mg/kg) and ropivacaine-xylazine combination (0.6 mg/kg-0.5 mg/kg) was evaluated by clinico-physiological and haemato-biochemical parameters in 6 goats after their administration in lumbosacral epidural space. The onset of analgesia was faster in animals in which ropivacaine-xylazine combination was used. The physiological parameters (pulse rate, respiratory rate and rectal temperature) did not show any significant change in all the animals in which ropivacaine was used as compared to animals in which ropivacaine-xylazine was used, which showed significant decrease in all the three physiological parameters. Among the haemato-biochemical parameters (Hb, TLC, DLC, PVC, TEC, glucose, total protein, ALP, ALT, total bilirubin, BUN and creatinine) Hb and PCV showed significant decrease in animals in which ropivacaine-xylazine was used. Blood glucose showed significant increase in both the groups. Total protein, ALP, ALT, BUN and creatinine showed significant increase in animals in which ropivacaine-xylazine was used while as these remain unaltered in the animals in which ropivacaine alone was used. Therefore, ropivacaine (0.6 mg/kg) may be used in clinical situations in the patients having impaired kidney and liver functions.

Key words: Ropivacaine, xylazine, anaesthesia

Introduction

One of the most common ways of analgesia in small ruminants, especially goats is epidural method as ruminants are generally not considered good subjects for general anaesthesia mainly because of hazards of regurgitation of ruminal contents and saliva into lungs. Therefore, epidural analgesia mainly employed in this species. Ropivacaine is a newer and potent long acting local anaesthetic agent, which has been used for epidural analgesia in dogs. (Otero and Bonafine, 2000) and buffalo calves (Amarpal et al., 2002). It has minimal effects on cardiovascular and renal functions. Xylazine is a potent alpha-2 agonist. The alpha-2 receptors are present in the smooth fibres of blood vessels. Xylazine is used as muscle relaxant, sedative and an analgesic drug. Epidural xylazine inhibits impulse conduction at adrenoreceptors in the spinal cord and CNS (Yakash, 1981). Epidural use of xylazine can enhance the analgesic effects of other agents given epidurally.

Research is going on mixing of two anaesthetic agents to take the advantage of both the drugs. The present study was, therefore, designed to evaluate efficacy of ropivacaine alone and ropivacaine-xylazine combination in goats.

Materials and Methods

The study was conducted on 6 healthy local non-descript breed of goats of same sex (male) weighing between 10-12 kg. All the goats were dewormed prior to experiment by administering the bolus Fenbendazole orally at the dose rate of 5 mg/kg of body weight. All the goats were maintained under similar standard managemental conditions and feeding schedule. Each experimental animal was subjected to 2 treatments and each treatment lasted for 3 days. An interval of 8 days after the first treatment was given before the onset of the next treatment. Each animal was subjected to two treatments at an interval of 8 days. In treatment I, ropivacaine alone was given @ 0.6 mg/kg b.wt. in the lumbosacral space and in treatment II...
ropivacaine - xylazine combination was given @ (0.6mg/kg - 0.5 mg/kg) b.wt. in the lumbo-sacral space.

The epidural catheter was placed at the lumbo-sacral space for the delivery of ropivacaine and ropivacaine-xylazine. The rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute) were recorded at 0, 20, 40, 60, 80,120,180, 240, 300, 360, 480 minutes. The “0” hr values from each animal was recorded immediately before the start of the treatment, as the control value. The onset and duration of analgesia and recovery was determined by the pin prick method.

The onset of analgesia was recorded by noting the time of loss of anal reflexes. The duration of analgesia was ascertained by noting the time of reappearance of anal reflexes. Complete recovery from analgesia was the interval between the onset of complete effect of analgesia and total regression of analgesia. The duration of analgesia was the interval between complete effect and the first sign of regression of analgesia. Five millilitre of blood was collected from each animal from jugular vein. About 2 ml of blood was poured in a sterile vial containing anticoagulant (EDTA) @ 2 mg/ml of blood for haematological studies. Remaining blood was collected in a centrifuge tube and was allowed for clotting. After clotting it was centrifuged @ 2500 rpm for 10 minutes and the serum was collected in sterile vials and kept at -20°C till biochemical estimation.

Eight blood samples from each animal for each treatment was collected at 0 hr.,15 min., 3 hrs., 6 hrs., 12 hrs., 24 hrs., 36 hrs., and 72 hours for estimation of hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differentials leukocyte count by standard methods. Blood glucose (GOD-POD method), total protein (Biuret method), ALP (Tris-Carb method), total bilirubin (Jendrassic and Grof method), BUN (Young’s method) and Serum Creatinine (GLDH-Urease method) were also estimated at same intervals. The data obtained during study was analyzed by employing completely Randomized Design (CRD) as described by Snedecor and Cochran (1994).

Results and Discussion

The onset of analgesia was faster in treatment II (1.26 minutes) as compared to treatment I (2.50 minutes) in which ropivacaine was used alone. Khan et al. (2002) also recorded faster analgesia with epidural bupivacaine-xylazine as compared to bupivacaine alone in dogs. Duration of analgesia was longer in treatment II (180 minutes) as compared to treatment I (110 minutes). This shows some synergistic effect of ropivacaine and xylazine when used in combination. Adetunji et al. (2002) also used Xylazine with bupivacaine and duration of analgesia recorded was 190.00 minutes. Singh et al. (2005) used 0.2% of Ropivacaine in healthy goats and duration of analgesia was 71.5±4.4 min. The animals who received ropivacaine recovered quickly as compared to animals who received ropivacaine and xylazine.

In treatment I the values of respiration rate fluctuated non significantly between 20 to 480 minutes. In treatment II, there was a significant (P<0.05) decrease in respiration rate from the control value and reached to its minimum value at 120 minutes after epidural administration of drugs. Thereafter, values started increasing and reached to at 480 minute. Significant decrease in respiratory rate is also reported by Kinjavedkar et al. (2000) and DeRossi et al. (2003) after epidural administration of xylazine in goats and claimed it to be due to depression of respiratory centre by these drugs. Adetunji (2002) also reported significant decrease in respiration rate after using bupivacaine-xylazine in goats. Singh et al. (2005) did not find any significant change in respiration rate after epidural administration of ropivacaine in goats. So in the present study, decrease in respiration rate in treatment II was observed which may due to depression of respiratory centre by xylazine (Lumb and Jones, 1984). There was no decrease in respiration in ropivacaine group suggestive of its superiority over other two groups.

In treatment I, there was non-significant (P>0.05) decrease in rectal temperature. In treatment II, there was a significant (P<0.05) decrease in rectal temperature from 0 to 80, there after rise in temperature was noticed and the value reached to pre treatment value at 480 minute. The present findings for rectal temperature is in accordance with Kinjavedkar et al. (2000) who observed the significance decrease in rectal temperature following subarachnoidally administered of xylazine in goat. Singh et al. (2005) used 0.2% ropivacaine in goats and they also observed non significant decrease in rectal temperature. In the present study the significant decrease in rectal temperature in treatment II, might be due to activation of Alpha-2 agonists and hypothalamus alpha-receptor, inhibiting the heat conserving mechanism. Reduced metabolic rate and muscle activities might have been resulted in production of less heat in body and depression of thermoregulation resulted in hypothermia (Ponder and Clark, 1980). Hence it can be concluded that ropivacaine does not have any adverse effect on thermoregulatory centre in hypothalamus.

There were non significant (P>0.05) alterations in pulse rate in treatment I. In treatment II, the values decreased significantly from 0 to 120 minute and
there after value started increasing from 180 minute and reached to pretreatment value at 480 minute. Singh et al. (2005) observed non significant changes in pulse rate after using epidural ropivacaine in goats. Dhage and Pawshe (2010) used epidural xylazine in goats and observed that there was significant decrease in pulse rate attributing inhibitory action of xylazine on cardiovascular system of goats.

Among haematological parameters (Hb, TEC, TLC, PCV and DLC), Hb and PCV showed significant (P<0.05) decrease in treatment II, while as other values remain unchanged while as in treatment I there was no alteration in these values during the study period. The result of epidural xylazine findings for Hb and PCV are in accordance with finding of Kinjavadker et al. (2000) and Pratab et al. (2000). The reduction in Hb and PCV after ropivacaine-xylazine administration might be due to shifting of fluid volume from extravascular compartment to intravascular compartment in order to maintain the normal cardiac output (Pratab et al., 2001).

Regarding biochemical parameters blood glucose was significantly increased in both the treatments up to 3 hours of post administration of drugs. In present study the increase in blood glucose level may be due to the release of adrenocorticotrophic hormones due to anaesthetic stress (Mirakhur et al., 1984) which might be responsible for hyperglycaemia in both the treatments.

There was non significant (P>0.05) increase and subsequent decrease in total protein values in treatment I. In treatment II, there was a significant (P<0.05) increase in total serum protein after 15 minutes to 6 hours of epidural analgesia and thereafter showed a decline trend and reached down to pretreatment value at 72 hours. Singh et al. (2005) reported non-significant changes in total protein value after epidural administration of ropivacaine in goats. Ropivacaine did not change, total protein values significantly at any time interval and hence the drug seemed to have no effect on protein metabolism. The transitory increase in total protein values in the present study might have been due to effect of Xylazine on liver during biotransformation of drug which might have caused increase in total protein levels (Fayed et al., 1989). There was non significant (P>0.05) increase in alkaline phosphatase level in treatment I. In treatment II, there was a significant (P<0.05) increase in alkaline phosphatase level up to 6 hours after that values started decreasing significantly up to 72 hours. Vickers et al. (1984) reported that there is a possibility of transient liver damage during biotransformation of Xylazine, which may be the cause of rise in ALP values in the present study.

There was non significant (P>0.05) increase in total bilirubin values in treatment I and II. Non significant rise in serum bilirubin levels were reported by Singh et al. (1999) also following the use of bupivacaine in combination with diazepam and orazepam in goats. In the present study, therefore, there is possibility that the metabolism of these drugs (Chang and Glazko, 1974) in the liver might have caused some disruption in the liver parenchymal cells leading to non significant increase in the serum bilirubin level.

In treatment I, there was non-significant (P>0.05) increase in ALT values. In treatment II, there was significant (P<0.05) increase in ALT values up to 6 hours. Chang and Glazko (1974) indicated that the local anaesthetic drugs are metabolised by the liver in most of the species and excreted in urine, during this there may be transient damage to liver. Pandey and Sharma (1994) reported significant increase in the level of serum ALT following parenteral administration of Xylazine-ketamine anaesthesia in goats. Therefore, in the present study there is possibility that during the process of metabolism of drugs there might have been some disruption in liver parenchymal cells which might have increased the cell membrane permeability leading to elevation in the level of ALT in the blood (Pandey and Rao, 2000). In treatment II, there was no damage to liver as indicated by the values. In treatment I, there was non significant (P>0.05) increase in blood urea nitrogen values after epidural administration of ropivacaine. In treatment II, there was significant (P<0.05) increase in BUN values up to 3 hours. There was non significant (P>0.05) increase in serum creatinine values in treatment I. However, there was significant increase (P<0.05) in serum creatinine values in treatment II up to 3 hours. Bisen et al. (1994) and Pandey and Rao (2000) reported significant elevation in serum creatinine levels following parenteral administration of opioids like pentazocine in goats. Jadon et al. (1985), Sharma et al. (1997) and Singh et al. (1999) recorded significant increase in the levels of blood urea nitrogen following epidural administration of Xylazine in goats. It is therefore, presumed that the increase in BUN and creatinine in treatment II could be as a result of decrease in glomerular filtration rate due to reduction in renal blood flow, disturbance in urine output leading to urinary retention and other alteration in urinary function. The decrease in glomerular filtration rate would result into increased concentration of creatinine in blood (Wright, 1965). Similarly any retardation in glomerular filtration rate will cause high concentration of blood urea nitrogen (Garner et al., 1997). Overall, it is concluded that all drugs used in two treatments produced good analgesia and were also good during surgical procedures and post operative pain alleviation which is most important demand of current research.
However, Ropivacaine was better as far as its effects on clinico-physiological and haemato-biochemical parameters were concerned. Most importantly, unlike other local anaethetics, Ropivacaine has no clinically relevant effects on respiratory, cardiovascular, renal and hepatic parameters. So, it is useful in patients with poor cardiopulmonary function including elderly and obese patients with impaired renal and hepatic function.

References
SEROPREVALENCE OF BRUCELLOSIS IN VETERINARY PROFESSIONALS FROM SOME DISTRICTS OF RAJASTHAN STATE

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ABSTRACT
In a preliminary study designed to find out seroprevalence of brucellosis in 1085 veterinary professionals (366 veterinary officers and 719 paraveterinary personnel) engaged in animal husbandry practices in ten districts of Rajasthan State (India), the sera were screened by Rose Bengal test (RBT) and then positive samples were subjected to tube agglutination test (TAT) for antibody titration. An overall 3.68% seroprevalence was recorded in veterinary professionals with 3.00% in veterinary officers and 4.03% in paraveterinary personnel. A higher prevalence of brucellosis among paraveterinary personnel probably reflects more exposure of these individuals to brucella infected cases. The reason for this could be assigned to the fact that paraveterinary personnel are either not fully aware of the nature of the disease or do not follow the measures to avoid the contract of the infection. Individuals from Jhunjhunu and Nagaur districts had very high seroprevalence. In these districts buffalo population predominates and there is much human-animal interaction. Our findings emphasize the need of an early implementation of a continuous surveillance system to monitor both human and animal populations, and of eradicating brucellosis by involving veterinary and medical professionals in tandem.

Key words: Brucellosis, sero-prevalence, veterinarian, para-veterinarian.

Introduction
According to World Health Organisation, brucellosis is one of the most common zoonoses worldwide and is considered a reemerging infectious disease of great economic importance in many areas of the world (Corbel, 2006). It affects both human and animal populations in many different countries with some of the countries with its endemic status. The disease is being regularly reported in human populations from countries like India (Renukaaradhya et al., 2002), Jordan (Nimri, 2003), Saudi Arabia (Almuneef et al., 2004), Iran (Roushan et al., 2004), Korea (Park et al., 2005), Germany (Dahouk et al., 2007), Italy (Marianelli et al., 2007), Greece (Makis et al., 2008), Latin America (Lucero et al., 2008), Bulgaria (Russo et al., 2009) and many others.

The genus Brucella is highly homogeneous with all members showing more than 95% homology in DNA-DNA pairing studies (Verger et al., 1985). Four species of genus Brucella are pathogenic for humans i.e. B. melitensis (contracted from sheep and goat), B. abortus (from cattle and other bovidae), B. suis (from pigs) and B. canis (from dogs) (Godfroid et al., 2002).

Brucella melitensis is the most important zoonotic agent, followed by B. abortus and B. suis (Marianelli et al., 2007). However, it was also reported that there was no evidence suggesting that B. melitensis is virulent than B. abortus or that infections due to B. abortus are less severe than the infection with B. melitensis (Dokuzoguz et al., 2005; Marianelli et al., 2007).

The disease is usually transmitted to humans by ingestion of unpasteurized dairy products or uncooked meat or by direct contact with infected animals. The occupational disease is contracted by exposure of veterinarians to infected animals especially aborted foetuses, fluids, membranes or urine (Nimri, 2003). Other methods of transmission, though rare, are by inhalation in laboratory workers (Memish and Mah, 2001) and by vertical transmission (Giannopoulos et al., 2002).

Among clinical signs fever, night sweats, fatigue, weight loss, arthralgia and myalgia are common. Deaths have also been reported due to brucellosis in humans (Dahouk et al., 2007). However, because of a wide spectrum of nonspecific clinical manifestations, healthcare providers may not consider brucellosis in their first differential diagnosis (Park et al., 2005) and isolation procedures are not routinely applied (Lucero et al., 2008). Because of these factors there is strong possibility of disease being considerably under-diagnosed. The true incidence of human brucellosis is unknown for most of the countries and no data are available for India (Smits and Kadri, 2005).

Because of paucity of literature on human brucellosis in India the present study was undertaken to know the status of brucellosis in trained veterinary professionals, a high risk group, in state of Rajasthan. The surveillance data analysis may also be used in knowing about the trends of epidemiology of the brucellosis (Dahouk et al., 2007).

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Materials and Methods

The period of study was between May 2007 and July 2010 and the population under study was from ten districts from Rajasthan state. The group of veterinary officers (366) was comprised of 356 males, and 10 females. The paraveterinary group (719) was comprised of 714 males, and 5 females. All the individuals ageing 25-60 years, were serving in Department of Animal Husbandry, Government of Rajasthan and were associated with handling, treatment and vaccination of the animals. Blood samples from all the individuals from a district were collected on a single day and were shipped on ice to laboratory after proper clotting for screening on next day.

Rose Bengal plate test was used as screening test and the sera which were found positive were subjected to standard tube agglutination test for titre evaluation. A titre of more than 1:160 was considered diagnostic (Jelastopulu et al., 2008; Mantur and Amarnath, 2008). No brucella cultivation was performed.

Results and Discussion

In the present investigation seroprevalence for brucellosis in veterinary officers was 3.00% and that in paraveterinary personnel 4.03% with an overall value of 3.68% (Table 1). In Jaisalmer, Bhilwara and Jaipur no single case of seropositive veterinary officers or paraveterinary personnel was recorded whereas highest percentage of seropositive individuals were from Jhunjhunu followed by Nagaur district. None of the female professionals were seropositive for brucella antibodies.

In the present investigation the seroprevalence of brucellosis was 3.00% in veterinary officers and 4.03% in paraveterinary personnel, both constituting a high risk population. In India milk is consumed usually after proper boiling and likewise other products are also made from boiled milk hence the chances of transmission of infection could have been other than through ingestion of milk or milk products. It is also suggested that respiratory transmission or direct contact transmission was responsible for the human infection rather than ingestion of the cattle meat or milk (Park et al., 2005). In a study conducted in Northern Tanzania to determine the risk factors for transmission of brucellosis to humans, brucellosis was found to be strongly associated with assisted parturition during abortion in cattle, sheep and goat (John et al., 2010). Similarly, direct contact (occupational contact with animals in field, handling animals, engaged in parturition of animals) was found to be significantly most important predisposing risk factor than indirect contact (ingestion of raw milk, raw meat, and laboratory personnel) (Kochar et al., 2007).

The higher seroprevalence in paraveterinary personnel could be due to the fact that they were either not fully aware of the nature of the disease or did not observe the precautions to avoid the contact of the disease. The awareness of brucellosis is low in disease-endemic areas, including knowledge of its transmission potential and its medical consequences (Makis et al., 2008).

In the present investigation a relatively higher seroprevalence among veterinary officers and paraveterinary personnel was recorded in two districts namely Jhunjhunu and Nagaur. These two districts possess more buffaloes in number which are high milk producers and livestock owners are caring much for these animals as they call veterinary professionals for any little veterinary care. The higher seropositivity of individuals in these districts may be result of the fact that either there is more human-animal interaction or the buffaloes are more significant source of infection in these areas (Corbel, 2006). In one study authors recorded 48.10% seropositive buffaloes at a farm in Nagaur district where abortion storm continued for more than two months (unpublished observation) as compared to that in cattle (39.80%) with abortion storm recorded at farm in Madhya Pradesh state by the same authors (Kataria et al., 2006). A considerable number of brucellosis cases in humans are expected in areas where infection remains endemic among animals (Taleski et al., 2002). It has also been estimated that the true incidence may be 25 times higher than the reported incidence due to misdiagnosis and underreporting (Smits and Kadri, 2005). Hence, in the subsequent survey programmes involving population at risk in these areas an increased incidence of brucellosis is expected like that was seen in Ireland (Allwright, 1979).

The most of the affected individuals in this study reported one or more of the various clinical manifestation viz. arthralgia, sweating, back pain, loss of weight, undue fatigue, headache, anorexia, however, the clinical manifestations of brucellosis in humans depend on various factors including size of the infecting inoculum, the route of infection, the patient’s age, duration of the disease, the species of the organism (Gotuzzo et al., 1982; Tasova et al., 1999). On the other hand, in the study some seropositive individuals did not report any clinical signs. Though asymptomatic brucella seropositive human individuals have also been reported (Almuneef et al., 2004), the positive agglutination reaction with the sera of individuals without clinical signs could have been a case of false-positive reactions due to cross-reactions between brucella antigen and antibodies against other gram negative bacteria like Yersinia, Escherichia, Vibrio, Salmonella etc. (Kittelberger et al., 1995; Godfroid et al., 2002). Further, the seropositive asymptomatic individuals were younger in age and might have contracted the disease recently. In asymptomatic individuals low antibody titre has been recorded than that in symptomatic individuals (Almuneef et al., 2004). Nevertheless, all the seropositive individuals were advised to see a medical physician.
Brucella melitensis biovar 3 was the main etiological agent most frequently isolated in human in Italy and it was suggested that sheep and goat populations were the principal cause of human brucellosis (Marianelli et al., 2007) whereas an outbreak of human brucellosis in Korea was reported due to B. abortus biovar 1 in livestock owners who had taken care of cattle and in a veterinarian who assisted calf delivery (Park et al., 2005). Hence, elimination of brucellosis is possible only by interventions that vigorously target animal reservoirs (Zinsstag et al., 2007). A study in western Greece also revealed a statistically significant decline in the incidence of human brucellosis after animal vaccination programme (Jelastopulu et al., 2008). Unfortunately in India vaccination against brucellosis is not usually practiced in animal population. Above all till date no cost-effectiveness studies have been done to see the financial benefits of livestock vaccination. Because brucellosis can infect animals and humans, the medical and veterinary communities should work closely together in clinical, public health, and research settings (Ryan, 2008).

Acknowledgements

We would like to thank all the veterinary and para-veterinary staff who participated in this study.

References

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Table 1: Seroprevalence of Brucellosis in veterinary professionals in different districts of Rajasthan State (India)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>District</th>
<th>Veterinary officers</th>
<th>Paraveternary personnel</th>
<th>Overall Seroprevalence</th>
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<tr>
<td></td>
<td>Total samples</td>
<td>Positive samples</td>
<td>Total samples</td>
<td>Positive samples</td>
</tr>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
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<td>Bikaner</td>
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<td>36 3 8.33</td>
<td>55 3 5.45</td>
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<td>2.</td>
<td>Jodhpur</td>
<td>36 0 0</td>
<td>38 1 2.63</td>
<td>74 1 1.35</td>
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<td>3.</td>
<td>Jaipur</td>
<td>32 1 3.12</td>
<td>8 1 12.50</td>
<td>40 2 5.00</td>
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<td>4.</td>
<td>Jhunjhunu</td>
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<td>5 1 20.00</td>
<td>46 6 13.04</td>
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<td>7.</td>
<td>Bhilwara</td>
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<td>118 0 0</td>
<td>153 0 0</td>
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<td>Nagaur</td>
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<td>99 12 12.12</td>
<td>139 16 11.51</td>
</tr>
<tr>
<td>9.</td>
<td>Alwar</td>
<td>53 1 1.88</td>
<td>138 10 7.24</td>
<td>191 11 5.75</td>
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<td>10.</td>
<td>Jaipur</td>
<td>80 0 0</td>
<td>193 0 0</td>
<td>273 0 0</td>
</tr>
<tr>
<td>Total</td>
<td>366 11 3.00</td>
<td>719 29 4.03</td>
<td>1085 40 3.68</td>
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COMPARATIVE STUDY ON CARCASS CHARACTERISTICS OF GUINEA FOWL (PEARL) AND DESI FOWL (KADAKNATH)

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ABSTRACT

Concept of utilizing guinea fowl as an alternative to chicken is gaining support in the population now-a-days. It has been found that guinea fowl is reared in different pockets of the country under backyard farming system. Kadaknath is an equally important breed utilized for backyard poultry farming and is known for its unique taste. Hence the study here is designed to produce a comparative picture of different carcass traits of a guinea fowl (Pearl) and a desi fowl (Kadaknath). Based on carcass yield and cut-up yield, Pearl was found to be superior to Kadaknath at three different age groups viz., 8, 12 and 16 weeks. The giblet yield comparison revealed high liver weight in Kadaknath whereas heart and gizzard weight was higher in Pearl. Increase in age of birds also resulted in the increase in above yields in both the fowls, but the changes observed in most of the parameters were found significant (P<0.05) only when the age increased from 8 to 12 weeks whereas as changes observed from 12 to 16 weeks were rarely significant (P<0.05).

Key words: Guinea fowl, pearl, kadaknath, desi fowl, carcass

Introduction

Guinea fowl (Pearl) is an interesting component of poultry in developing countries like India and is gaining attention as an alternative poultry resource. It is primarily raised for meat production under backyard system and is popular for its gamey flavour and has ready acceptability without any prejudice. Guinea fowl (Lavender) can be an alternative to chicken for providing variety to non-vegetarian diets (Sharma et al., 2007) Kadaknath is an important breed of desi fowl, popular for backyard poultry farming. Its meat is black in colour and is known for its unique flavour. As guinea fowl is also emerging as an alternative species for backyard poultry farming, therefore, it is of interest to explore this bird regarding dressed and drawn weights and proportion of edible portions in the dressed carcass. There was significant difference between live weight, wing weight and neck weight of exotic and desi turkey (Kumaravelu et al., 2010). These parameters will help in ascertaining the economic benefits associated in the rearing of these two fowls. Hence the study here is designed to compare the carcass yield, by-product yield, proportion of cut-up parts, giblet yield between Pearl (guinea fowl) and Kadaknath (desi fowl). To overcome the effect of age the study is planned at three different age groups viz., 8, 12 and 16 weeks.

Materials and Methods

Guinea fowl (Pearl) and desi fowl (Kadaknath) of different age groups viz., 8, 12 and 16 weeks were procured from Instructional Poultry Farm (IPF) of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. A total of 30 birds comprising 15 guinea fowls and 15 desi fowls (5 from each age group) were utilized for the study. The observations were recorded while slaughtering the birds following standard scientific protocol. Statistical analysis of the data was done using ANOVA technique according to the method described by Snedecor and Cochran (1967) by Completely Randomized Design (CRD).

Results and Discussion

Carcass yield

There was a significant (P<0.05) difference between the pre-slaughter weight of Pearl and Kadaknath at 8 and 12 weeks of age, but at 16 weeks a non-significant difference was found. With the increase in age the pre-slaughter weight of Pearl increased significantly (P<0.05) from 8 to 12 weeks only whereas in Kadaknath the increase was significant (P<0.05) from 8 to 12 and 12 to 16 weeks both. Hence 12 weeks of age can be considered to be an optimum age for the slaughter of guinea fowls as growth rate reduces after that. Kumar et al. (1995) also reported the slaughtering age of guinea fowl as 12 weeks of age. The pre-slaughter weight for 12 week Pearl (1240.80 g, Table 1) was very near to that reported by Ayorinde (1987), however, live weights reported by Singh et al. (1991) for guinea fowl were lower than the present findings. The two types of birds differed significantly (P<0.05) for bled weight at 8 and 12 weeks of age where Pearl was superior to Kadaknath, however, at 16 weeks of age a non significant difference was found. A significant (P<0.05) difference was observed for defeathered weight between Pearl and Kadaknath at 8

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Table 1: Comparison of carcass and by-product yield (g) between Pearl (Guinea fowl) and Kadaknath (Desi fowl) (Mean ±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Birds</th>
<th>8 week</th>
<th>12 week</th>
<th>16 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-slaughter weight</td>
<td>Pearl</td>
<td>874.00±41.03</td>
<td>1240.80±30.23</td>
<td>1350.00±16.25</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>642.00±18.07</td>
<td>1118.26±35.53</td>
<td>1392.60±64.39</td>
</tr>
<tr>
<td>Bled weight</td>
<td>Pearl</td>
<td>850.00±40.18</td>
<td>1222.20±30.56</td>
<td>1318.80±15.40</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>611.00±15.19</td>
<td>1077.60±37.26</td>
<td>1349.80±62.22</td>
</tr>
<tr>
<td>De-feathered weight</td>
<td>Pearl</td>
<td>803.00±35.59</td>
<td>1141.60±25.82</td>
<td>1208.00±15.61</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>587.04±15.85</td>
<td>1019.68±30.93</td>
<td>1307.84±63.84</td>
</tr>
<tr>
<td>Eviscerated weight</td>
<td>Pearl</td>
<td>536.20±23.57</td>
<td>776.12±24.12</td>
<td>851.82±11.81</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>364.08±17.70</td>
<td>628.56±38.33</td>
<td>698.84±39.44</td>
</tr>
<tr>
<td>Skin weight</td>
<td>Pearl</td>
<td>60.48±4.58</td>
<td>74.64±6.38</td>
<td>77.88±5.92</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>33.40±4.01</td>
<td>87.32±10.15</td>
<td>112.48±15.27</td>
</tr>
<tr>
<td>Head weight</td>
<td>Pearl</td>
<td>24.68±1.62</td>
<td>38.36±1.38</td>
<td>42.52±2.1</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>38.84±1.16</td>
<td>38.64±2.12</td>
<td>42.60±2.9</td>
</tr>
<tr>
<td>Shank weight</td>
<td>Pearl</td>
<td>33.12±1.67</td>
<td>39.00±0.94</td>
<td>40.32±1.10</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>30.60±1.15</td>
<td>38.88±0.75</td>
<td>40.04±4.0</td>
</tr>
</tbody>
</table>

Means with different superscripts column wise in capital alphabet (A, B) between Pearl and Kadaknath for single parameter differ significantly (P<0.05).
Means with different superscripts row wise in small alphabet (a, b, c) between different age groups differ significantly (P<0.05).

Table 2: Comparison of cut-up and giblet yield (g) between Pearl (Guinea fowl) and Kadaknath (Desi fowl) (Mean ±SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Birds</th>
<th>8 week</th>
<th>12 week</th>
<th>16 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck weight</td>
<td>Pearl</td>
<td>26.86±1.84</td>
<td>41.24±2.75</td>
<td>45.72±2.00</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>23.00±1.67</td>
<td>33.40±2.41</td>
<td>38.04±1.07</td>
</tr>
<tr>
<td>Wing weight</td>
<td>Pearl</td>
<td>78.64±2.52</td>
<td>88.72±2.46</td>
<td>97.76±4.00</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>56.36±1.78</td>
<td>69.72±5.44</td>
<td>76.52±4.83</td>
</tr>
<tr>
<td>Back weight</td>
<td>Pearl</td>
<td>91.08±5.47</td>
<td>129.24±1.94</td>
<td>137.46±3.63</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>60.44±1.60</td>
<td>122.56±11.02</td>
<td>136.75±9.53</td>
</tr>
<tr>
<td>Breast weight</td>
<td>Pearl</td>
<td>181.80±10.13</td>
<td>278.24±8.04</td>
<td>311.20±8.95</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>106.18±10.10</td>
<td>188.68±12.60</td>
<td>229.96±15.81</td>
</tr>
<tr>
<td>Drumstick weight</td>
<td>Pearl</td>
<td>74.80±4.28</td>
<td>107.00±2.56</td>
<td>109.48±1.62</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>56.84±0.98</td>
<td>94.08±3.87</td>
<td>97.20±6.09</td>
</tr>
<tr>
<td>Thigh weight</td>
<td>Pearl</td>
<td>83.00±3.05</td>
<td>131.40±5.80</td>
<td>149.86±3.96</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>61.16±2.39</td>
<td>99.76±4.70</td>
<td>118.44±6.53</td>
</tr>
<tr>
<td>Liver weight</td>
<td>Pearl</td>
<td>15.52±1.47</td>
<td>21.40±1.18</td>
<td>22.58±0.94</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>15.82±1.26</td>
<td>22.90±2.95</td>
<td>37.60±3.09</td>
</tr>
<tr>
<td>Heart weight</td>
<td>Pearl</td>
<td>4.52±0.27</td>
<td>7.06±0.26</td>
<td>7.28±0.39</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>3.20±0.11</td>
<td>5.52±0.59</td>
<td>6.50±0.52</td>
</tr>
<tr>
<td>Gizzard weight</td>
<td>Pearl</td>
<td>20.04±1.69</td>
<td>24.20±0.90</td>
<td>26.04±0.95</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
<td>18.44±1.00</td>
<td>20.68±1.65</td>
<td>20.44±0.95</td>
</tr>
</tbody>
</table>

Means with different superscripts column wise in capital alphabet (A, B) between Pearl and Kadaknath for single parameter differ significantly (P<0.05).
Means with different superscripts row wise in small alphabet (a, b, c) between different age groups differ significantly (P<0.05).
and 12 weeks of age, but at 16 weeks age a non-significant difference was there. In eviscerated weight category Pearl and Kadaknath differed significantly (P<0.05) at all the three age groups, Pearl having higher weight at each age group. This may be due to higher proportion of skin and other by-products in Kadaknath as compared to Pearl. With the increase in age of Kadaknath a significant (P<0.05) increase in bled weight and defeathered weight was found. In Pearl though the increase in bled weight, defeathered weight and eviscerated weight from 8 to 12 week was significant (P<0.05) but it was non significant from 12 week to 16 weeks of age. This may have been due to higher growth rate in muscle part at lower ages than at higher.

By-product yield
The two birds differed significantly (P<0.05) for skin weight at 8 and 16 week of age, but at 12 week the difference was non significant. Lower skin weight was observed in Kadaknath at 8 week of age but at 12 and 16 weeks, Kadaknath was having more skin weight than Pearl, this may be due to high subcutaneous fat deposition with the increase in age. With the increase in age, non significant increase in skin weight was observed in majority of cases. Head weight differed significantly (P<0.05) at 8 weeks of age in both the birds but at 12 and 16 weeks of age a non significant difference was observed between them. No major influence of age was found on the weight of heads in both the birds (Table 1). The two birds differed marginally for shank weight at three different age groups and the difference was non significant. Significant (P<0.05) change in shank weight was observed in both the birds with the increase in age from 8 to 12 weeks. Sachdev et al. (1990) found that different weight group birds had different shank weight.

Cut-up yield
Neck weight was distinct at 12 and 16 weeks age and was significantly (P<0.05) higher in Pearl but difference was non-significant at 8 weeks of age (Table 2). There was an overall significant superiority (P<0.05) of wing weight, breast weight, drumstick weight and thigh weight observed for Pearl at 8, and 16 weeks of age as compared to that of Kadaknath. Higher wing weight of Pearl can be attributed to strong wing muscle development in birds required for flight (Ayorinde, 1991). The difference between the birds with respect to back weight was significant (P<0.05) only at 8 weeks of age with 60.44 g and 91.08 g for Kadaknath and Pearl, respectively, which became almost equal at 16 weeks of age (Table 2). Since Kadaknath had lowest back weight at 8 week, above result indicates a rapid increase in back weight of ‘Kadaknath’ with increase in age.

With the change in age of birds from 8 to 12 weeks there was significant (P<0.05) increase in weight of neck, back and drumstick, however increase in neck weight from 12 to 16 week was found non significant. Sachdev et al. (1990) was of the view that with increase in body weight, significant change occurs in back and drumstick weight of the birds. Wing weight and thigh weight also showed increasing trend with increasing age of the birds (Table 2). Brake et al. (1993) reported significant effect of age on the of back, neck, thigh and wing weight of broilers. However, with increase in age of birds there was significant (P<0.05) increase in breast weight for both species except the increase observed from 12 week (278.24 g) to 16 week (311.20 g) in Pearl (Table 2). Mahapatra et al. (1986) also reported an increase in proportion of breast weight as age of guinea fowl increased.

Giblet yield
Weights of giblet are given in Table 2. Liver weight was found to be almost equal in the birds at 8 and 12 weeks of age. But at 16 week, a distinct difference was observed, making liver weight of Kadaknath (37.60 g) more than that of Pearl (22.58 g) and producing a significant (P<0.05) difference between them. Weight of heart and gizzard was found more in Pearl as compared to Kadaknath in all the age groups.

With the increase in age of birds there was a significant (P<0.05) change in weight of liver except the change observed from 12 week (21.40 g) to 16 week (22.58 g) in Pearl (Table 2). Increase in age of birds resulted in significant (P<0.05) change in weight of heart from 8 to 12 week. Heart contributed minimum to giblet weight and the same was reported by Brah and Sandhu (1985). Increase in age of birds resulted in overall increase in weight of their gizzard, but this change was found significant (P<0.05) only from 8 weeks (20.04 g) to 12 weeks (24.20 g) in Pearl. Brake et al. (1993) also found significant effect of age on weight of liver, heart and gizzard.

References
PCR TECHNIQUE AS A TOOL FOR IDENTIFICATION OF DOMESTIC ANIMALS

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ABSTRACT

The present work was undertaken to differentiate various species of domestic animals by PCR technique. High quality DNA was extracted from muscles samples by using a phenol-chloroform method. The genomic DNA was evaluated for its quality by using submarine agarose gel electrophoresis. The PCR assay was standardized for amplification of DNA fragment with species specific primers of 16s rRNA gene sequence of cattle, buffalo, sheep and pig. The DNA samples from all the species of animals were amplified under standard conditions. The PCR amplification assay was used to differentiate various species of domestic animals on the basis of band patterns. The PCR reaction for species specific primers with DNA extracted from muscles samples of cattle, buffalo, sheep and pig revealed bands at 603; 603; 374 and \(<100\) base pairs, respectively.

Key words: PCR, RFLP, muscle, domestic animals.

Introduction

There are ever increasing incidences of crime like killing of cows for meat purpose or substitution of costly meat with cheaper one. Similarly, the presence of unwanted or unknown animal species in food is of great concern from public health, economic, religious and legal point of view. Substitution of an inferior animal species in a food product is referred as food fraud and has both economic and legal ramifications.

In a country like India, the identification of species, origin of meat and meat products acquires immense importance in forensic science because of vetero-legal cases and religious reasons.

Various methods have been developed for the identification of species origin of meat and meat products. But these methods have their own limitations and are not hundred per cent reliable and accurate.

The DNA based molecular techniques are being preferred to exploit the DNA rather than protein content of fresh or processed meat for identification of species origin of meat and meat products, due to its stability at high temperature and also due to conserved structure within all tissues of an individual.

The first DNA based molecular technique used, that successfully differentiated meat species was DNA hybridization (Chikuni et al., 1990; Ebbenhoj and Thomson 1991; Buntjer et al., 1999). The DNA hybridization is a quite reliable and sensitive technique, but is complicated and time consuming and is replaced by Polymerase Chain Reaction (PCR) based techniques.

The PCR technique is quite reliable, accurate and authentic but requires species-specific or conserved oligonucleotide primers for identification of species. The increased complexity of these techniques made this approach less realistic for application in routine quality control methods.

Materials and Methods

The muscle samples of cattle, buffalo, sheep and pig were collected from recognized slaughter house and after natural death. In the present study fresh muscle tissues were collected to evaluate applicability of PCR assay. PCR was carried out at Veterinary college, Nagpur.

Oligonucleotide Primer Pairs

In the present study, published primer pairs (Ahmed et al., 2006) based on the mitochondrial gene sequences of cattle, buffalo, sheep, and pig were used. The primer pairs were synthesized from NEUMAX SCIENTIFIC, MUMBAI.

DNA Extraction from muscle samples

Genomic DNA was isolated from the muscle samples by phenol chloroform extraction method as described by Sambrook and Russel (2001) with slight modifications.

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Checking Quality of DNA

Horizontal submarine agarose gel electrophoresis was used to check the quality of genomic DNA using 0.8% (w/v) agarose gel. A 0.8% agarose (w/v) suspension in 1 x TBE buffer was made and heated on an electric heater until the agarose was completely melted to give a clear transparent solution. After cooling to 60°C, ethidium bromide (10 mg/ml) at 5 µl per 100 ml of agarose solution was added and mixed gently. The agarose was poured into a leveled casting tray and the gel was made to about 4 mm in thickness. The agarose was allowed to set at 4°C temperature till it solidified and subsequently the comb and adhesive tapes were gently removed.

For loading the sample, 5 µl diluted DNA was taken and after mixing it with 2 µl of 6 x gel loading dye, it was loaded into the well of agarose gel, which was submerged in the electrophoresis tank containing 1x TBE buffer. A DNA marker was also run in one of the well. Electrophoresis was performed at 80V for 80-90 min. After electrophoresis, the gel was visualized under UV transilluminator and documented by gel documentation system.

Standardization of Polymerase Chain Reaction (PCR) Assay

The reaction mixture was prepared in a 500 µl PCR tube in a total volume of 50 µl containing 5 µl of 10x PCR buffer (with KCl), 2 µl of MgCl₂ (2 mM), 1 µl of dNTP mix (10 mM each), 0.50 µl of Taq DNA polymerase (2 units), 2 µl each of forward and reverse primer (20 pmol), 1 µl of DNA template (20-30 ng) and nuclease free water (to make up the reaction volume to 50 µl). For second PCR assay, 1 µl, 1/20th diluted DNA fragments of first PCR assay were taken. The PCR tube containing the reaction mixture was flash spun on a micro-centrifuge to get the reactants at the bottom. The reactions were performed in thermal cycler with a heated lid. The amplification conditions used were as follows.

**Reaction**

1. Initial denaturation at 94 °C for 5 min.
2. A. Denaturation at 94 °C for 30 sec.
   B. Annealing temperature as shown in table for specific primer sequence of a species.
   C. Extension at 72 °C for 30 sec. Step A to C was repeated for 35 cycles.
3. Final extension at 72 °C for 10 min.

All the ingredients were taken using filter tips to avoid any cross-contamination. Every-time negative control (without template DNA) was put to make sure that there was no contamination in PCR system. Finally, the PCR product was kept at -20 °C for further use.

Results and Discussion

In the present study, the yield of genomic DNA estimated ranged between 10-30 µg from fresh muscle samples of almost all the species under study. The extracted DNA samples were stored at -20 °C for further use. An equal amount of DNA from each of the species was diluted with desired concentration of DNase/RNase free water for its use in PCR.

Standardization of PCR Assay

The PCR reaction was standardized by using 1.0 u of Taq DNA polymerase, 25 pmol dNTP, 25 pmol of random primers, 2.5 µl of 10x Taq DNA polymerase buffer and 50 mg DNA. This reaction mixture was run on the PCR. Present study revealed that the PCR reaction for species specific primers on DNA samples of cattle, buffalo, sheep and pig yielded bands at 603; 603; 374 and <100 base pairs respectively (Fig. 2). These band patterns obtained at various base pairs in different species of animals, allowed a clear cut demarcation for identification of pig, sheep and cattle and buffalo together.

The observation recorded during the present work regarding PCR reaction for species specific primers on DNA samples of cattle, buffalo, sheep and pig found at different base pairs are in agreement with the findings recorded by Ahmed and Abdel Rahman (2006). They also reported the band patterns at 603; 603; 374 and ≤100 base pairs respectively (Fig. 2). These band patterns obtained at various base pairs in different species of animals, allowed a clear cut demarcation for identification of pig, sheep and cattle and buffalo together.

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In agreement with the findings of the present study, Mane et al. (2006) reported similar findings. They stated that the primer RPN2 showed polymorphism with unique and species specific banding pattern in cattle, pig and chicken meat by RAPD-PCR method.

It has been also noted that the band patterns for cattle and buffalo were found at same base pair of 603 bp. This indicated that the PCR assay was not just sufficient to differentiate between Cattle and buffalo.
Buffalo species. It was therefore necessary to undertake the RFLP for the identification of samples of cattle and buffalo.

Abdel Rahman (2006) concluded that the similar band pattern of cattle and buffalo suggests the same evolutionary history and are derived from the same ancestor. Similar observations were also recorded by Jain et al. (2007). They used cytochrome b gene for detection of species origin of meat samples from cattle, buffalo, sheep and pig.

References
DIAGNOSTIC STUDIES ON TRAUMATIC PERICARDITIS IN CATTLE

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ABSTRACT

Traumatic pericarditis was studied in 25 cases of cows and bull, which were diagnosed on the basis of history, clinical signs, haematological test, radiography or ultrasonography-wherever feasible and glutaraldehyde test. The definitive diagnosis of traumatic pericarditis was made on the basis of history and clinical findings which were showing typical signs of the disease. The clotting time of whole blood in glutaraldehyde test indicated the chronicity of the disease. Thoracic radiography and ultrasonography revealed fluid in the pericardial sac in the form of increased radiodensity and anechoic area respectively. All animals of present study showed neutrophilia and lymphocytopenia while TLC values were within the normal range.

Key words: Cattle, traumatic-pericarditis, diagnosis

Introduction

Traumatic pericarditis in cattle is almost always attributable to a reticular foreign body that has penetrated the reticular wall, diaphragm and pericardial sac. It is an inflammation of the pericardium with accumulation of serous or fibrinous inflammatory products. The lead signs of pericarditis are tachycardia, muffled heart sounds, asynchronous abnormal heart sounds, distension of the jugular veins and submandibular, brisket, and ventral abdominal oedema. The glutaraldehyde test and other laboratory haematological examination are important diagnostic tools. Radiographs of the thorax and reticulum often show a foreign body cranial to the reticulum. Ultrasonography is the method of choice for diagnosis and characterisation of pericardial effusion (Braun, 2008). Cows with TRP shows reduced milk production, anorexia, dullness, tympany and digestive disorders (Senna et al., 2003). In view of the insidious nature and deceptive manifestations, traumatic syndrome often remains undiagnosed before sufficient and almost irreparable damage has been inflicted to the animal (Misra, 1972). It is important that the clinician examine the cardiovascular system with care during the routine clinical examination of the bovine patient. Early detection of cardiovascular disease may enable appropriate remedial action be taken and an accurate prognosis of the patient’s condition to be offered (Jackson and Slater, 1997).

In view of above studies and occurrence of traumatic pericarditis in cattle causing production losses and mortality, a project was undertaken to diagnose the hardware disease in cattle.

Materials and Methods

Traumatic pericarditis was studied in 25 cases of cows and bull which were brought to the clinics of Department of Veterinary Surgery and Radiology, CVAS, Bikaner. These cases were diagnosed on the basis of history, clinical signs, haematological test, radiography or ultrasonography- wherever feasible and glutaraldehyde test.

Diagnosis

(A) History

All owners of animals of present study were subjected to a detailed history related to occurrence of brisket oedema, pregnancy, appetite, tympany, milk yield and other important signs noted by the owner.

(B) Clinical examination

It was done by a gross examination of animal during standing and locomotion and by a close-up examination of animal in the trevis. These animals were examined in relation to general appearance, loss of appetite, tympany, milk yield and other important signs noted by the owner.

(C) Haemato-biochemical examination

Twenty four animals of the present study were subjected to haematological examination in terms of total leukocyte count, differential leukocyte count, haemoglobin, packed cell volume, total erythrocyte count and erythrocyte sedimentation rate by

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aspirating out 5 ml blood from jugular vein in a test tube containing EDTA. These parameters were estimated by standard methods described by Schalm et al. (2000) and Puri and Kataria (2003).

The glutaraldehyde test was conducted in 6 animals in the present study as per the method described by Brink et al. (2005). This test was conducted on 3 ml jugular blood, which was aspirated out in a test tube containing EDTA.

(D) Radiological and Ultrasonographic examination

In present study 5 animals were subjected to a lateral thoraco-abdominal radiograph with an ultrasonographic examination done in 10 animals of present study. A Shimadzu-350 machine and convex transducer was used on the 4th intercostal space from the right side.

Results and Discussion

Diagnosis

(I) History

Traumatic pericarditis was diagnosed in 25 cases, out of which 24 were female and 1 was male cattle. These animals were aged from 2 to 10 years. Out of 24 cows 5 were pregnant, 14 parturited 3 days to 4.5 months back and 5 cows did not show the history of either pregnancy or parturition. In animals of present study, 23 cases had brisket oedema which was 1 to 50 days old but in majority of cases it was 1 to 3 weeks old. The animals were partially off-feed since last 7 to 45 days (18 cases) and these were completely off-feed since last 2 to 15 days (13 cases). However, in animals of present study, 8 cases which were initially partially off-feed became completely off-feed later on. There was a sharp drop in milk yield in 16 animals of present study. However, the remaining 3 were dry and 5 were non-pregnant among cows. The predominant signs recorded by owners were anorexia and brisket oedema in 17 animals of present study. However, 3 animals showed tympany, anorexia and brisket oedema and 3 animals showed respiratory distress, reluctance to move together with anorexia and 2 animals showed only brisket oedema. Ramakrishna (1993) also reported higher incidence of traumatic pericarditis in pregnant or recently calved animals due to increased intraabdominal pressure towards the thorax. He said there occurs brisket oedema of the jaw, dewlap and ventral abdominal region extending up to the udder which is indicative of congestive heart failure. He also reported anorexia, drop in milk yield and reluctance to walk in cases of traumatic pericarditis in cattle.

In 3 cases tympany was reported which was persistent from 20 days in one case and was recurrent from 2 months in 2 cases.

(II) Clinical examination

All animals of present study were examined for general appearance in standing position and for stiffness during motion. Out of 25 cases only 5 showed a normal general appearance and remainder showed anxiety and dull or depressed appearance. The brisket oedema of a variable extent was seen in 23 animals of present study. Three animals of present study showed stiff gait, reluctance to move and did not sit for extended periods. A positive jugular pulse was seen in 9 cases of present study whereas 15 cases showed only engorgement and one case did not show either of these. The pulsation of jugular was evident even without palpation. Ramakrishna (1993) also described engorgement and pulsation of jugular veins in cases of traumatic pericarditis.

Animals of present study showed a variable spectrum of rectal temperature. Fifteen animals showed temperature between 101 to 102.9°F, 9 animals showed between 103 to 104°F and one animal showed 105°F. Respiration of affected animals showed marked variation as normal respiration, tachypnoea and dyspnoea were seen in 4, 17 and 4 cases, respectively. The different types of heart sounds recorded on auscultation were tinkling (2 cases), splashing (3 cases), frictional (3 cases) and muffled (17 cases). Tachycardia was observed in all cases of present study. Ramakrishna (1993) also described occurrence of pyrexia, increased pulse rate and abdominal respiration in cases of traumatic pericarditis in cattle. He reported pericardial frictional sounds in initial stages as inflamed parietal and visceral surfaces of pericardium rubbed each other due to minimal fluid and increased fibrin in the pericardial sac. Sounds become faint or muffled later on due to the accumulation of more fluid in the pericardium. Splashing sound are heard if gas is present.

(III) Ancillary diagnostic procedures

The glutaraldehyde test was done in 6 cases of present study with a 1.25% solution. The clotting time was between 2 to 3 minutes in 4 cases, 8 to 11 minutes in 2 cases suggestive of chronic and mild chronic infection respectively. Braun et al. (2007) recorded clinical findings in 28 cattle with traumatic pericarditis and found reduced clotting time in glutaraldehyde test in 26 animals and leucocytosis in 22 animals.

The radiographic examination was done in 5 cases of present study. The lateral radiograph of thoraco-abdominal region revealed evidence of fluid in pericardial sac in these cases. However, presence of fluid in pericardial sac masked other radiological details of thoracic area. In 2 cases there was evidence of a radiopaque metallic foreign body in the cardiac region. Ramprabhu et al. (2003) opined...
that in cases of traumatic reticuloperitonitis and allied syndromes in cattle, radiography is able to detect metallic foreign bodies but unable to detect any tissue reaction. Ramakrishna et al. (1980) did radiography of 10 cows suspected for traumatic pericarditis and found radiopaque foreign bodies, enlarged cardiac silhouette and adhesions between pericardium, diaphragm and reticulum.

The ultrasonographic examination in 10 cases revealed the presence of fluid in pericardial sac which was marked by a thick anechoic area surrounded by a distinctly echogenic tissue. However, the anechoic area representing fluid disappeared following removal of pericardial fluid by pericardiocentesis. Senna et al. (2003) did ultrasonographic examination of 8 buffaloes with traumatic pericarditis and showed the presence of anechoic fluid filling the pericardial spaces which ranged between 2.52 to 2.65 cm. In 7 cases, there was an echodense structure (fibrin or exudates) in the echofree pericardial space. However, ultrasonography was unable to detect foreign bodies.

Haematological parameters studied revealed mean Hb levels up to the 8.12 gm% and it ranged from 5 to 11.5 gm%. However, in animals of present study 11 animals had Hb lesser than the minimum range of reference value of Hb, i.e. 8 gm%. The mean PCV was 28.23 vol% and it ranged from 20 to 35 volume %. The mean TEC values were 5.075 million/cumm and it ranged from 2.3 to 14.3 million/cumm. In animals of present study, 13 animals showed TEC lower to minimum range of reference value, i.e. 5 million/cumm and one animal showed higher to maximum range of TEC, i.e. 10 million/cumm. The mean TLC value was 6.98 thousand/cumm and it ranged from 4 to 11.9 thousand/cumm. In animals of present study, the TLC values were found within normal range of reference values, i.e. 4-12 thousand/cumm but all animals showed neutrophilia and lymphocytopenia. The mean eosinophils count were 3.46% and it ranged from 1 to 6%. In 3 animals present study, the eosinophils were found within normal range of reference values, i.e. 4-12 thousand/cumm but all animals showed neutrophilia and lymphocytopenia. The mean monocytes count were 7.25% and it ranged from 4 to 15%. The monocytes were found lower to minimum reference value, i.e. 5%, in 2 animals of present study, whereas these were higher to maximum range of reference value, i.e. 8% in 6 animals. The mean basophil counts were 0.625% and it ranged from 0 to 2%. The basophils were found higher to the maximum range of reference value in 12 animals. The mean ESR value was 0.25 mm/hr and it ranged from 0 to 2 mm/hr. Radostits et al. (2000) reported a marked leucocytosis and shift to left in haematological examination in cases of traumatic pericarditis. Senna et al. (2003) recorded decreased erythrocyte count of traumatic pericarditis affected cows. In animals of present study there was no leucocytosis but neutrophilia and lymphocytopenia was present in all animals and the TEC was less than normal value in almost 50% of the cases.

Present study concludes that the history and physical examination of the animal form the foremost important basis for the diagnosis of traumatic pericarditis in the cattle and the radiography, ultrasonography and haematobiochemical tests are important non-invasive supplementary diagnostic methods which also gives information regarding the severity of the disease.

References
Pregnancy toxaemia in goat occurs due to insufficient nutrition associated with carrying of multiple kids in the last stage of pregnancy or in the early lactation. Major cause is metabolic disorder due to negative energy balance characterized by hypoglycaemia and hyperketonaemia. The clinical picture comprises neurological manifestations and weakness.

Case history and investigations

A Black Bengal doe of one and half years old in its first gestation was presented in the polyclinics of IVRI with the history of advanced pregnancy (142 days), anorexia, dry and scanty faeces with sternal recumbency and lateral deviation of head for last one day. Clinical examination revealed normal body temperature (101.3°F) and pulse rate (76/min) with fruity odour from mouth. Abdominal palpation revealed the presence of more than one foetus. The case was suspected for pregnancy toxaemia.

Haematological parameters viz Hb, PCV, TEC, TLC were estimated as per method described by Schalm (1975) and biochemical parameters viz serum glucose, total protein, albumin, ALT, AST and alkaline phosphatase were estimated by using span diagnostic kit. Ross test was conducted to detect ketone bodies in the urine (Benjamin, 1998). Doe was treated with 10% dextrose and Ringers lactate @ 10 ml/kg b. wt. i/v for two days. Vitamin B complex (Tribivet M/s. Neovet) 2 ml i/m and appetizer (Ruchamax, Ayurvedet) 5 g bid p/o was given for 5 days. Owner was advised to give 250 g molasses daily till the date of parturition.

Results and Discussion

After two days of treatment, doe was bright and active with posture towards normalcy. Lateral deviation of head in this case might be due to cog-wheel type of clonic contraction of the cervical muscle (Radostitis et al., 2005). Presence of ketone body in the urine showed purple reaction (++) to Ross test. Haematological examination showed Hb 7.4 gm%, PCV 23.6%, TEC 6.2 x 10^6/µl, TLC 2500/µl and serum biochemical values of glucose 36 mg/dl, total protein 4.96 gm/dl, albumin 3.57 gm/dl, globulin 1.39 gm/dl, A:G 2.56, ALT 66 IU/L, AST 53 IU/L and alkaline phosphatase 432 IU/L. Haematological parameters towards their lower limit in the present case might be due to long term negative energy balance associated with pregnancy. Upper level of liver function enzymes might be due to fatty infiltration and degeneration of the liver (West, 1996). Treatment with dextrose and ringers lactate was carried out to combat hypoglycaemia and acidosis, respectively, associated with pregnancy toxaemia (Pastor et al., 2001). Ruchamax and vitamin B complex helped to improve normal body condition. Study revealed that pregnancy toxaemia can be checked by early diagnosis and prompt treatment in goats even in the field condition.

References

THERAPEUTIC MANAGEMENT OF BABESIOSIS IN CROSSBRED COWS

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Bovine babesiosis is an important disease of cattle caused by piroplasma protozoan parasites of Babesia spp. which are transmitted by tick vectors. Babesiosis is also called as Redwater or Cattle tick fever or Texas fever (Radostits et al., 2008). Babesiosis occurs almost all over the world. It is a major threat to dairy development in many tropical and sub-tropical countries, especially when exotic breeds are used (Zint et al., 2003). A Babesia organism infects red blood cells of host and is clinically characterized by anaemia, jaundice and haemoglobinuria. Intestinal and ruminal motility increases leading to spasms of the anal sphincter which produce pipestem diarrhoea (Collins et al., 1970). The major species of babesia which affects cattle in tropical and subtropical countries are Babesia bigemina and Babesia bovis while Babesia major and Babesia divergens occur in temperate regions. The tick species responsible for transmission of disease are Boophilus spp., Rhipicephalus spp., Haemophysalis spp. and Ixodes spp. (Radostits et al., 2008).

Case history and observations

Case 1: A 7 years old cross breed cow (300 kg) was brought the teaching veterinary hospital, College of Veterinary and animal Science, Pantnagar, Uttarakhand with history of reduced feed intake from last 2 days. Owner complained that the cow was passing red coloured urine and diarrhoea. The clinical examination of cow revealed heavy tick infestation. The cow was having fever (104°F), suspended rumen motility, increased heart rate (86/min) with slight dehydration. The conjunctival mucus membrane was slightly pale. The urine was red coffee coloured. A 10 ml of blood sample was collected in EDTA vial for haematology. A blood smear prepared from peripheral blood (ear tip) was stained with Giemsa’s stain and examined under oil immersion. The smear revealed presence of comma shaped Babesia organism, while microscopic examination of faecal sample was negative for parasitic eggs. Haematology revealed TLC 6000/cmm, haemoglobin 3.6 gm%, DLC (N 50%, L 44%, M 2%, B Nil, E 4%).

Treatment and Discussion

Case 1: The cow was treated with Chlorpheniramine 10 ml i/m, Conciplex 10 ml i/m Normal Saline- 3 litre i/v for single time. Diminazene aceturate (Nilbery) was given at the dose rate of 1 ml/20 kg body weight as a single dose by deep route in cervical neck muscles. Iron supplement viz. Imferon 10 ml was given biweekly. The cow was examined on 3rd day of treatment clinically. Significant improvement was observed in vital parameters. Temperature reduced to 102°F, heart rate was 50/min. Feed intake of the cow increased to appreciable amount as per the owner. The colour of urine was also changed from coffee colour to normal clear transparent colour.

Case 2: Treatment was done by Berenil 20 ml deep i/m , Belamyl, 10 ml and Chlorpheniramine maleate, 10 ml i/m, antidiarroheal powder was given orally at the dose rate of 50 gm at 12 hours interval for 3 days. Streptochrome was given i/m 10 ml once along with iron preparation, Imferon 10 ml i/m biweekly. There was gradual improvement in the faecal consistency. The peripheral blood smear was examined 5 days post therapy revealed absence of babesia organisms.

In both the cases of babesiosis, rational approach to therapy of bovine babesiosis included babesicides, antihaemorrhagic drugs with iron supplement along with nonspecific supportive therapy like dextrose, purgatives, fluid therapy and B complex vitamins and iron preparations in order to enhance erythropoiesis in recovered patients (Gray and Murphy, 1985). Animals with acute anaemic anoxia due to severe haemoglobinuria needs blood transfusion (Sherlock et al., 2000). Quinuronium derivatives, acridine derivatives, aromatic diamidines and imidocarbs are the some of babesicidal drugs commonly used in treatment of bovine babesiosis (Radostitis et al., 2008). Berenil (Diminazene diaceturate) a babesicide is a most commonly used compound for the treatment of bovine and ovine babesiosis (Vial and Gorenflot, 2006).

References

A CASE OF LYMPHOMA IN A DOG-CHEMOTHERAPY TREATMENT IN CONJUNCTION WITH PREDNISOLONE, OMEGA-3 FATTY ACIDS AND IRON SUPPLEMENTS

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Lymphoma or lymphosarcoma is one of the most common cancers in dogs and is characterised by the growth of malignant tumour in lymph tissue, but can spread to any organ in the body. Main cause is genetic in nature, though some environmental factors have also been suspected to cause the disease (Morrison and Wallace, 1998). In one study use of herbicide 2,4-D have been found as a causative agent (Zahm and Blair, 1992).

Lymphoma has been classified in to four categories according to the area of growth and the affected organs- multicentric lymphoma, gastrointestinal lymphoma, mediastinal lymphoma, and extranodal lymphoma (Cynthia and Scottline, 2009).

It has been reported that 85% of documented cases were affected by multicentric lymphoma (Chand Khanna, 1996). This lymphoma affects lymph nodes and may or may not involve other organs. Eventually the multicentric lymphoma infiltrate the organs and cause death by organ failure.

Lymphoma has a very high remission rate i.e. survival rate is decreased without proper treatment. Affected dogs survive for about 2 months without any treatment while the survival rate may go up to 60-90% with proper combination of anti-cancer medicine and intensive chemotherapy.

Case study
A boxer, 5 years old, female dog was brought to the clinic after 2 months of onset of symptoms. During this period she was on antibiotics and anti-inflammatory drugs as prescribed by the other veterinarian. History revealed anorexia, fever, weight loss, laboured breathing, restlessness and lumps over neck, armpits, back and behind knees. The lumps were quite large on visual examination and were found to be hard and round on physical examination. Blood sample was evaluated for haematology and biochemistry. All parameters were within the normal range except Total leukocyte count and monocyte, which were found to be above the normal values.

Dog was given chemotherapy (Inj. Vincristine 0.5 mg/m² i/v once a week for seven weeks. Long term chemotherapy is very effective in treatment of lymphoma (Simon et al., 2006). Chemotherapeutic drugs are toxic to red blood cells and RBC count may drop rapidly to cause anaemia. Lymphoma and anaemia shortened survival time in dogs (Miller et al., 2009; Abbo and Lucroy, 2007). Oral iron supplement was given to prevent this condition. Chemotherapy drugs also suppress the bone marrow to make animals more prone to get infections. To combat this effect, immunity boosters were given to the dog. Along with this she was put on high protein diet (protein starve the cancer and provide good support to dog’s health system whereas carbohydrates feed the cancer and causes progression in the tumour’s growth) and omega 3 fatty acid supplements.

In conjunction with chemotherapy, oral corticosteroids (Predinosolone @ 2 mg/kg/day per os initially and then tapered to 1 mg/kg/day per os over a period of time) was given as well. Prednisolone alone can regress the lump in 50% cases (Chand Khanna, 1996) but it gives short term improvement, which may vary from 1-2 months (Morrison and Wallace, 1998). Use of prednisolone alone as a single treatment option can make the cancer resistant to other chemotherapeutic agents, so it should be used only when other treatment options cannot be used, corticosteroids helped in improving the appetite, activity and attitude of the animal.

After one week of treatment the lumps reduced to 1/3rd of the original size and all blood parameters were within the normal range. After second chemotherapy, the jaw lumps reduced to 1/7th of the initial size and lumps over back, neck, armpits and behind knees disappeared. Dog was very active physically and appetite was absolutely normal.

Blood haematological values and blood biochemistry values were evaluated on monthly basis and were found to be within the normal range. Remission was noticed after four months of last chemotherapy injection. The lumps over jaw were enlarged enough to make the dog anorectic, restless and she had laboured breathing. Total leukocyte count and monocyte were found above the normal range. Thoracic radiograph revealed the radio-opaque impressions near heart suggestive of cancerous growth in thoracic cavity and abdominal radiograph also had similar impressions over the kidney and liver area.

Dog was on liquid diet along with i/v fluid therapy. But she was restless and lethargic and uneventfully collapsed within one week of remission of lymphoma.

Summary
A combination of chemotherapy, corticosteroids, immune boosters, high protein diet, iron tonics and omega 3 fatty acid supplements gave good quality life to dog for about 6 months. But a relapse of lymphoma proved to be fatal to dog.

References
Chand Khanna, (1996) JAVMA.
Introduction
Congestive heart failure is the most common form of heart failure, which can result from a number of cardiac and extra cardiac diseases. Dilated cardiomyopathies are second most common cause of death in dogs (Rao et al., 2007). The classical treatment for the control of signs due to congestive heart failure (CHF) includes diuretics, vasodilators and antiarrhythmic drugs. Pimobendan is a novel drug with phosphodiesterase-inhibiting and calcium-sensitizing effects which increase myocardial contractility, promote arterial and venous dilatation and is effective for the treatment of congestive heart failure in dogs. (Haggstrom et al., 2008). Present article reports the clinical efficacy of Pimobendan in management of CHF along with the standard protocol of treatment.

Case history and observation
A 10-year-old male Irish setter dog weighing 35 kg was brought to outpatient department of TVCC, DUVASU, Mathura with complaint of chronic coughing, reduced exercise tolerance, anxiety and restlessness during the night, dyspnoea, orthopnoea, weight loss, progressive abdominal distension and reluctance to lie down. Paroxysmal cough was present resulting in the production of white, foamy, blood tinged sputum. Mucous membranes were muddy to somewhat cyanotic and pulmonary auscultation revealed increased respiratory sounds with crackles. Abdominal distension was evident with a positive fluid wave test and shifting dullness, indicating ascitis. On percussion of abdomen, hepatomegaly was suspected. Femoral pulse was prominent and easily palpable, but exhibited a jerky “collapsing” character with irregularity and tachycardia (100 beats/minute). Area of auscultation of heart was enlarged extending from 2nd to 9th intercostal space.

Tentative diagnosis
On the basis of history and clinical examination, the case was tentatively diagnosed as suffering from congestive heart failure. Thoracic radiography was performed in lateral recumbency as an integral part of the diagnosis (Root and Bahr, 2002). On lateral radiographic views, the long axis of the heart was measured from the ventral border of the left main stem bronchus to the cardiac apex. The short axis was measured at the widest point of the cardiac image on a line perpendicular to the long axis at the level of the caudal vena cava. The measurements were made using a metric ruler and sum of two measurements (long and short axis) was 12.5 vertebra heart score (VHS). This score was found to be higher than normal in case of dogs (Buchanan, 2000). Cardiomegaly confirmed the heart disease (Lamb and Boswood, 2002). The other observations included pulmonary oedema as a diffuse increase in interstitial density in the hilar or caudal lung field with hepato-splenomegaly.

Haematology and serum biochemistry analysis revealed neutrophilia with leucocytosis (94% Neutrophil, 06% lymphocyte, TEC-23,030/cu mm), augmented values of liver function test (Serum bilirubin- 2.3 mg/dl, Conjugated-1.5, Unconjugated-0.8, SGOT- 82.45 IU/ml, SGPT- 92.56- IU/ml, alkaline phosphates- 97.36 IU/ml, total protein-4.8 gm/dl, albumin- 1.9 gm/dl, globulin- 2.9 gm/dl). Electrolyte status was almost within normal range (Na- 141 mEq/L, K- 4.9 mEq/L).

Treatment and Discussion
The primary goal of treating CHF is to manage the clinical signs by reducing the formation of oedema and effusion and to increase cardiac output. Treatment was started with Tab. Lacilactone 50 @ 2.5 mg/kg b. wt bid orally, Tab. Enalapril @ 5 mg/kg b.wt bid orally, Tab. Digoxin @ 0.02 mg/kg b. wt bid orally on first day followed by 0.009 mg/kg b.wt bid orally, Tab. Higado plus (Silymarin, L-ornithine) 1 tab bid orally, Catnitor (Carnitine) 500 mg was also given 1 tab od orally and sodium restricted diet was recommended along with restricted exercise. On reevaluation of clinical signs after 7 days, there was appreciable improvement in the systemic and pulmonary congestion. Appetite of the dog also returned to near normal. After one month of therapy, signs of systemic and pulmonary congestion reappeared. At this time Pimobendan (Vetmedin®) was added @ 0.25-0.3 mg/kg per os bid. to the
previous treatment regimen (Kleemann and others 1998; Caro et al., 2009) and an improvement was seen within 3 days of therapy in the form of improved tolerance to exercise, reduced coughing and increased appetite. After 15 days of carrying out the new treatment regimen, dog was evaluated by radiography and serum biochemistry, VHS was found to be 12.0 with reduced degree of pulmonary congestion. Serum biochemistry was back in normal reference range, similar results have been reported by Caro et al. (2009). Further radiographic evaluation was done after 2 month, VHS was reduced to 11.5 this time and dog was clinically fit without any congestive signs. At present, the dog is clinically well and routinely coming for follow up.

Therapy of cardiac insufficiency is based on two principles including reduction of the heart work and strengthening of its contractility. Pimobendan being phosphodiesterase III inhibitor, sensitizes the cardiomyocytes to intracellular calcium and functions as a positive inotrope without increasing myocardial oxygen demand, resulting in balanced arteriovenous dilation. Mixtures of the d- and l-isomers of Pimobendan have been shown to cause stereospecific increases of the calcium sensitivity of cardiac myofilaments, specifically at the regulatory calcium binding sites of troponin C (Solaro et al., 1989), besides having vasodilatory properties mediated by the phosphodiesterase III effects on vascular smooth muscle. It is recommended to be used in conjunction with other standard therapies such as furosemide and an ACE inhibitor and can be safely used in combination with additional medications such as spironolactone, digoxin and other anti-arrhythmics (beta-blockers, calcium channel blocker). In combination these medications are currently considered as standard care in cases of CHF. Ouellet, et al. (2009) found similar clinical results of pimobendan and reported that patients did not develop arrhythmias and the heart frequency remained stable. Reduction in the VHS score during therapy was in agreement with Richard et al. (2007).

Improvement in clinical biochemistry was similar to Lombard et al. (2006), who reported improvement in biochemical parameters especially with respect to renal and hepatic function.

This study indicates that the new inodilator or calcium-sensitiser drug Pimobendan has shown convincing evidence of good tolerability, safety and clinical efficacy in randomised, blinded multicenter clinical trials. It appears therefore that this new drug represents a new pillar in the heart failure therapy of dogs. What remains to be tested are its safety and efficacy when applied in less severe stages of heart failure, convincing evidence of non-arrhythmogenesis, and elucidation of its influence on pathologic remodelling of the heart during these diseases (Lombard, 2006).

References
POST-PARTURIENT UTERINE PROLAPSE IN A GOAT-A CASE REPORT

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Introduction

Prolapse of the uterus is a common complication of third stage of labour in cow and ewes (Arthur et al., 1996). In the ruminant species, the uterine prolapse is generally a complete eversion of gravid horn (Nokes, 2009) yet it occurs less frequently in sows and rare in mares and bitches. The uterine prolapse is quite common in sheep and the incidence may be higher during the lambing season though this condition is much less common in goats and mare (Jackson, 2004). This condition usually occurs immediately or with in few hours after the delivery of last kid. However, in goats uterine prolapse following delivery is rarely encountered (Palanisamy et al., 2007). The uterine prolapse in goats may be complete with both the horns protruding out from vulva or may be limited to uterine body. In present case report, a complete eversion of uterine body and uterine horns, hanging out of vulvar lips up to hock joints and its successful management in a non-descript is discussed here.

Case history and observations

A non-descript pluriparous doe aged about 6½ years and delivered kids two days prior was brought to Teaching Veterinary Clinical Complex, Hisar, with post partum complication of uterine prolapse, soon after difficult kidding. The goat delivered two kids having optimum size and weight. On examination, the prolapsed mass was soiled, inflammed, oedematous and intact foetal membranes. The goat was apparently healthy and was in standing position. The prolapsed mass was hanging out from the vulva up to hock region. Rectal temperature recoded was 103°F. Increased pulse rate and respiratory rate was there. The placental cotyledons were attached to maternal caruncles and moderate bleeding was noticed. Clinically, the goat was discomfort, restless and panic. There was a mild internal haemorrhage due to rupture of uterine vessels. Uterus appeared as congested and contaminated mass (Fig.1).

Clinical management and treatment

Considering the severity of the case and the owner’s agreement, the management of prolapsed mass was proceed with epidural anaesthesia injecting 5 ml of lignocaine hydrochloride. After 10 minutes of it, the prolapsed mass washed with lukewarm saline solution by wearing sterile polythene sleeves in both hands. The everted mass was elevated to the level of vulva to relieve the urine (Selvaraju et al., 2010). The foetal membranes were detached manually with finger tips from maternal caruncles avoiding bleeding. After this whole mass and vulvar lips are again washed by luke warm normal saline solution and finally washed with 1:1,000 potassium permangnate solution (KMnO₄). A combination of lignocaine jelly and soframycine ointment on exposed mass was applied. Then with the help of fingers the uterus with uterine horn was retracted to its original anatomical position by slight lubrication with liquid paraffin. A single furea bolus was placed intra uterine. To avoid further prolapse, a stay suture was applied on vulvar lips and antiseptic dressing was advised for five days. Intramuscular injections of 10 I.U. of oxytocin and 150 ml of calcium borogluconate administered through intravenous route. Five ml of Chromostate was given intramuscularly at the time of replacement to cheek bleeding. To avoid further complication, the goat was administered 500 ml fluid therapy of Dextrose (5%) along with metronidazole @ 10 ml/1 kg b.wt. Then 1 gm Cefstan, 3 ml of meloxiccan, 3 ml of Avil and 3 ml of Vit. B complex injected intramuscularly as supportive therapy. Oral Calcium with laxative diet also advised for 10 days. The stay suture from vulvar lips get removed after 5 days.

Discussion

The occurrence of uterine eversion in goat has been reported by Dhaliwal et al. (1986), Baruah and Borgohain (1996), Palanisamy et al. (2007) and Gupta et al. (2010) The common complication of uterine prolapse may be haemorrhages, shock, septic metritis, decreased milk yield, sucking problems, infertility or death. Sometimes in delayed cases, partial contraction of cervix interferes with repositioning, resulting in reoccurrence of prolapse. The retention of foetal membranes and uterine inertia which resulted following abortion might be the cause for uterine prolapse in this case as described by Baruah and Borgohain (1996). Interrupted horizontal matters stay sutures were applied on vulvar lips to prevent re-occurrence of uterine prolapse and thus reoccurrence was not
noticed further. After 5 days sutures were removed and treatment was stopped. Resultant doe died after ten days of kidding. Uterine prolapse is predisposed to a violet tenesmus and retention of foetal membranes as reported by Roberts (2004).

Reference
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USE OF NEOSTIGMINE IN THE TREATMENT OF PUPS SUFFERING FROM IVERMECTIN TOXICITY- A CLINICAL CASE STUDY

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Ivermectin is a macrolide antibiotic of avermectins class of chemicals produced from a fungus *Streptomyces avermitilis*. It has a wide margin of safety and broad spectrum of activity against many nematodes and arthropod parasites. Ivermectin toxicity has been reported in many animal species due to inadvertent use of the product. Major concern of Ivermectin toxicity is in Collie breed of dogs (Paul et al., 1987), which seems to have greater penetration of Ivermectin and toxicity than adults. Toxicity signs and symptoms in young animals are more sensitive to Ivermectin for the normal dose if Ivermectin is needed for toxic conditions. Prognosis of the treatment depends upon species, breed, age of the animal and dose of Ivermectin. At least ten times increase in mental alertness and helps in reversing Ivermectin-induced CNS depression, weakness, ataxia and stupor may be related to GABA agonistic action of Ivermectin in CNS and drooling may be related to GABA-mediated cholinergic effects. Neostigmine is not a specific antidote of Ivermectin toxicity rather it is a reversible acetylcholinesterase inhibitor and increases level of acetylcholine in the CNS and is related to the mental alertness and arousal. Therefore, Neostigmine may be taken as physiological antagonist of Ivermectin in CNS and cause increase in mental alertness and helps in reversing Ivermectin-induced CNS depression. Atropine sulphate was used to counter GABA-mediated cholinergic effects of Ivermectin and peripheral cholinergic effects of neostigmine. Use of dexamethasone and DNS solution is a part of general line of treatment in shock and toxic conditions. Prognosis of the treatment depends upon the severity of clinical symptoms and individual response.

Case history and observations

Three pups (German Shepherd, Pomeranian and Rottweiler) of thirty days, thirty-five days and thirty-seven days of age, respectively, were brought to the canine section of Teaching Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary and Animal Science, Hisar for treatment. History revealed that the owners injected a high dose of Ivermectin to their pups for the control of ectoparasites. Upon clinical examination, weakness, ataxia, tremors, drooling, shallow respiration, tachycardia and hypothermia were observed. On the basis of history and clinical symptoms it was diagnosed that the pups were suffering from Ivermectin toxicity.

Treatment and Discussion

The pups were administered Neostigmine @ 0.025 mg/kg b.wt., i/v at twelve hourly interval, for three days. Dexamethasone @ 0.05 mg/kg b.wt., i/m, o.d. for three days followed by 0.025 mg/kg b.wt., i/m, once a day, for next two days, atropine sulphate @ 0.045 mg/kg b.wt., s/c, once a day for three days and Dextrose Normal Saline 70 ml slow i/v, o.d., for three days. Good management care was provided with the above therapy and the same was suggested to the owners of the pups. Two of the three pups showed improvement after six hours of the treatment. Complete recovery was observed in five days. Rottweiler pup did not respond to the above treatment and died after three hours of treatment. Ivermectin is effective against arthropods and nematodes, but not against trematodes and cestodes. The drug acts as GABA agonist (Pong and Wang, 1982) and trematodes and cestodes apparently lack a GABA system. In mammals, Ivermectin exerts toxicity by potentiating the release and binding of GABA in the CNS (Campbell and Benz, 1984; Bennett, 1986; Barragry, 1987). Although Ivermectin does not readily crosses the BBB, there are several reports of Ivermectin-induced CNS toxicosis in the Collie dogs (Paul et al., loc cit and Barragry, loc cit), and in other domestic animals (Houston et al., 1987). Some adverse effects seen in dogs, horses, and cattle given Ivermectin may be due to GABA-mediated cholinergic effects (Bassudde, 1989) which seem to increase serum pseudocholinesterase levels. Passage of Ivermectin through BBB is related to high dose of Ivermectin or mutation in p-glycoprotein (Yang, 2008). P-glycoprotein is a large transmembrane protein in BBB encoded by multigene drug resistance (mdr-1) gene and functions as a drug transport pump at BBB to transport a variety of drugs from brain to blood. Ivermectin sensitive Collies have altered mdr-1 gene expression compared with the unaffected Collies. In the present study, CNS depression, weakness, ataxia and stupor may be related to GABA agonistic action of Ivermectin in CNS and drooling may be related to GABA-mediated cholinergic effects. Neostigmine is not a specific antidote of Ivermectin induced toxicity rather it is a reversible acetylcholinesterase inhibitor and increases level of acetylcholine in the CNS and is related to the mental alertness and arousal. Therefore, Neostigmine may be taken as physiological antagonist of Ivermectin in CNS and cause increase in mental alertness and helps in reversing Ivermectin-induced CNS depression. Atropine sulphate was used to counter GABA-mediated cholinergic effects of Ivermectin and peripheral cholinergic effects of neostigmine. Use of dexamethasone and DNS solution is a part of general line of treatment in shock and toxic conditions. Prognosis of the treatment depends upon the severity of clinical symptoms and individual response.

References

The facial nerve (cranial nerve VII) passes through the petrous temporal bone, and then exits the skull through the stylomastoid foramen and splits into auricular, palpebral, and buccal branches. The common causes of damage to the nerve are fracture of the petrous temporal bone, guttural pouch mycosis and damage to the peripheral nerve at the mandible (Radostits et al., 2000). Clinical signs of facial nerve paralysis vary with the location, severity and chronicity of the lesion. A lesion of the buccal branch of the facial nerve which supplies motor fibres to the lips results into the loss of prehensile capacity of the horse (Smith, 1996). The present case report communicates the facial nerve paralysis in a horse leading to cachexia and death.

A dark bay coloured 5 years old male horse (Case No. 1824) was presented to the Teaching Veterinary Clinics of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, with a primary complaint of inability to take feed for last 25 days with gradual loss of weight. The horse had been defaecating and urinating normally, however, it had not been dewormed for last 6 months. The client owned 5 horses with a tendency to fight each other. There was history of fight with this horse.

Clinical examination of the horse revealed it to be dull, depressed and emaciated. The rectal temperature was 100°F with congested conjunctival mucus membrane and severe dehydration. The lower lip of the horse was drooping. The animal was thoroughly examined to rule out any abnormality of teeth, which were found to be normal. When offered green fodder, the horse, despite of visible signs of hunger, exhibited signs of inability to prehension. This phenomenon of showing interest in the feed misled the owner about the horse that it is taking feed but still loosing the weight.

The haematological findings included Hb 13.5%, TLC 13,970/cumm, neutrophils 90%, lymphocyte 10%, ESR 90 mm/hr and platelets 1,08,000 cells/ml. Fecal and blood examination for any parasite resulted negative. Serum biochemistry revealed total bilirubin 4.4 mg/dl, AST 691 U/L, ALT 38 U/L, AKP 144 U/L, BUN 8.4 mg/dl, creatinine 1.2 mg/dl, Ca 10.4 mg/dl, P 3 mg/dl and creatine kinase 1338 U/L. The case was diagnosed as facial nerve paralysis based on clinical findings and the cause could be damage to the peripheral nerve. Other two causes were ruled out on the basis of clinical findings. In guttural pouch mycosis of horse, the most common sign is epistaxis, spontaneous and severe haemorrhage and repeated bouts may precede a fatal haemorrhagic episode. Other cause of facial nerve paralysis was ruled out as there was no history or sign of fracture of the petrous temporal bone.

The horse was treated with normal saline solution @ 5 litre i/v, Dextrose normal saline solution @ 5 litre i/v, Biotrim @ 20 ml bid i/m, Crys 40 @ 40 lacs IU bid i/m, Tribivet @ 15 ml od i/m, Brotone @ 40 ml bid per os, Yeasacc 2 boli bid per os and Hitek bolus @ 1 bolus (80 mg) once per os. The horse

Fig. 1: Drooping of lower lips

Fig. 2: Cachexia in horse
could survive only 3 days during the treatment regimen.

Trauma is a common cause of facial nerve paralysis in all species. Tyler et al. (1993) reviewed case records of 450 horses with signs of neurological diseases and found that basisphenoid/basioccipital bone fractures were the most common form of cranial trauma and facial nerve paralysis the most common cranial nerve injury. Halter injuries and prolonged lateral recumbency in horses may injure the buccal branches of the facial nerve on the side of the jaw and cause unilateral or bilateral paresis or paralysis of the lips and nostrils (Radostits et al., 2000). Improper healing of injury after a blow (such as a kick from another horse) to the face, even though the skin may not be broken, can also result in facial nerve paralysis (Radostits et al., 2000). In the present case, there was no clear history of injury but close proximity of horses and their predisposition towards fight might have lead to the conclusion of an obscure injury to the horse. The horse had droopy lower lip with inability to prehend the feed. Radostits et al. (2000) described paresis or paralysis of the lips and nostrils with a lesion of the buccal branch of the facial nerve, as it courses along the surface of the masseter muscles. In acute lesions, the paralyse side of the lip may hang down and expose the gums. When the horse eats or drinks, food and fluids may fall from the lips rendering the horse hungry. The horse succumbed to death because of severe emaciation. The present case report concludes suggesting close watch on horses kept in near proximity.

References
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STUDY ON PREVALENCE OF YOKE GALL IN KARNATAKA STATE IN BULLOCKS

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ABSTRACT

Prevalence of yoke gall was studied under three different situations i.e., number of yoke gall cases that receive farmers attention for treatment, number of yoke gall cases that were sold in cattle market and number of bullocks with yoke gall that were slaughtered. Thus, the study was conducted under three categories as clinical cases, among animals sold in cattle market and among the animals disposed for slaughter.

Key words: Prevalence, yoke gall, Karnataka, bullocks

Introduction

Yoke gall refers to inflammatory swelling of skin and subcutaneous tissue on the dorsal aspect of the neck in draught animals. This condition results due to continuous friction of yoke placed on the neck of the animal while pulling the bullock cart or ploughing implements. Yoke gall is one of the commonest surgical affection in bullocks. According to Prof. N. Ramaswamy, former chairman of animal welfare board of India and present director, CARTMAN, one million cattle are slaughtered every year due to yoke gall condition.

However, yoke gall is not such a disease that cannot be cured. Because, it is a work induced disease and can recur if animal is repeatedly used, the farmers tend to sell their bullocks to others or for slaughter purpose instead of providing a prolonged treatment during their busy time of ploughing or harvesting. Another reason for disposal of large number of animals in absence of effective and well informed network of animal health care in remote villages.

Though yoke gall is a very common condition, limited work has been done on this problem and hence the literature on exact incidence covering a large area is lacking. Hence, to know the extent of this problem in the areas, where active agricultural operations take place, study was conducted in a large area covering over 2,50,000 animals, which were treated for different diseases. As clinical incidence alone is not sufficient to describe an existing problem in an area, attempts were made to describe the prevalence of yoke gall in non clinical situations i.e., when the farmers have not provided the treatment or resorted to sell or slaughter the animals.

Materials and Methods

The present study on prevalence of yoke gall was done in three different situations i.e., among clinical cases, among animals sold in cattle market and animals slaughtered. Prevalence of yoke gall among the clinical cases was studied during the last 5 years (2000-2004) in 9 Veterinary hospitals of 9 different districts in North, Central and South Karnataka regions viz., Veterinary college hospital, Bidar; Veterinary dispensary, Tengali, Gulbarga Dist.; Veterinary hospital, Raichur; Veterinary hospital, UAS, Dharwad; Veterinary dispensary, Sambra, Belgaum Dist.; Veterinary hospital, Kunigal, Tumkur Dist.; Veterinary dispensary, V.C. Farm, Mandya Dist.; Veterinary hospital, Shidlagatta, Kolar Dist. and Veterinary hospital, Doddaballapur, Bangalore (rural) District. A total of 2,61,882 clinical cases were presented to 9 veterinary hospitals during 5 years (2000 to 2004). Out of 2,61,882 cases 70,896 cases were brought for the treatment of surgical disorders. The prevalence of yoke gall was evaluated both out of all types of diseases and out of surgical disorders.

Prevalence of yoke gall among the animals sold in cattle market called "Mylara" in Bidar dist., which were sold for various reasons though out the year. The study was conducted in different seasons of the year for 10 times in different Sunday markets and prevalence of yoke gall was evaluated out of all types of animals such as cattle and buffaloes and total bullocks exhibited (Photo 1,2,3). A total of 2,225 animals were exhibited for sale in cattle market, out of which 1,100 were bullocks. The prevalence of yoke gall was evaluated out of 2,225 cattle and buffaloes exhibited for sale. Prevalence of different types of yoke gall was also evaluated.

Prevalence of yoke gall among the animals disposed for slaughter was studied at Karnataka meat and poultry marketing Corporation (KAMPCO), Bangalore and by examining the animals before they were taken to private slaughter houses of Chitguppa town (Bidar district) on every Wednesday of each week (Photo 4,5). A total of 1,148 animals were disposed forslaughter. Prevalence of yoke gall among the clinical cases was estimated from 9 Veterinary hospitals during 5 years (2000-2004).

REFERENCES

1. Part of M.V.Sc. Thesis. Corresponding author. Email: manjunathpatil.2010@rediffmail.com; Mobile No: 09449236868.
slaughter, out of which 825 were bullocks at KAMPCO, Bangalore city.

A total of 2,143 cattle and buffaloes were disposed for slaughter at private slaughter houses, Chitguppa and out of which 1,230 animals were bullocks. These animals were examined for presence of yoke gall on their bodies to know its prevalence. Prevalence of different types of yoke gall among the animals disposed for slaughter was also studied.

Results and Discussion

Prevalence of yoke gall among clinical cases and also among surgical cases only, presented to 9 veterinary institutions is given in Table 1. Out of 2,61,882 cases, 70,896 cases were surgical cases. A total of 2,081 cases were affected with yoke gall in the present study. The percentage of prevalence among the total cases (medical and surgical) was 0.79% and among the surgical cases, it was 2.94%. This showed that yoke gall shares 1% prevalence when all medical and surgical ailments are considered and among all surgical problems it constitutes 3% and hence is one of the important disorders in farm animals.

Rahman and Ahmed (1975) reported 2.7% of incidence of yoke gall in one hospital of Bangladesh. This incidence is slightly higher than the overall prevalence of 0.79% recorded in the present study. The reason for this is when incidence is studied in only one hospital, the incidence of yoke gall may be less or more depending upon the intensive agricultural practices and use of bullock for draft purpose. An incidence of 27.89% out of all surgical cases mentioned by Kale (1997) involved very short duration and small locality and hence do not match with the prevalence observed in the present study and the other report of Rahman and Ahmed (1975).

The highest (7.57%) prevalence of yoke gall was observed at veterinary dispensary, V.C. farm (Mandya dist.) where intensive paddy and sugar cane cultivation using bullocks around river Kaveri and Krishna Raja Sagara Dam. The least (0.48%) prevalence was observed at veterinary hospital, Doddaballapur (Bangalore rural district) due to more industrialization, reduction in the availability of agricultural land and urbanization.

The year wise prevalence of yoke gall in bullocks among the total cases and also among the surgical cases presented to 9 veterinary institutions are given in Table 2. The highest prevalence of yoke gall was observed during the year 2004 (0.93%) and the least was observed during the year 2003 (0.68%). There was negligible variation regarding the occurrence of yoke gall among different years and this may be attributed to average rainfall during that year and extent of use of bullocks for land cultivation.

The season wise prevalence of yoke gall in animals are presented in Table 3 and Fig. 4. The prevalence in summer was 465 (22.34%), in rainy season was 962 (46.23%) and in winter season was 654 (31.43%). This suggested that prevalence of yoke gall is highest during monsoon due to increased agricultural activities using bullocks as draft power. The prevalence was also more in winter due to continued agricultural activities during growing and harvesting stages of crops. Also the acute cases which do not respond for treatment continue as sub acute and chronic yoke galls during winter and thus more prevalence may be seen during the season when compared to summer. As most of the cultivation is rain dependent in the state, less prevalence was noticed in summer. Adequate literature is not available to discuss these results with other studies.

Age wise prevalence of yoke gall was analysed only at university college hospital, Bidar where records of exact age of the animals were mentioned. The age wise prevalence of yoke gall at Veterinary college, hospital, Bidar from 2000-04 is presented in Table 4 and Fig. 2. The highest prevalence (48.42%) was observed in the age group of 6-8 years followed by the age group of more than 8 years (34.74%). The least prevalence (16.84%) was observed in the animals below the age of 5 years. This suggested that the bullocks are more extensively used for draft purpose after 5 years and the prevalence increases due to tender skin of bullocks at this age. Though very young animals are much more prone to yoke gall, it was more commonly observed in young adults due to extent of work received by them. It was also high in aged bullocks (34.74%), probably due to untreated or unsuccessfully treated persisting chronic yoke galls.

Kale (1997) observed 49.75% incidence in bullocks of 9-12 years of age, 27.94% between 4-8 years of age and 22.29% above 12 years of age.

Sex wise prevalence of yoke gall was considered only in veterinary hospital, Kunigal (Tumkur district) of southern Karnataka, where yoke gall cases occurred both in bullocks and cows. This was the only hospital where the prevalence of yoke gall was seen in female animals. The prevalence is given in Table 5.

Out of 2,61,882 clinical cases, yoke gall was seen in 1,840 (88.42%) male animals and 241 (11.58%) female animals. This suggested that mainly bullocks are used in Karnataka for draft power. In only at one location (Tumkur district) 241 females (66.39%) were found to have yoke gall as against 122 males (33.61%). At this location, cows were used for land cultivation and hence yoke gall was found in these animals. Literature is very scanty to compare these results with other studies.

Prevalence of various types of yoke gall in animals is presented in Table 6 and Fig. 3. Analysis of 2,081 cases of yoke gall in the present study revealed that acute yoke gall occurred in 835 (40.13%) animals, sub acute in 566 (27.19%) animals and chronic type of yoke gall in 680 (32.68%) animals. The study revealed that acute type of yoke gall is most predominant (40.13%) followed by chronic (32.68%) and sub acute
(27.19\%) types of yoke gall. Higher prevalence of acute yoke gall was statistically significant (P<0.01) when compared to sub acute and chronic types. There was no statistical difference (P>0.05) between the prevalence of sub acute and chronic type of yoke gall.

Prevalence of yoke gall among animals marketed at cattle market is given in Table 7. A total of 2,225 animals were presented to Mylara cattle market for sale on ten different Sunday markets. Out of 2,225 animals, 1,100 animals were bullocks. After thorough examination, it was found that among 1,100 bullocks exhibited for sale, 38 animals were affected with yoke gall. The prevalence of yoke gall among the bullocks exhibited for sale was 3.46%.

Different types of yoke gall were also studied in animals marketed at cattle market and their prevalence at Mylara cattle market is given in Table 7. A total of 38 bullocks were found affected with different types of yoke gall. Among 38 animals, 10 were found to have acute yoke gall, 20 were found to have sub acute and 8 were found to have chronic tumour type of yoke gall. The prevalence of acute yoke gall was 26.32%, sub acute was 52.63% and chronic was 21.05%.

Prevalence of yoke gall among the animals disposed to slaughter houses is given in Table 8. A total of 33 bullocks were found affected with different types of yoke gall. Among 33 animals, 6 were found to have acute yoke gall, 10 were found to have sub acute and 17 were found to have chronic tumour type of yoke gall. The prevalence of acute yoke gall was 18.18%, sub acute was 30.30% and chronic was 51.52%.

In study on prevalence of yoke gall at private slaughter house, Chitguppa (Bidar district) a total of 2,143 cattle and buffaloes were disposed for slaughter. Out of which 1,230 animals were bullocks. After thorough examination, it was found that, among 1,230 bullocks disposed for slaughter, 65 bullocks were affected with yoke gall. The prevalence of yoke gall among the bullocks disposed for slaughter was 5.29%.

Different types of yoke gall was also studied in animals disposed to slaughter houses and their prevalence at private slaughter houses, Chitguppa (Bidar district) is given in table no. 8. A total of 65 bullocks were found affected with different types of yoke gall. Among 65 animals, 10 were found to have acute yoke gall, 20 were found to have sub acute and 35 were found to have chronic tumour type of yoke gall. The prevalence of acute yoke gall was 15.39%, sub acute was 30.77% and chronic was 53.85%.

### Table 1: Prevalence of Yoke Gall among Clinical Cases (2000-2004)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of Veterinary Institution</th>
<th>Total No. of All Clinical Cases Treated in 5 yrs</th>
<th>Total No. of Surgical Cases Treated in 5 yrs</th>
<th>Total No. of Yoke Gall Cases</th>
<th>Percentage of Yoke Gall Cases among Clinical Cases Treated in 5 yrs</th>
<th>Percentage of Yoke Gall Cases among Surgical Cases Treated in 5 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Veterinary College Hospital, Bidar (Bidar district)</td>
<td>10,960</td>
<td>5,588</td>
<td>95</td>
<td>0.87</td>
<td>1.70</td>
</tr>
<tr>
<td>2</td>
<td>Veterinary Dispensory, Tengali (Gulbarga district)</td>
<td>10,006</td>
<td>5,285</td>
<td>205</td>
<td>2.05</td>
<td>3.88</td>
</tr>
<tr>
<td>3</td>
<td>Veterinary Hospital, Raichur (Raichur district)</td>
<td>44,351</td>
<td>6,935</td>
<td>460</td>
<td>1.04</td>
<td>6.63</td>
</tr>
<tr>
<td>4</td>
<td>Veterinary Hospital, UAS Dharwad (Dharwad district)</td>
<td>29,150</td>
<td>5,165</td>
<td>102</td>
<td>0.35</td>
<td>1.98</td>
</tr>
<tr>
<td>5</td>
<td>Veterinary Dispensary, Sambra (Belgaum district)</td>
<td>17,176</td>
<td>2,303</td>
<td>28</td>
<td>0.16</td>
<td>1.22</td>
</tr>
<tr>
<td>6</td>
<td>Veterinary Hospital Kunigal, (Tumkur district)</td>
<td>48,108</td>
<td>14,980</td>
<td>363</td>
<td>0.76</td>
<td>1.22</td>
</tr>
<tr>
<td>7</td>
<td>Veterinary Dispensary, V.C.Farm, Mandya (Mandya district)</td>
<td>29,622</td>
<td>8,834</td>
<td>669</td>
<td>2.26</td>
<td>7.57</td>
</tr>
<tr>
<td>8</td>
<td>Veterinary Hospital, Shidadghatta (Kolar district)</td>
<td>49,533</td>
<td>14,879</td>
<td>126</td>
<td>0.25</td>
<td>0.85</td>
</tr>
<tr>
<td>9</td>
<td>Veterinary Hospital, Doddaballapur (Bangalore (rural) district)</td>
<td>22,976</td>
<td>6,927</td>
<td>33</td>
<td>0.14</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,51,882</strong></td>
<td><strong>70,896</strong></td>
<td><strong>2,081</strong></td>
<td><strong>0.79</strong></td>
<td><strong>2.94</strong></td>
<td><strong>2.94</strong></td>
</tr>
</tbody>
</table>

The prevalence of yoke gall among the bullocks disposed for slaughter was 4%. Different types of yoke gall were also studied in animals disposed to slaughter houses and their prevalence at KAMPCO, Bangalore is given in Table 8. A total of 33 bullocks were found affected with different types of yoke gall. Among 33 animals, 6 were found to have acute yoke gall, 10 were found to have sub acute and 17 were found to have chronic tumour type of yoke gall. The prevalence of acute yoke gall was 18.18%, sub acute was 30.30% and chronic was 51.52%.
### Table 2: Prevalence of yoke gall in different years

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Year</th>
<th>Total no. of all clinical cases treated</th>
<th>Total no. of surgical cases treated</th>
<th>Total no. of yoke gall cases</th>
<th>Percentage of yoke gall among clinical cases</th>
<th>Percentage of yoke gall among surgical cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2000</td>
<td>48,818</td>
<td>13,077</td>
<td>377</td>
<td>0.77</td>
<td>2.88</td>
</tr>
<tr>
<td>2.</td>
<td>2001</td>
<td>51,584</td>
<td>13,205</td>
<td>415</td>
<td>0.81</td>
<td>3.14</td>
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<tr>
<td>3.</td>
<td>2002</td>
<td>50,292</td>
<td>13,797</td>
<td>395</td>
<td>0.79</td>
<td>2.86</td>
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<tr>
<td>4.</td>
<td>2003</td>
<td>55,117</td>
<td>14,169</td>
<td>375</td>
<td>0.68</td>
<td>2.65</td>
</tr>
<tr>
<td>5.</td>
<td>2004</td>
<td>56,071</td>
<td>16,648</td>
<td>519</td>
<td>0.93</td>
<td>3.12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2,61,882</td>
<td>70,896</td>
<td>2,081</td>
<td>0.79</td>
<td>2.94</td>
</tr>
</tbody>
</table>

### Table 3: Prevalence of yoke gall in different seasons

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Season</th>
<th>No. of yoke gall cases</th>
<th>Prevalence of yoke gall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Summer</td>
<td>465&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.34</td>
</tr>
<tr>
<td>2.</td>
<td>Rainy</td>
<td>962&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.23</td>
</tr>
<tr>
<td>3.</td>
<td>Winter</td>
<td>654&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.43</td>
</tr>
</tbody>
</table>

a. b: Numericals bearing superscript a and b differ significantly (P<0.01) from each other.

The data was analysed after angular transformation of observation.

### Table 4: Age wise prevalence of yoke gall at Veterinary college hospital, Bidar (Bidar district)

<table>
<thead>
<tr>
<th>Age</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>Total</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 years</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>16</td>
<td>16.84</td>
</tr>
<tr>
<td>6 to 8 years</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>46</td>
<td>48.42</td>
</tr>
<tr>
<td>&gt; 8 years</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>33</td>
<td>34.74</td>
</tr>
</tbody>
</table>

### Table 5: Prevalence of yoke gall in male and female at Veterinary hospital, Kunigal (Tumkur district)

<table>
<thead>
<tr>
<th>Year</th>
<th>Male No. of cases</th>
<th>Prevalence (%)</th>
<th>Female No. of cases</th>
<th>Prevalence (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>12</td>
<td>32.43</td>
<td>25</td>
<td>67.57</td>
<td>37</td>
</tr>
<tr>
<td>2001</td>
<td>25</td>
<td>38.46</td>
<td>40</td>
<td>61.54</td>
<td>65</td>
</tr>
<tr>
<td>2002</td>
<td>13</td>
<td>27.08</td>
<td>35</td>
<td>72.92</td>
<td>48</td>
</tr>
<tr>
<td>2003</td>
<td>22</td>
<td>25.88</td>
<td>63</td>
<td>74.12</td>
<td>85</td>
</tr>
<tr>
<td>2004</td>
<td>50</td>
<td>39.06</td>
<td>78</td>
<td>60.94</td>
<td>128</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>33.61</td>
<td>241</td>
<td>66.39</td>
<td>363</td>
</tr>
</tbody>
</table>

### Table 6: Prevalence of different types of yoke gall among clinical cases

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Types of yoke gall</th>
<th>No. of yoke gall cases</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acute</td>
<td>835&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.13</td>
</tr>
<tr>
<td>2.</td>
<td>Subacute</td>
<td>566&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.19</td>
</tr>
<tr>
<td>3.</td>
<td>Chronic</td>
<td>680&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.68</td>
</tr>
</tbody>
</table>

a. b: Numericals bearing superscript a and b differ significantly (P<0.01) from each other.

The data was analysed after angular transformation of observation.
Table 7: Prevalence of yoke gall among the animals marketed in cattle market.

<table>
<thead>
<tr>
<th>Total animals exhibited for sale</th>
<th>Prevalence of yoke gall in all animals (%)</th>
<th>Total bullocks exhibited for sale</th>
<th>Prevalence of yoke gall among bullocks exhibited for sale (%)</th>
<th>Prevalence of different types of yoke gall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,225</td>
<td>1.71</td>
<td>1,100</td>
<td>3.46 (38)</td>
<td>26.32 (10) 52.63 (20) 21.05 (8)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate number of yoke gall cases.

Table 8: Prevalence of yoke gall among the animals disposed to slaughter houses.

<table>
<thead>
<tr>
<th>Name of slaughter house</th>
<th>Total No. of animals (cattle &amp; buffaloes slaughtered)</th>
<th>Prevalence of yoke gall in all animals (%)</th>
<th>Total No. of bullocks slaughtered</th>
<th>Prevalence of yoke gall among bullocks disposed for slaughter (%)</th>
<th>Prevalence of different types of yoke gall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karnataka meat &amp; poultry marketing corporation (KAMPCO) Bangalore</td>
<td>1,148</td>
<td>2.88</td>
<td>825</td>
<td>4 (33)</td>
<td>18.18 (6) 30.30 (10) 51.52 (17)</td>
</tr>
<tr>
<td>Private slaughter houses Chitguppa (Bidar district)</td>
<td>2,143</td>
<td>3.03</td>
<td>1,230</td>
<td>5.29 (65)</td>
<td>15.39 (10) 30.77 (20) 53.85 (35)</td>
</tr>
<tr>
<td>Total</td>
<td>3,291</td>
<td>2.98</td>
<td>2,055</td>
<td>4.77 (98)</td>
<td>16.33 (16) 30.61 (30) 53.06 (52)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate number of yoke gall cases.

Fig. 1: Seasonal prevalence of yoke gall

Fig. 2: Age wise prevalence of yoke gall at Veterinary College hospital, Bidar (Bidar district)
Fig. 3: Prevalence of different types of yoke gall

- Chronic: 32.68%
- Acute: 40.13%
- Subacute: 27.19%

Fig. 4: Comparative assessment of prevalence (%) of yoke gall in animals brought for treatment, sold and disposed for slaughter.

- Animals treated: 0.79%
- Animals sold: 1.71%
- Animals slaughtered: 2.97%

Fig. 5: Comparative assessment of prevalence (%) of yoke gall in animals brought for treatment, sold and disposed for slaughter.
Photographs of bullocks suffering from yolk gall, going to various markets for selling.

The prevalence of yoke gall was compared among clinical situations and in the population of animals marketed and slaughtered and the results are given in Fig. 4 and 5. The results revealed that the prevalence of yoke gall among the animals treated for total medical and surgical ailments, among the animals sold in cattle market and among the animals disposed for slaughter was 0.79%, 1.71% and 2.97%, respectively. This showed that the prevalence of yoke gall varies from 0.79% to 2.97%, which is quite significant in a population and yoke gall is an important disease in draft animal.

The prevalence of yoke gall among the animals brought for treatment was less (0.79%) suggesting that all the existing cases in a population are not brought for treatment. The prevalence was found slightly more among the animals brought to cattle markets for selling (1.71%) or among the animals disposed for slaughter (2.97%) suggesting that more and more cases are added to these two groups every year due to continued use of animals for farm work, inability of the farmers to provide treatment or unsuccessful treatment for all the types of yoke gall. Hence when the farmers decide to sell their animals in exchange of healthy and new animals, slightly more prevalence of yoke gall is found under this type of study.

Among the different types of yoke gall, acute yoke gall was significantly (P<0.01) prevalent (40.13%) under clinical conditions when compared to sub acute (27.19%) and chronic (32.68%). The higher prevalence of acute yoke gall among the total yoke gall cases treated (835 out of 2,081) suggested that farmers prefer to provide treatment at acute stage of the disease, when it is recent and interfering with their farm work during monsoon season. The more sub acute (52.63%) and chronic (21.05%) yoke galls were found in cattle market in the present study suggest that farmers decide to sell such animals in exchange of good animals rather than depending on such animals for farm work. Similarly, more chronic (53.06%) and sub acute (30.61%) yoke gall cases among the animals disposed for the slaughter suggested that when the treatment is not successful or economical or not worthy due to their old age and chronicity of the disease, farmers prefer to dispose them off. Hence, slightly higher prevalence was found under above two situations when compared to fresh cases seen under clinical conditions.

References
EMERGING STATUS OF FOOTROT IN SHEEP IN HARYANA

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ABSTRACT
Emergence of footrot in sheep was observed for the first time in Haryana and adjoining areas of Rajasthan as five outbreaks were reported and investigated in different places. Animals of all age groups were affected, with morbidity of 30-40%. Affected animals showed good response to antibiotics like enrofloxacin and gentamicin.

Key words: Sheep, footrot

Introduction
Footrot, is a hoof infection that is commonly found in sheep, goat and cattle. It is characterized by an exudative inflammation with a strongly characteristic odor; this is followed by necrosis of the epidermal tissues of the hoof, leading in some cases to the complete separation of the horn (Katitch, 1979). The clinical presentation of footrot is separation of the hoof horn from the sensitive tissue of the claw with a greyscum present in the resulting cavity with a characteristic odour (Green and George, 2007). Severe virulent ovine foot rot was identified as an important disease of sheep and an increasing cause of lameness in UK (Harwood et al., 1997).

Footrot caused by Dichelobacter nodosus. The accepted pathogenesis for disease is that when the interdigital skin of the foot is damaged or wet for a prolonged period, it may be invaded by the ubiquitous soil and faecal bacterium Fusobacterium necrophorum. In isolation, F. necrophorum may cause inflammation of the interdigital skin (i.e. interdigital dermatitis) and produce a number of toxins which cause necrosis of the superficial layer of the interdigital skin and enable the establishment of other bacteria, including D. nodosus (Beveridge, 1941; Graham and Egerton, 1968). Virulent, invasive, D. nodosus digests the living dermis, feeding on collagen. A foul smell, typical of anaerobic bacterial activity, accompanies the development of clinical signs including an accumulation of grey pasty scum between the sensitive dermis and epidermal horn and ultimately separation of the hoof horn from the underlying dermis occurs (Egerton et al., 1969). Typically, affected sheep are obviously lame and prolonged lameness is painful (Ley et al., 1994).

In India there are about 38 million sheep producing 60 million pounds of wool per annum. Sheep mainly reared by poor people with no land and those belonging to scheduled castes and backward caste, as a source of their livelihood. Aim of this report is to show the emerging status of this contagious disease in sheep in the districts of Haryana.

During the year 2008, a total of five outbreaks of footrot in sheep were attended and investigated. Disease was diagnosed on the basis of clinical signs, symptoms and the geological conditions of grazing areas. Most of the affected animals presented for examination were lame with swelling between the claws of the hoof. In severely affected animals there was sloughing of the skin between the claws and maggot infestation was present. In most of the affected animals white thick pus like discharge was oozing from the affected foot with a characteristic foul smell. Retrospective study was conducted to know the cause of outbreaks and it was found that rains started quite early in this part of the country i.e in mid May and continued till August end with heavy rains in between. This condition resulted in water logging in many areas with damp environment conditions. Sheep of the region are mainly reared by weaker sections and nomads and it was noted that they used to graze them on waste lands. Since most areas were inundated with water and sheep were forced to stand in waterlogged areas. All these conditions facilitate the occurrence of foot rot. Whittington (1995) described that the causative organism of footrot survives best in a warm (>10 ºC), damp environment.

Data reveals that foot rot seems to be emerging disease in Haryana and its border areas of Rajasthan. Out of total 5 outbreaks reported, 2 were from border areas of Haryana and one each from Hisar, Jind and Bhiwani. All the age groups (adult and young) were affected. Isolation was also attempted from the foot lesions of affected animals. Morbidity was recorded as 30-40%, where as mortality was low in those flocks who followed the recommended treatment.

To control the disease farmers were advised to keep the sheep flock at dry area. Treatment of...
affected animals include fomentation of foot lesions with Copper sulphate (bluestone) solutions (10%) and parental administration of enrofloxacin was given @ 10 mg/kg body weight for 4 days. Most of animals responded positively to the treatment, while animals with sloughing off hoof and maggot infestation remained affected and later on died. Egerton and Parsonson (1966) reported that systemic antibiotic treatment leads to a reduction in inflammation of feet with footrot and response to treatment is rapid, with lame sheep becoming sound within 3-4 days. From the present report it was proposed that footrot disease in sheep is showing its emergence in the regions of Haryana, with most of the affected flocks kept in poor managemental conditions.

References
THERAPEUTIC EVALUATION OF GLUCOSE AND INSULIN IN SUBCLINICAL KETOTIC BUFFALOES

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ABSTRACT

In the present investigation Dextrose 25% alone and Dextrose 25% with insulin therapy was evaluated in subclinical ketotic buffaloes. The biochemical study revealed significant decrease in the level of blood glucose, serum insulin and increase in level of serum triglycerides in subclinical ketosis as compared to normal healthy buffaloes. After institution of therapy with (T₁) Dextrose 25 % (500 ml i/v OD) and (T₂) Dextrose 25 % plus insulin (@ 0.4 IU/kg body weight s/c OD), the significant restoration of all altered biochemical parameters were observed towards normalcy. The dropped milk yield was significantly improved in both the groups (T₁ and T₂) after therapy. However, the complete restoration of milk yield could not be achieved. It is concluded that both the treatments were found effective in subclinical ketosis. However, the group treated with Dextrose 25% with insulin showed apparently better improvement in milk-yield than group treated with Dextrose 25% alone.

Key words: Subclinical ketosis, glucose and insulin, buffaloes.

Introduction

Subclinical ketosis is an economically important metabolic disorder commonly occurs in high yielding lactating animals. It is caused by impaired glucose metabolism leads to excessive production of ketone bodies during peak lactation (Ambore et al., 2001). Brockman (1979) reported that the insulin is the primary modifier of lipid and ketone metabolism while glucagon has secondary effect when insulin concentrations are low. The low insulin, furthermore, can impair maximal ketone body utilization, thus, exacerbating the hyperketonaemia.

Thus, the present study was undertaken to evaluate the therapeutic role of insulin along with glucose in subclinical ketosis.

Materials and Methods

Total 88 lactating buffaloes during 6th to 8th weeks after parturition in and around Akola district and Buffalo Instructional Farm, PGIVAS, Akola were screened for subclinical ketosis on the basis of low plasma glucose level (<35 mg/dl), Rothera’s test in urine and Ross test in milk. Out of which 12 buffaloes were found positive for subclinical ketosis. These subclinical ketotic buffaloes were selected and divided randomly into two equal groups. One additional group of normal healthy buffaloes showing negative test for subclinical ketosis with normal plasma glucose level was kept as a normal control (C). One group of (T₁) of subclinical Ketotic buffaloes was treated with Dextrose 25% @ 500 ml i/v OD. Another group of (T₂) subclinical Ketotic buffaloes was treated with Dextrose 25 % @ 500 ml i/v OD with injection of insulin @ 0.4 IU/kg b. wt. s/c OD.

The blood samples were collected from jugular vein in vial containing sodium fluoride (1%) for estimation of plasma glucose (enzymatic GOD-POD method) by Span diagnostic kits on Auto analyzer (Autochem-2011). The serum samples were collected for estimation of serum triglycerides (mg/dl) by using Span diagnostic kits on Auto analyzer (Autochem-2011) and serum insulin (mcU/L) by using ELISA kit (Accu-bind) supplied by Monobind Inc. USA. The biochemical parameters were determined on ‘0’ day (before treatment) and on 3rd day post treatment. The milk yield was recorded daily during the experiment period. The data was analyzed statistically by using FRBD as described by Snedecor and Cochran (1994).

Results and Discussion

The average values of biochemical parameters in normal healthy control and subclinical ketotic groups at different intervals are given in Table 1. In subclinical ketotic groups, the blood glucose level before initiation of treatment (‘0’ day) varied between 31.77±0.57 to 31.80±0.58, indicating reduction in blood glucose level in subclinical ketosis as compared to normal healthy control group (C). Similar observations were also recorded by Ambore

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and Singh (2003) and Teli and Ali (2007). The decrease in blood glucose level in subclinical ketosis might be due to insufficient carbohydrate supplementation during the peak lactation leads to negative energy balance (Baird, 1982). The group treated with Dextrose 25 per cent (T₁) and with insulin plus Dextrose 25% (T₂) showed significant improvement in blood glucose level on 3rd day post treatment as compared to pre treatment values ('0' day) of respective groups (Table 1). The improvement in blood glucose level observed to be at par towards normal level on 3rd day post treatment indicated the effectiveness of both the treatment. Similar improvement in blood glucose level after administration of 25 per cent Dextrose was also observed by Singh et al. (2002). The administration of insulin along with Dextrose might have been facilitated cellular uptake of glucose, suppresses fatty acid mobilization and stimulate hepatic gluconeogenesis (Radosits et al., 2000). Teli and Ali (2007) also recorded improvement in plasma glucose level after administration of insulin along with Dextrose 25% in subclinical Ketotic buffaloes.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Control</th>
<th>T₁</th>
<th>T₂</th>
<th>Pooled mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood glucose</td>
<td>51.210 ± 1.20</td>
<td>50.881 ± 1.14</td>
<td>51.04a ± 0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Serum triglycerides</td>
<td>68.772 ± 1.25</td>
<td>63.022 ± 1.21</td>
<td>65.89c ± 1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Serum insulin</td>
<td>21.501 ± 0.62</td>
<td>23.311 ± 0.70</td>
<td>22.42b ± 0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mcU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In subclinical ketotic groups (T₁ and T₂), serum triglyceride level significantly increased as compared to normal healthy control group (C). These findings are in agreement with the findings of Ahuja (2003) and Ali et al. (2006), who also reported increase in serum triglyceride level in subclinical ketotic buffaloes. The increase in serum triglyceride level in subclinical ketosis could be attributed to the negative energy balance, low serum concentration of glucose and insulin resulted into mobilization of adipose tissue with consequent increase in serum triglyceride level (Borghese, 1994). The free fatty acid produced from mobilization of fat are transported to the liver and oxidized to produce acetyl-Co-A and NADH. Thus, there is increase in serum triglyceride level in ketotic buffaloes (Chakrabarti, 2006). The group treated (T₁) with Dextrose 25 per cent and insulin plus Dextrose (T₂) showed significant improvement in serum triglyceride level on 3rd day post treatment as compared to pre-treatment levels of respective groups, indicated effectiveness of both the treatment. Similar observations were also recorded by Ali et al. (2006).

In subclinical ketotic buffaloes the serum insulin level was lowered as compared to level observed in normal control group (C). Ahmad et al. (2005), Ali et al. (2006) and Teli and Ali (2007) also reported decrease in serum insulin level in subclinical ketotic buffaloes. The decrease in the serum insulin levels could be attributed to the diminished ability of alpha-cells of endocrine pancrease to synthesize and release insulin (Dokovic et al., 1998). In group T₁ and T₂, the serum insulin level was significantly improved on 3rd day post treatment as compared to
pretreatment level of corresponding group (Table 1). The results obtained are in close agreement with Ahmad et al. (2005) and Ali et al. (2006), who also recorded improvement in the level of serum insulin after administration of Dextrose 25 per cent plus insulin.

In subclinical ketosis, the average milk yield was dropped to 5.75±0.62 (T₁) and 6.91 ± 0.49 kg/day (T₂), indicated average loss of production by 2.75±0.19 and 2.92±0.78 kg/day per animal in T₁ and T₂ group, respectively, as shown in Table 2. The percentage fall in milk yield in subclinical ketosis at 6-8 weeks of lactation was 32.35 per cent and 29.70 per cent in T₁ and T₂ group, respectively. In Ketosis the capacity of animal to supply the lactogenic precursors to mammary gland is reduced than the capacity of the gland to produce due to homeorhetic drive for production (Lean et al., 1992). Moreover, elevated blood ketones also results in decrease in milk production (Anderson and Lundstorm, 1985).

Both the treated groups (T₁ and T₂) showed increasing trend in milk yield from 1st day post treatment and increased significantly on 3rd day post treatment as compared to pre-treatment milk yield of respective groups. An average of 1.61±0.11 kg milk per animal per day (21.87%) and 2.0±0.24 kg milk per animal (22.44%) were regained in animal after recovery with dextrose 25 per cent treatment (T₁) and Dextrose plus insulin therapy (T₂). However, complete restoration of milk yield was not achieved in both the groups. Both the treatments regained the milk production after 3rd day post treatment. However, restoration of milk yield in group T₂ was apparently more than group T₁ (Table 2). Ahmad et al. (2005) and Jain et al. (2002) reported regained in milk yield in ketotic buffaloes treated with Dextrose 25% plus insulin therapy within 2 days, indicated Dextrose plus insulin therapy was more efficacious based on early significant increase in milk yield.

The overall study indicated that both the treatments found equally effective in restoring altered biochemical parameters towards normalcy with regained milk yield after 3rd day post treatment. However, restoration of milk yield in group treated with Dextrose 25% plus insulin recorded appreciable improvement in milk yield and early taking of concentrate feed than the buffaloes treated with Dextrose 25% alone.

### Table 2: Lactation Kinetics of subclinical ketotic buffaloes

<table>
<thead>
<tr>
<th>Milk Yield per buffalo (Kg/day)</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁</td>
</tr>
<tr>
<td>Before ketosis</td>
<td>8.50±0.96</td>
</tr>
<tr>
<td>During ketosis</td>
<td>5.75±0.62</td>
</tr>
<tr>
<td>Drop</td>
<td>2.75±0.19</td>
</tr>
<tr>
<td>Per cent Drop</td>
<td>32.35%</td>
</tr>
<tr>
<td>Regain after treatment</td>
<td>1.61±0.11</td>
</tr>
<tr>
<td>Per cent regain at recovery</td>
<td>21.87%</td>
</tr>
</tbody>
</table>

### References


CLINICAL MANAGEMENT OF PRIMARY HYPoadrenocorticism IN A MONGREL MALE DOG

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Introduction
Hypoadrenocorticism (or Addison’s disease) is an uncommon disease of canine occurring mostly in young to middle aged female dogs (Klein and Peterson, 2010; Reusch, 2000). Diagnosis of Addison’s disease poses a great challenge due to vague and non-specific clinical signs that are often attributable to multiple organ system dysfunctions. The disease is often confused with primary gastrointestinal disease, renal failure or a neurological problem (Melian and Peterson, 1996). The present report describes diagnosis and successful management of hypoadrenocorticism in a mongrel dog.

Case description
A 3.5 years old male mongrel dog weighing 17.5 kg was presented at Teaching Veterinary Hospital, G.A.D.V.A.S.U., Ludhiana with the complaint of vomition, mild diarrhoea and profound weakness for the past one week. The deworming and vaccination status was updated and there were no history of prior sickness. Clinical examination revealed dehydration, subnormal rectal temperature (100.6°F), heart rate 80/minute and congested mucous membranes. Blood sample was submitted for estimation of haemato-biochemical parameters.

The dog was treated with intravenous fluid (Ringers lactate, and Dextrose 25% solution), parenteral antibiotics, antacid, antiemetic and multivitamin supplements in recommended doses. However, treatment failed to respond and the condition further deteriorated. The next day animal was presented in comatose condition with weak femoral pulse and heart rate 48 beats per minute. Electrocardiogram (Fig. 1) revealed absence of P wave (suggesting atrial standstill), QRS interval 0.12 second (increased), R wave amplitude 2.8 mV, negative T wave (amplitude 1.0 mV) and QT interval 0.26 second. The mean electrical axis was +90°. During administration of intravenous fluid profound bradycardia developed and therefore, atropine sulphate and dexamethasone sodium succinate was given intravenously to save the life. Evaluation of peripheral blood picture revealed leukocytosis (TLC 40,730/cumm) with absolute neutrophilia (71%) and eosinophilia (10%). However, platelet count (2.99 x 10⁵/cumm) and haemoglobin concentration (14.4 g/dL) were within the normal range. Serum biochemical parameters revealed hyponatraemia, hypokalaemia, altered Na:K ratio, and increased ALT, creatinine, BUN, GGT and AKLP values (Table 1). Based upon clinical signs, biochemical reports and ECG findings, diagnosis of primary hypoadrenocorticism associated with secondary renal and hepatic function impairment was given.

Intravenous normal saline solution and sodium bicarbonate (7.5%, 20 ml) were given for two days. Fludrocortisone (@ 13 µg/kg body weight; Tablet Floricot, 100 mcg, Samarth Pharma, Mumbai) was prescribed to be given orally once daily. Marked improvement in clinical condition of the dog was observed after two days and hence it was decided to continue fludrocortisone therapy and go for regular monitoring. During this period, the dog appeared almost normal but appeared calm and quiet and preferred to remain indoors. One day owner himself decided to stop the medicine, thinking that the dog is now alright. The very next day, dog suddenly fell down and lost consciousness for some time. The owner reported the event on telephone and he was advised to give fludrocortisone and bring the dog next day for investigation. When presented, the dog had normal rectal temperature (101°F), and ECG findings (Fig 2). Level of all enzymes related to kidney and liver functions were almost within the normal range. However, only slight improvement in Na, K levels and Na:K ratio was observed (Table 1). Therefore, fludrocortisone was advised to be given twice daily at the same dose rate, i.e. @ 13 µg/kg body weight. After few days, owner reported marked improvement in attitude and activity of the animal. It was advised to continue the medicine and bring the dog fortnightly for examination. But the owner did not turn up and after a lapse of two months he only reported that the dog is doing well after receiving the medicine on regular basis.

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Discussion

Hypoadrenocorticism is an uncommon disease that is clinically manifested in different forms including gastrointestinal signs to overt shock, severe bradycardia and acute collapse (Herrtage, 2005). Hypoadrenocorticism may be either primary or secondary. Primary hypoadrenocorticism usually results from immune mediated destruction of adrenocortical layers resulting into deficiency of mineralocorticoids (aldosterone) and glucocorticoids (cortisol). Secondary hypoadrenocorticism is less common form which results from the failure of the pituitary gland to secrete ACTH (Kintzer and Peterson, 1997). In secondary hypoadrenocorticism, changes in serum electrolytes are minimal because aldosterone secretion is preserved. Hyperkalaemia and hyponatraemia are the most consistent serum biochemical abnormality in dogs with primary hypoadrenocorticism (Kintzer and Peterson, 1997; Peterson et al., 1996). It can occur either independently or together. Hyperkalaemia occurs in up to 95% of dogs with primary hypoadrenocorticism and none of the dogs with secondary hypoadrenocorticism (Feldman and Nelson, 2004). However, hyponatraemia can occur in primary as well as in small per cent of dogs suffering from secondary hypoadrenocorticism (Feldman and Nelson, 2004; Kintzer and Peterson, 1997). Aldosterone deficiency results into failure of kidneys to conserve sodium and to excrete potassium (DiBartola, 2006). At the time of presentation, serum chloride concentration was reduced, while Ca and P concentrations were within the normal range. All abovementioned biochemical changes were suggestive of a case of primary hypoadrenocorticism.

Azotaemia, as observed in the present case, is a common finding at the time of initial diagnosis in cases of primary hypoadrenocorticism (Klein and Peterson, 2010a). It occurs due to decreased glomerular filtration rate, but it disappears rapidly if appropriate fluid and hormonal therapy is given (Feldman and Nelson, 2004). Mild to moderate increase in ALT may occur in 30 to 50 % of cases (Herrtage, 2005). The definitive cause remains unknown, but it may result from poor cardiac output, hypotension and poor tissue perfusion (Kintzer and Peterson, 1997; Herrtage, 2005). Alternatively, it may be secondary to immune mediated diseases (Feldman and Nelson, 2004). In present case also, increase in level of plasma enzymes related to hepatorenal functions were increased at the time of diagnosis, but they all returned to normal levels after the fluid therapy. This further indicated absence of any primary hepatic or renal disorders. Metabolic acidosis commonly accomplishes which further exacerbates hyperkalaemia, as it cause a shift of potassium from the intracellular space to the extracellular space in exchange for hydrogen ions (DiBartola and Autran
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private (Melian and Peterson, 1996; Klein and Peterson, 2000b). Fluodrocortisone is a synthetic glucocorticoid with significant mineralocorticoid activity (Plumb, 2005). It has been found quite effective in cases of Addison’s disease (Kintzer and Peterson, 1997).

In present case also, we observed very good response of the drug when given @ 13 µg/kg body weight twice daily. However, we could not follow up the case after one month due to non-cooperation of the owner.

From the present case, it can be concluded that, any dog showing profound weakness, subnormal rectal temperature and heart rate and changes in blood electrolyte concentrations should be evaluated for Addison’s disease. The diseased dog can lead normal lives with proper medication and regular monitoring.

Reference

De Morais, 2006). In present case also, bicarbonate therapy was given for management of hyperkalaemia. The most deleterious effects of hyperkalaemia occur on myocardial tissue due to reduced excitability, slowed conduction and cardiac standstill (Guyton and Hall, 2000; Di Bartola and Autran De Morais, 2006). Severe bradycardia along with absence of P-wave (atrial standstill) observed in present case may be due to increase in blood potassium level. QRS interval increases and a sinoventricular rhythm may appear. In addition to hyperkalaemia, ECG is also influenced by concurrent hyponatraemia and metabolic acidosis (Klein and Peterson, 2010a). In present case also, ECG initially showed signs of atrial standstill, but it returned to almost normal after improvement in biochemical parameters. This indicated ECG changes were secondary to blood electrolyte imbalances.

The recommended therapy for hypoadrenocorticism includes Fluodrocortisone acetate and deoxycorticosterone

Table 1: Serum biochemical and hormonal profile of the dog

<table>
<thead>
<tr>
<th>Parameter (unit) (Reference range)*</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/L) (140-154)</td>
<td>109</td>
<td>143</td>
</tr>
<tr>
<td>K (mEq/L) (3.8-5.6)</td>
<td>6.8</td>
<td>7.7</td>
</tr>
<tr>
<td>Na : K ratio (27-40:1)</td>
<td>16.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Cl (mEq/L) (102-117)</td>
<td>91</td>
<td>104.7</td>
</tr>
<tr>
<td>Ca (mg/dL) (8.7-11.6)</td>
<td>9.8</td>
<td>-</td>
</tr>
<tr>
<td>P (mg/dL) (2.9-6.2)</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td>ALT (U/L) (8-57)</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>Creat (mg/dL) (0.5-1.6)</td>
<td>3.1</td>
<td>1.3</td>
</tr>
<tr>
<td>BUN (mg/dL) (8-26)</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>GGT (U/L) (1.0-9.7)</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>AKP (U/L) (10.4-101)</td>
<td>165</td>
<td>75</td>
</tr>
<tr>
<td>Cortisol (ng/mL) (10-50)</td>
<td>1.09</td>
<td>1.24</td>
</tr>
<tr>
<td>TSH (IU/mL) (0.14)</td>
<td>181.1</td>
<td>0.03</td>
</tr>
<tr>
<td>T4 (µg/dL) (1.2-3.60)</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>T3 (ng/mL) (0.48-1.54)</td>
<td>3.92</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*Kahn and Line (2005); †Silverstein and Hopper (2009)
OEDEMATOUS MASTITIS IN CAMELS (CAMELUS DROMEDARIUS)

F. C. Tuteja, S. K. Ghorui and B. L. Chirania
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ABSTRACT

Oedematous mastitis in camels is a managemental problem, due to inadequate exercise after calving. This inadequate exercise results in persistent post parturient udder oedema for longer periods and interferes with the normal milk let down and milking process. In some cases, it leads to a chronic oedematous condition of udder with cessation of milk. In very advanced cases, it leads to flabby udder with loosening and wrinkling of udder skin and there is complete stoppage of milk. Examination of 40 clinical oedematous mastitis quarters revealed, infections with major mastitis pathogen i.e. Staphylococcus aureus. Most of the fresh calvers were clear from such infections. In the apparently healthy animals increase in level of intramammary infections, occurs with the advancement of lactation. In vitro antibiotic sensitivity revealed tetracycline the effective drug against majority of the bacterial isolates.

Key words: Oedematous, mastitis, camel, etiology, antibiotic.

Introduction

Camel is proving to be a good source of milk in the Thar desert of India. Camel continues lactating even under stress conditions like drought, when the production of other milch animals ceases. Mastitis in camels was thought to occur rarely, by now it has been reported from almost all camel rearing countries (Barbour et al., 1985; Abdurahman, 1996; Guliye et al., 2002; Khedid et al., 2003; Tuteja et al., 2003 and Mohammed et al., 2005). Mastitis is the cause of hazardous effects on human beings consuming raw milk and animal production (Makovec and Ruegg, 2003; Hegazi et al., 2004; Al-Majali et al., 2008). Parturient udder oedema may lead to development of oedematous type of mastitis in camels. This is a managemental problem due to inadequate exercise of the animals. Besides roughness, udder and teats of camel are soft compared to other domesticated animals. Post parturient udder oedema is more severe in case of camels and persists normally for 3-4 days. Thereafter a sort of udder involution starts and udder becomes normal in 15-20 days. The involution of udder is faster, if the animals are exercised after calving. Daily walking of the animals either for normal browsing in the desert fields or migration of livestock etc. provides sufficient exercise. Most of the camel owners are well aware of this managemental tool and authors too have learnt this from them. If this type of oedema persists in animals of organized farm, then animals are made to walk in the field.

Materials and Methods

Oedematous condition of udder occurs more severe in dairy camels compared to camels with only suckling calves. Occurrence of this condition was observed both in the field conditions as well as at organized dairy farm. In district Udaipur, Rajasthan, where camels are reared mainly for the milk and this particular area is known as camel milk belt, this condition was more severely observed. At a single camel dairy out of the 10 lactating camels, six developed oedematous mastitis with varying degree of severity. In this area, movement of the animals is comparatively restricted due to land use by cultivation.

Gross examination of oedematous mastitis cases: Observations of 17 such clinical cases were recorded as per the history provided by the farmers and gross examination of the udder was carried out.

Bacteriological examination: Aseptically collected quarter milk samples from 66 lactating camels from field as well as from an organized herd were examined bacteriologically by using five per cent sheep blood agar and MacConkey’s lactose agar following standard procedures (Brown et al., 1981). These animals were divided into three groups:

Group 1: 40 quarter milk samples of 17 cases with clinical symptoms of oedematous mastitis.

Group 2: 80 quarter milk samples of 20 fresh calvers within one month of calving along with some animals having normal post parturient udder oedema, and

Group 3: 115 quarter milk samples of 29 apparently healthy lactating animals in the mid lactation.

The resulting growth from the respective plate of media was purified and identified based on colony morphology, Gram staining reaction, fermentation of lactose on MacConkey’s lactose agar, haemolysin production on five per cent sheep blood agar. Staphylococci and Streptococci were differentiated based on catalase test. Gram positive and catalase positive cocci were differentiated into Micrococci and
Staphylococci based on oxidase test. Staphylococci were further differentiated as Staph aureus and Staph epidermidis based on the coagulase test (Gibbs and Skinner, 1966).

In vitro antibiotic sensitivity: 58 bacterial isolates comprising Staph aureus (41), Staph epidermidis (10) Corynebacterium spp. (5) and Micrococcus spp. (2) obtained from intramammary infections in camels were subjected to in vitro chemotherapeutic sensitivity to six antimicrobials using the disc diffusion method (Bauer et al., 1966). The concentration of different antimicrobial discs was Tetracycline (30 µg), Co trimoxazole (25 µg), Cefuroxime (30 µg), Cloxacillin (1 µg) Cephaloxime (30 µg) and lincomycin (2 µg). The susceptibility was interpreted as sensitive, intermediate and resistant according to the zone size interpretation chart supplied by the manufacturer.

Results and Discussion

Gross examination of oedematous mastitis cases: Initial physiological udder oedema shows moderate oedematos fluid in the udder and teats. During initial stages of development of mastitis, there is severe enlargement and oedema of the udder. This gives appearance of complete milk letdown condition of the udder. Holding the teats with palm shows dryness of the teat skin. Animal feels pain while milking or suckling by the calf and actual milk letdown is reduced (Fig. 1). Further it progress into tightness of the udder and shrinkage of teats. At this stage, there is difficulty in holding the teats with hand and milk letdown is almost negligible. Clots, flaks and pus like secretions are observed in such cases. Then starts bluish discoloration of the teats and some quarters may become blind at this stage (Fig. 2). Finally there is loosening and wrinkling of the udder skin and whole of the udder gives bluish cyanotic appearance. At this stage, there occurs complete stoppage of milk. Udder becomes cold to touch and sloughing of the udder skin is rarely observed in such cases. It looks as if a leather bag has been tied over the udder and touching the skin reveals hard leathery skin covering a huge tight mass. (Fig. 3). Sometimes either right half or left half of the udder are severely affected then the opposite site.

Bacteriological examination: Majority of the clinical oedematous mastitic quarters were infected with major mastitis pathogen i.e. Staph aureus. Whereas, fresh calvers were mostly clear from such organisms. This indicates that oedema causes retention of milk, which subsequently results in entrance and precipitation of infectious agents. Further the toxins produced by Staph. aureus results in damage of the udder tissue. Low level of infections observed in the middle of lactation in apparently healthy quarters may be due to slow entrance of infectious agents through the teat canal, level of these infections increases, with the advancement of lactation (Table 1 and 2).

In vitro antibiotic sensitivity: In considering overall sensitivity, tetracycline (94.82%) was found to be the
The highly vascular nature of the mammary gland makes the tissue more prone to develop localized oedema due to an increase in blood and lymphatic flow. The developing bovine mammary gland undergoes extensive growth and physical changes during late gestation, which likely contributes to oedema development. The oedematous and swollen udder is more prone to physical injury and damage as well. In cases where udder oedema is severe or continues for long periods of time, damage to the suspensory apparatus of the udder can cause permanent damage, such as a pendulous udder, thus leading to a more chronic form of the disorder (Vestweber and Al-Ani, 1985). Dentine and McDaniel (1983) found that some degree of udder oedema was noted in 97 per cent of cows around the time of parturition. Mild to moderate cases of udder oedema makes effective and complete milking difficult due to the swelling of the teats and other structural changes. Clinical mastitis in early lactation is more common in cows with oedematous udder than cows without oedema. (Slettbakk et al., 1995 and Waage et al., 2001). Udder oedema was associated with clinical mastitis in the first 30 days postpartum (Van Dorp et al., 1999). Regarding oedematous mastitis in camels, almost similar clinical condition of mammary gland has been explained by Hawari and Hassawi (2008) that camels with obvious signs of inflamed udders had a mean lactation of about four months. The visible signs of inflammation included acute and oedematous swelling of the udder and formation of pus in the mammary exudates resulting in a visible alteration of the milk. Congestion of udder at parturition is a physiological phenomenon but it may be sufficiently severe to cause the oedema of the belly, udder and teats (Al-Ani and Vestweber, 1986). It can result due to compression of mammary vein by the large foetus, causing mammary or ventral oedema in late pregnancy (Ibrahim et al., 1998). Muhammad et al. (2005) reported parturient udder oedema in a 10 year old dromedary camel (Camelus dromedarius) with soft and cold swelling of the udder.

High number of blind teats observed in the clinical cases of mastitis, is in agreement with Abdurahman (2006) that 3.3 per cent camels had blind teats and 9.4 per cent had clinical mastitis. Abera et al. (2009) observed that taking clinical mastitis and blocked teats into account; only 57.9% of the camels have four teats for milk production. They concluded mastitis a major problem in traditionally managed camels and deserve further attention owning to its potential impact on milk production affecting food security.

Predominance of Staphylococci in camel mastitis has also been reported by (Tuteja et al., 2003., Bhatt et al., 2004 and Abdurahman, 2006)). High sensitivity observed with tetracycline in this study is in agreement with our previous study against subclinical mastitis isolates from camels (Tuteja et al., 2003) and has been reported by Hawari and Hassawi (2008). Low sensitivity with tetracycline observed in other species of the animals may differ from this species because antibiotics are less frequently used in case of camels due to more traditional ethno-veterinary practices used by the camel owners, low susceptibility of this species to diseases owning to low use of antibiotics and less survival of infectious agents in extreme hot and cold of the desert. The period around calving, two weeks before calving and two weeks after calving, is often the highest risk period for mastitis infections to occur. Many of these infections can be prevented by implementing some simple management changes of cleanliness etc. Giving moderate exercise in terms of walking to relieve oedema and subsequently development of mastitis in case of camels in the post calving periods may give fruitful results.

Table 1: Cultural examination of quarter milk samples

<table>
<thead>
<tr>
<th>Type of animals</th>
<th>No of quarters examined</th>
<th>Quarters Culturally positive</th>
<th>Per cent positive</th>
<th>Blind quarters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>40</td>
<td>40</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>Group 2</td>
<td>80</td>
<td>3</td>
<td>3.75</td>
<td>-</td>
</tr>
<tr>
<td>Group 3</td>
<td>115</td>
<td>15</td>
<td>13.04</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2: Relative frequency of different type of microorganisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of isolates</td>
<td>Per cent</td>
<td>No of isolates</td>
<td>Per cent</td>
</tr>
<tr>
<td>Staph aureus</td>
<td>38</td>
<td>95</td>
<td>2</td>
<td>13.33</td>
</tr>
<tr>
<td>Staph epidermidis</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>46.67</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>26.67</td>
</tr>
<tr>
<td>Micrococcus spp.</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>13.33</td>
</tr>
<tr>
<td>Total no of isolates</td>
<td>40</td>
<td>15</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: In vitro antibiotic sensitivity of bacterial isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No of isolates tested (58)</th>
<th>Overall sensitive</th>
<th>Per cent sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staph aureus 41</td>
<td>Staph epidermidis 10</td>
<td>Corynebacterium spp. 5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>38</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>28</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>20</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>19</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>24</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Cephaloxime</td>
<td>25</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

References
ABDOMINOCECTESIS AND PERITONEAL FLUID ANALYSIS IN CLINICAL CASES OF BOVINE OBSTRUCTIVE UROLITHIASIS

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ABSTRACT

Thirty clinical cases of complete bovine obstructive urolithiasis, 15 with intact and 15 with ruptured urinary bladders were used to standardize the technique of abdominocectesis and to determine the gross and biochemical alterations in peritoneal fluid in relation to blood biochemical changes for future references. Abdominocectesis using 20 gauge 5 cm hypodermic needle mounted over a 5 ml disposable sterile syringe at the site slightly dorsal and caudal to the umbilicus in calves suffering from complete obstructive urolithiasis was found successful, easy to perform, safe, quick and reliable. However, some complications like inadvertent enterocentesis, rumenocentesis and gross bleeding were recorded with no after consequences. The quantity of peritoneal fluid obtained varied between 0.9 and 2.1 ml taking 10 seconds to 2 minutes. Colour of the peritoneal fluid obtained varied from straw coloured to pale yellow to yellow to blood tinged (reddish) to red. Thirteen peritoneal fluid samples were cloudy/turbid with slight opacity and 14 peritoneal fluid samples were clear and transparent. Only three samples clotted on standing at room temperature. The protein content of peritoneal fluid samples was less than 3.0 g/dl with higher values in the peritoneal fluid samples collected from the cases with ruptured urinary bladder. Peritoneal fluid urea nitrogen-to-blood urea nitrogen ratio was 2.17:1 and peritoneal fluid creatinine-to-blood creatinine ratio was 3.00:1, in the cases with ruptured urinary bladder, indicative of uroperitoneum. Abdominocectesis and peritoneal fluid analysis can be performed easily in calves for the diagnosis of uroperitoneum and peritonitis.

Key words: Abdominocectesis, calves, peritoneal fluid analysis, uroperitoneum, urolithiasis

Introduction

Abdominocectesis is used successfully in many species to aid in the diagnosis of abdominal disorders (Anderson et al., 1995). Peritoneal fluid in adult cattle has great variability. Peritoneal fluid constituents can be affected by stage of pregnancy, disease, and location of the lesion with respect to the site of peritoneal fluid collection (Anderson et al., 1995; Radostits et al., 2000). Peritoneal fluid analysis is a repeatable and informative procedure to assist in the assessment of severity of abdominal lesions, and may help to decide whether or not to perform abdominal surgery (Ducharme and Lowe, 1988). Peritoneal fluid analysis has been reported for cattle with displacement of the abomasum, metritis, omental bursitis, peritonitis, and ascites (Oehme 1969; Wilson et al., 1985; Kopcha and Schultze, 1991; Hirsch and Townsend, 1982). Changes in peritoneal fluid constituents after exploratory celiotomy and omentopexy and after laparoscopy have been described (Anderson et al., 1993 and 1994). The peritoneal fluid constituents of clinically normal young calves have also been described (Anderson et al., 1995); however, no detailed information regarding the changes in peritoneal fluid constituents in calves suffering from complete obstructive urolithiasis is available. This study was thus undertaken to explore the easy, safe and quick method of abdominocectesis and to record the gross and biochemical changes in peritoneal fluid of calves suffering from obstructive urolithiasis for future references.

Materials and Methods

Thirty male cattle calves, suffering from complete obstructive urolithiasis, 15 with intact urinary bladder and 15 with ruptured urinary bladder, presented for treatment at Teaching Veterinary Clinical Service Complex, Faculty of Veterinary Sciences and Animal Husbandry (F.V.Sc & A. H.), Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar, formed the material of the study. At the time of admission, all the animals were subjected to complete pre-operative evaluation including physical, clinical, radiographic, sonographic, haematobiochemical and peritoneal fluid examination.

Two sites were used for the collection of peritoneal fluid. First site: slightly dorsal and caudal to the umbilicus. Second site: centre of the inguinal region. Initially first site was used for the collection of the peritoneal fluid, in the event of failure second site was used. The animals were restrained in left lateral recumbent position with right hind limb pulled dorsally and caudally. The sites were prepared aseptically and not infiltrated with any local anaesthetic. A 20 gauge 5 cm hypodermic needle mounted over a 5ml disposable sterile syringe was introduced and directed slightly caudally and toward the midline while maintaining it parallel to the internal abdominal wall once the peritoneal cavity was entered. Gentle suction was applied and the peritoneal fluid
obtained was placed in a 2 ml tube containing tri-potassium EDTA.

Peritoneal fluid obtained was quantified. Its physical characteristics like, colour, smell consistency and clotting were recorded. The peritoneal fluid obtained was centrifuged at 1500 g for 10 minutes. The supernatant was preserved at -20°C for biochemical estimations. Creatinine, urea nitrogen and total protein content of the peritoneal fluid were estimated by standard procedures.

For biochemical studies, 5 ml of blood was collected in sterile heparinised vials from jugular vein of obstructive urolithiasis cases using aseptic syringes. The blood samples were centrifuged immediately at 2000 g for 30 minutes so as to separate out the plasma which was preserved until further analysis. Blood urea nitrogen, Creatinine and total protein were estimated by standard procedures.

The data thus obtained was classified and subjected to statistical analysis and inferences drawn.

Results and Discussion

The site of abdominocentesis in animals varies according to species. Sites for abdominocentesis in adult cattle include the right and left flanks (Kopcha and Schultz, 1991), right and left ventral paramedians (Kopcha and Schultz, 1991), cranial and lateral to umbilicus (Wilson et al., 1985), and caudal to the sternum (Oehme and Noordsy, 1970). During these procedures animals are sedated, locally infiltrated at the site with local anesthetic, bluntly stabbed at the site for entering into the peritoneal cavity, and cannulated or catheterized to collect the peritoneal fluid (Wilson et al., 1985). Anderson et al. (1995) also sedated the calves for easy restraining, infiltrated with local anaesthetic and stab incised the site for easy entry into the peritoneal cavity and introduced the female catheter for obtaining the peritoneal fluid. Teat cannulas had also been used to obtain the peritoneal fluid in calves (Wilson and Mac Williams, 1998). During this study no sedatives were used for restraining the animals in lateral recumbency for abdominocentesis, at both the sites, one slightly dorsal and caudal to the umbilicus and another in the centre of the inguinal region. The animals were of smaller size and of lesser age, hence could be handled comfortably without sedation. The sites of abdominocentesis were neither anesthetized nor stab incised for entry into the peritoneal cavity, which was possible because of smaller gauge needle used during this study. The use of small gauge needle for abdominocentesis hence obviated the use of catheters and cannulas, which might have otherwise contaminated the peritoneal fluid sample with gross bleeding. These findings resemble with those of Al-Rukibat et al. (2006), who also used 20-gauge 1.5 inch needle successfully to aspirate the peritoneal fluid without any premedication and stab incising the site in sheep.

In 28 (94.66%) cases the first site technique was successful in obtaining the peritoneal fluid. The second site additionally used in this study for failed two cases could not either yield result. The first site was therefore reliable and successful. Cattle have a low volume of peritoneal fluid and failure to obtain a sample is not considered to be abnormal (Wilson et al., 1985; Oehme and Noordsy, 1970; Hansow et al., 1992; Radostits et al., 2000). Failure to obtain fluid does not preclude possibility of peritonitis. Dehydrated animals have also less peritoneal fluid (Radostits et al., 2000). All the animals of this study were suffering from dehydration, varying from moderate to severe form, which could be responsible for low volume of peritoneal fluid per sample and no peritoneal fluid in 2 cases.

Time taken to collect sufficient quantity of peritoneal fluid varied between 10 seconds and 2 minutes, which is lesser than the early reports of Wilson et al. (1985), who took 5 seconds to 10 minutes to obtain 0.5 ml of peritoneal fluid. The technique of the present study is thus quick.

Complications like inadvertent enterocentesis in one and contamination of sample with gross bleeding in another one case were recorded during this study, while performing abdominocentesis at first site. Inadvertent rumenocentesis was recorded during abdominocentesis at second site, which was confirmed by detecting the ciliated rumenal protozoa on the slide made directly from the sample. The technique most likely to cause bowel penetration is the use of a sharp needle instead of the blunt cannula (Radostits et al., 2000). The observations are in total conformity with those of Al-Rukibat et al. (2006), who observed enterocentesis in 5 and contamination of 3 peritoneal fluid samples with gross bleeding at the time of abdominocentesis. In another study penetration of rumen and abomasum in adult cattle had been reported during the procedure of abdominocentesis performed at a site 10 cm cranial and 10 cm to the right side of the umbilicus using teat cannulas for the aspiration of the peritoneal fluid (Wilson et al., 1985). Inadvertent penetration of a particular viscus during abdominocentesis depended upon the site of abdomino-centesis. Contamination of one peritoneal fluid sample with gross bleeding observed during this study could either be due to puncture of a blood vessel or from bleeding into the peritoneal cavity from rupture of urinary bladder (Radostits et al., 2000). Furthermore, there were no undue complications resulting from the procedure that required treatment or special care. The findings match with those of Wilson et al. (1985). The technique of abdominocentesis used in this study obviated the use of sedation, local infiltration of the site with any kind of anaesthetic, blunt stabbing of the site for easy entry into the peritoneal cavity, and introduction of catheters and cannulas to obtain the peritoneal fluid. Hence this technique proved easy to perform, simple-avoiding the use of catheters and cannulas, cheap, quick, safe and reliable.

Peritoneal fluid typically is examined for colour,
turbidity, RBC count, total nucleated cell count (TNCC), differential cell count, and total protein and albumin concentrations (Duncan et al., 1994). The use of peritoneal fluid as an aid to diagnosis of abdominal disease has been well documented (Hirsch and Townsend, 1982; Wilson et al., 1985; Kopcha and Schultze, 1991; Duncan et al., 1994). Peritoneal fluid estimation for urea nitrogen and creatinine is especially made in the cases of obstructive urolithiasis to diagnose uroperitoneum.

In adult normal cattle, a normal flow is 1-5 ml per sample. The quantity of peritoneal fluid obtained during the study varied from 0.9 to 2.1 ml per sample in the cases with intact urinary bladder and no seepage of urine into the peritoneal cavity. In general small amounts of peritoneal fluid can be obtained from the abdominal cavity of the most normal animals. Furthermore, it is more difficult to perform abdominocentesis in ruminants compared to horses because of the fact that ruminants have an extensive omentum that may block the needle used for the procedure (Al-Rukibat et al., 2006). Earlier workers also obtained varied quantities of peritoneal fluid from different species, 1 ml in cows (Anderson et al., 1994), 1-1.5 ml in goats (Dehghani et al., 2000), and 2 ml in sheep (Al-Rukibat et al., 2006). The total quantity of peritoneal fluid in horses is 3-5 ml (Brownlow et al., 1981), but the quantity of peritoneal fluid present in the normal ruminants is not known (Al-Rukibat et al., 2006). A positive free-flowing paracentesis with 10-20 ml/sample indicates excess abdominal fluid (Hirsch and Townsend, 1982; Radostits et al., 2000). The positive free flow occurred in 17 cases, 15 with ruptured urinary bladder and two with intact urinary bladder but having seepage of urine into the peritoneal cavity. Free flow was actually due to excessive accumulation of urine into the peritoneal cavity (uroperitoneum) either due to the rupture in urinary bladder or seepage of urine form intact urinary bladder. In otherwise tentatively diagnosed cases of obstructive urolithiasis, free flow of fluid at abdominocentesis can serve as one of the criteria for the diagnosis of

<table>
<thead>
<tr>
<th>Table 1: Physical characteristics of peritoneal fluid samples obtained from obstructive urolithiasis cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Scan t</td>
</tr>
<tr>
<td>No. of animals</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>39.2</td>
</tr>
<tr>
<td>FF = free flow; Py = pale yellow; Sc = straw coloured; Rd = reddish; Bt = blood tinged; Y = yellow; C = clear; T = transparent; Tb = turbid; Cld = cloudy; O = opaque</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Mean ± SE and range of different biochemical parameters of peritoneal fluid and blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
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<tr>
<td>------------</td>
</tr>
<tr>
<td>Total proteins (g/dL)</td>
</tr>
<tr>
<td>Blood</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
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<tr>
<td>Blood</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
</tr>
<tr>
<td>Blood</td>
</tr>
</tbody>
</table>

Ranges a-c: values superscripted a-c are significantly (P<0.01) different from the corresponding values of other group.

References:
1. Anderson et al., 1995
2. Benjamin, 1985
3. Kaneko et al., 1997
4. Values with superscript a-c are significantly (P<0.01) different from the corresponding values of blood.
Peritoneal fluid in normal bovines varies from straw coloured to yellow (Radostitis et al., 2000). Wilson et al. (1985) obtained yellow coloured fluid from normal cows. In other studies, the peritoneal fluid obtained was pale yellow in sheep (Al-Rukibat et al., 2006) and goat (Nazifi et al., 1999). Colour of the peritoneal fluid obtained during this study varied from straw coloured to pale yellow to yellow to blood tinged (reddish) to red. Straw coloured peritoneal fluid was obtained from 11 (39.20%), pale yellow 7 (25.00%), yellow 3 (10.71%), blood tinged 6 (21.42%) cases. The blood tinged and reddish colour imparted to the peritoneal fluid could be due to haemorrhage from the recent rupture of the bladder.

In cattle, the normal peritoneal fluid is crystal clear and watery; and the pathological samples could be cloudy, and turbid with slight opacity (Oehme, 1969; Radostits et al., 2000). During this study cloudy/turbid with slight opacity peritoneal fluid samples were collected in 11 cases with ruptured urinary bladder and in 2 cases with intact urinary bladder. Clear and transparent peritoneal fluid was obtained in 14 cases (10 with intact urinary bladder and 4 with ruptured urinary bladder). Addition of cellular debris, increase in its cellular and fibrin constituents could be responsible factors for the increase in viscosity and consequent decrease in transparency of these fluid samples. The change in viscosity and transparency of peritoneal fluid could be due to mild peritonitis induced by the presence of urine in the peritoneal cavity because of rupture in urinary bladder and seepage of urine from intact but highly distended urinary bladder. Clear and transparent peritoneal fluid had also been obtained earlier in normal sheep and goats (Al-Rukibat et al., 2006; Nazifi et al., 1999).

Of the 27 samples, only 3 samples clotted on standing at room temperature. All these samples were turbid and slightly opaque and obtained from the ruptured urinary bladder cases. As the peritoneal fluid shifts from being a transudate to an inflammatory exudate, its viscosity increases and high viscous fluid may clot (Radostits et al., 2000). This could be the reason for the highly viscous peritoneal fluid samples to clot on the standing because such samples have high fibrin content. These findings are in consonance with those of Nazifi et al. (1999), who did not observe any clot formation in the peritoneal fluid samples from normal goats on standing at room temperature. Contrarily, Wilson et al. (1985) reported that all normal and most diseased peritoneal fluid samples from cattle clotted easily and did not find any relationship between clotting of samples and their protein contents.

Peritoneal fluid is a dialysate of serum; therefore, most of its biochemical values reflect those of serum (Adamu et al., 1991). Changes in peritoneal fluid constituents of calves suffering from complete obstructive urolithiasis are depicted in table.

Total protein level estimation in the peritoneal fluid has been reported to be useful in clinical examination of various abdominal crisis (Saini et al., 1992). Normal protein content in peritoneal fluid is less than 3 g/dl (Whitney et al., 1999; Kopcha and Schultze, 1991), although higher peritoneal fluid protein contents have been reported from apparently healthy adult cattle up to 4 g/dl (Anderson et al., 1995), 4.6g/dl (Wilson et al., 1985), and 4.2 ± 0.9 g/dl (Anderson et al., 1994) and calves up to 3.3 g/dl (Burton et al., 1997), 3.8 g/dl (Anderson et al., 1995), or 6.4 g/dl (Mendes et al., 2005). The overall mean ± SE protein of peritoneal fluid samples obtained in 27 cases (12 intact and 15 ruptured urinary bladder) was 2.67 ± 0.11g/dl with a range of 1.4 - 3.8 g/dl. The protein concentration was non-significantly higher in the peritoneal fluid samples collected from the cases with ruptured urinary bladder (2.72±0.21 g/dl (1.7-3.8 g/dl)) than in the samples collected from the cases with intact urinary bladder (2.60±0.14 g/dl (1.4-3.1 g/dl)). All the values for protein in the peritoneal fluid were within normal reference range for calves i.e., 2.5±0.9 g/dl, i.e. less than 3 g/dl, thus confirming the observations of Wilson and Mac Williams (1998), who also recorded less than 3g/dl protein content in the peritoneal fluid of normal calves. The findings are also in agreement with those of Nazifi et al. (1999), who also reported less than 3 g/dl protein content in the peritoneal fluid of clinically normal adult goats. However, total protein concentrations in peritoneal fluid of normal sheep was reported to be lower i.e. 1.7±0.74 g/dl than cattle and goats. The non significantly higher protein content in the urinary bladder ruptured cases could be due to mild peritonitis induced by the infiltration of peritoneal cavity with urine. The values for protein in 3 peritoneal fluid samples, which clotted on standing, were 3.63±0.1 g/dl (3.0 - 3.8 g/dl), higher than the overall values and greater than the normal reference range, which could be due to the increased fibrin and cellular constituents, as all these samples were highly viscous. Cystocentesis, reportedly performed in these cases in the field before their presentation to faculty clinics, could be one of the reasons for increased viscosity of these samples and consequent clotting.

Evaluation of peritoneal fluid urea nitrogen and creatinine is performed for the diagnosis of uroperitoneum in the cases of obstructive urolithiasis. Thus, comparison of peritoneal fluid urea nitrogen and creatinine and blood urea nitrogen and creatinine is essential (Grindem et al., 1990).

The mean±SE values of urea nitrogen in plasma and peritoneal fluid were 48.03±1.68 mmol/L and
93.77±6.93 mmol/L, respectively. The value of urea nitrogen in peritoneal fluid was significantly (P<0.01) higher than the plasma value with a ratio of 1.95:1. The values of peritoneal fluid urea nitrogen were significantly (P<0.01) higher in the cases of ruptured urinary bladder than in the cases of intact urinary bladder with the respective values of 116.32±6.03 mmol/L and 65.66±8.15 mmol/L. In the cases with ruptured urinary bladder the mean±SE values of urea nitrogen in plasma (53.40±2.13 mmol/L) was significantly (P<0.01) lower than the peritoneal fluid values (116.32±6.03 mmol/L) with a ratio of 2.17:1. Likewise in the cases with intact urinary bladder the values of urea nitrogen in peritoneal fluid were significantly (P<0.01) higher than the plasma values with the peritoneal fluid urea nitrogen-to-plasma urea nitrogen ratio of 1.5:1 (Table 2). All the peritoneal fluid urea nitrogen values were very high than the normal reference range.

The mean ± SE value of creatinine in peritoneal fluid (1069.27±95.47 µmol/L) was significantly (P<0.01) higher than the plasma value (453.49±22.1 µmol/L) with a ratio of 2.35:1. Peritoneal fluid creatinine values were significantly (P<0.01) higher in the cases of ruptured urinary bladder than in the cases of intact urinary bladder. In the cases with ruptured urinary bladder the mean±SE peritoneal fluid creatinine values (1414.00±83.98 µmol/L) were significantly (P<0.01) higher than the plasma values 470.28±33.59 µmol/L with peritoneal fluid creatinine-to-plasma creatinine ratio of 3.00:1. Likewise in the cases with intact urinary bladder the values of creatinine in peritoneal fluid were significantly (P<0.01) higher than the plasma value and the peritoneal fluid creatinine-to-plasma creatinine ratio was 1.43:1 (Table 2). All the peritoneal fluid creatinine values were very high than the normal reference range.

As expected, the peritoneal fluid urea nitrogen and creatinine-to-blood urea nitrogen and creatinine ratios were significantly (P<0.01) lower in the cases with the intact urinary bladder than in the ruptured urinary bladder cases. However, the mean±SE urea nitrogen and creatinine values in the peritoneal fluid were significantly (P<0.01) higher than the mean±SE values of blood urea nitrogen and creatinine, which could be due to seepage of urine into the peritoneal cavity either from the intact but highly distended urinary bladder or from subserosal rupture of urinary bladder.

When urinary bladder is ruptured and urine invades the peritoneal cavity a number of movements in fluid and electrolytes are expected. Urea and creatinine are high in urine and thus they move from the high concentration area in the peritoneal space to the interstitial and intravascular components. Creatinine, which is a larger molecule, diffuses at slower rate than urea. But, kidneys in the cases of ruptured urinary bladder apparently remain functional to excrete and concentrate these products in the urine and hence in the peritoneal cavity (Smith, et al., 1983). Peritoneal fluid creatinine-to-blood creatinine ratio greater than 2:1 indicates leakage of urine into the abdominal cavity (Bohn and Callan, 2007).

During this study, peritoneal fluid urea nitrogen-to-blood urea nitrogen ratio was 2.17:1 and peritoneal fluid creatinine-to-blood creatinine ratio was 3.00:1, in the cases with ruptured urinary bladder. Donecker and Bellamy (1982), Sockett et al. (1986), Larson (1996) and Van Metre (2004) also recorded peritoneal fluid: serum creatinine ratio of 2:1 or greater and considered it as diagnostic for uroperitoneum in both adult and perinatal mammal.

References

CRYOPRESERVATION OF MARWARI STALLION SEMEN USING PRIMARY AND SECONDARY SEMEN EXTENDERS- A COMPARISON

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ABSTRACT

In most of the species, except equines single extender is used for cryopreservation of semen. Semen ejaculates (16) were obtained from 4 adult Marwari stallions and were subjected to gel removal immediately after collection and then mixing the semen with primary extender to remove the seminal plasma. The aim of the present study was to evaluate different primary and secondary semen extenders in terms of post-thaw semen quality in Marwari horses. Pre-freezing motility and post-thaw motility showed significant (P<0.05) differences between primary and secondary extenders, respectively. It was observed that A5, A2 and A6 could be used as primary extenders and B2, B5 and B6 as secondary extenders in the semen freezing process. But, citrate-EDTA and lactose-glucose-EDTA-egg yolk extender was recommended as primary and secondary extender, respectively for semen freezing of Marwari stallions and to get optimal post thaw motility.

Key words: Marwari, stallion, extenders, post thaw motility and freezing

Introduction

Artificial insemination (AI) using cryopreserved semen is well in use in all the species including equines in different parts of the world but with limited success. One of the limiting factors in the use of cryopreserved semen in equines is the reduced fertility (Squires et al., 2004). Also, equine semen is far less tolerant of the freezing and thawing process than bull semen. Furthermore, not all stallion semen freezes alike (Pal et al., 2011). No single technique or magic formula exists to freeze all equine semen. Semen from some stallions fairs better than others with certain freezing media. Some stallion’s semen does not freeze well and may require extensive testing and laboratory procedures in attempting to preserve the spermatozoa (Pal et al., 2011). Keeping in view the above facts, need was felt to study the efficacy of different primary and secondary semen extenders in terms of post-thaw semen quality in Marwari horses.

Materials and Methods

Semen was collected from four adult, apparently healthy Marwari stallions using Colorado model of artificial vagina (AV) equipped with a disposable liner as per the standard method at four different occasions. All the Marwari stallions were maintained under uniform standard conditions of feeding and management at Equine Production Campus, National Research Centre on Equines, Bikaner, India. The stallions were kept at distance for visual stimuli to get proper erection before mounting for ejaculation in the AV. An oestrus mare was used as dummy. Soon after the semen collection, the semen was passed through sterilized gauze filter to remove the gel and microscopic and macroscopic analysis was performed. The semen having progressive motility more than 60% was processed for cryopreservation.

Semen extenders

Six different primary and secondary extenders were used for washing of spermatozoa and freezing, respectively using split ejaculate technique. The composition of primary and secondary extenders is mentioned as below:

Primary extenders:
A1: BSA Primary extender (Samper, 1995)
A2: Citrate EDTA extender (Cochran et al., 1984)
A3: Glucose EDTA extender II (Cochran et al., 1984)
A4: Skimmed milk and sugar extender (Piao and Wang, 1988)
A5: Sucrose solution 11% (Piao and Wang, 1988)
A6: HF-20 extender (Nishikawa, 1975)

Secondary extenders:
B1: Skim milk egg yolk extender (Samper, 1995b)
B2: Lactose-glucose-EDTA-egg yolk extender (Cochran et al., 1984)
B3: Glycine egg yolk extender (M Boyle, Cambridge, 1996)
B4: Skim milk and sugar extender (Piao and Wang, 1988)
B5: Sugar based extender (Piao and Wang,

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B6: HF-20 extender (Nishikawa, 1975)

Gel free semen was divided in six parts and each part was mixed with above said primary extenders in the ratio of 1:1 and centrifuged at 2000 rpm for 4-5 min at 8-10°C. The supernatant was aspirated off and the sperm pellet was dissolved and diluted in respective secondary extender keeping 100-120x10^6 spermatozoa per ml of diluted semen. Equilibration time of 2 hour was given to all samples. The equilibrated semen was filled in the Polyvinyl chloride (PVC) straws of 0.5ml capacity (IMV make) and sealed with filling-sealing machine. The semen-filled straws were put in the programmable Bio-med planner for further lowering of the temperature, finally plunged into liquid nitrogen (LN2), and kept stored in LN2 as described by Pal et al., (2011). Frozen straws were thawed at 37°C for one minute in water bath immediately wiped off, cut open and emptied into a clean pre-warmed vial (37°C), which was maintained at 37°C and evaluated for post-thaw motility. The data was analyzed by standard statistical methods (Snedecor and Cochran, 1989).

Results and Discussion

In most of the species, except equines single extender is used for cryopreservation of semen whereas in equines, primary extender is used for washing of the spermatozoa as seminal plasma is detrimental for the spermatozoa if not removed immediately after the collection and secondary extender containing cryoprotectant is used for semen freezing. Secondary extender is meant for nourishment of spermatozoa and to provide protection against cold shock.

In the study, pre freezing motility (PFM) was observed as 53.75±1.29, 65.63±1.32, 55.63±1.32, 59.38±1.99, 75.0±1.33 and 64.38±1.32 per cent with A1, A2, A3, A4, A5 and A6 primary extenders, respectively. PFM showed significant (P<0.05) differences between extenders. Primary extenders A1 & A3, A2 & A6, and A3 & A4 did not show any significant differences between them in term of motility. However, extender A5 was significantly (P<0.05) superior to all others in the study. Highest PFM % was observed in A5 and lowest in A1 extender. The results indicated that A2, A5 and A6 could be used as primary extenders as the PFM is maintained which is the success of any semen freezing process.

The secondary extender contains glycerol as a cryoprotectant in order to protect spermatozoa from cold shock. In the study, Marwari stallion semen was cryopreserved using six secondary extenders. Post thaw motility (PTM) was observed as 18.75±0.99, 35.31±1.10, 13.13±1.14, 15.63±1.32, 24.69±1.10 and 26.25±0.99 per cent with B1, B2, B3, B4, B5 and B6 secondary extenders, respectively. PTM of stallion semen showed significant (P<0.05) differences between secondary extenders. Secondary extender B1 & B4, B3 & B4 and B5 & B6 did not show any significant differences between them in terms of PTM. However, secondary extender B2 was significantly (P<0.05) superior to all others. Highest PTM % was observed in B2 and lowest in B3 secondary extender. In equines, semen containing 25% PTM is acceptable for AI. Hence, secondary diluent B2, B5 and B6 could be used for the freezing of Marwari stallion semen.

Based on results obtained in the study, it is evident that A2, A5 and A6 could be used as primary extenders and B2, B5 and B6 as secondary extenders in the semen freezing of Marwari stallions. But, based on post thaw motility of Marwari stallion semen, citrate-EDTA and lactose-glucose-EDTA-egg yolk extender were most suitable and recommended as primary and secondary extender, respectively for semen cryopreservation of Marwari stallion semen under arid conditions of Rajasthan.

References

MANAGEMENT OF OUTBREAK OF PPR IN A FARM

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ABSTRACT

Total of 30 goats were affected with Peste Des Petits Ruminants (PPR) in goat flocks in near by Village of Pantnagar. History revealed high morbidity and mortality in near by flocks. Animals were not vaccinated. Clinical examination revealed high fever, coughing, sneezing and serous discharge from the eyes and nostrils in some animals including kids. Profuse blood tinged diarrhoea developed in some cases. History, clinical signs and postmortem findings were being suggestive of Peste Des Petits Ruminants. Therapeutic management was carried out by antibiotics to stop secondary bacterial infections along with symptomatic treatment which helped to control this drastic disease.

Introduction

Peste des Petits Ruminants is a highly acute contagious viral disease of goats and sheep that is clinically similar to rinderpest and is characterized by fever, erosive stomatitis, diarrhoea, conjunctivitis, gastroenteritis, and pneumonia. The name is French for “disastrous disease of small ruminants”. Goats are usually more severely affected than sheep. PPR occurs in an epizootic form, it may have morbidity of 80-90% and mortality between 50 and 80 % (Lefevre and Diallo, 1990). It is considered to be one of the main constraints in improving small ruminant productivity (Stem, 1993). PPR, also known as goat plague, PPR is now endemic in India (Nanda et al., 1996; Singh et al., 2006). The presence of PPR can have a serious impact on livestock production and trade. Economic losses are due to loss of production, death, abortion and cost of controlling the disease. The presence of the disease can limit local trade and export. The transmission of virus requires close contact between susceptible and infected animals in the febrile stage (Braider, 1981). The discharge from eyes, nose, mouth and the loose faeces contain large amount of the virus. Fine infected droplets are released into the air from these secretions and excretions, particularly when infected animals cough and sneeze (Taylor, 1984 and Bundza et al., 1988).

History and observations

A goat flock of 30 goats near by village of Pantnagar were exposed. History revealed high morbidity and mortality in near by flocks. Animals were not vaccinated with PPR. Around 8 animals in the same flock died. All animals were having history of high fever, anorexia, profuse nasal discharge, breathing problems, restlessness and diarrhoea. Some pregnant animals had abortion. Routine clinical examination revealed high fever (above 40°C), accompanied by dullness, coughing, sneezing and serous discharge from the eyes and nostrils in 18 animals including 10 kids. In some cases nasal and ocular discharges became mucopurulent. There was matting the eyelids and partially occluding the external nares. Profuse mucoid and blood tinged diarrhoea was found in 8 cases. Dyspnoea and coughing was also observed with discrete necrotic lesions in the mouth and extended over the entire oral mucosa, forming diphtheric plaques. Foetid odour from the animal’s mouth was also felt. Auscultation revealed pneumonia. There was profound halitosis and the animals were unable to eat because of a sore mouth and swollen lips (Rajak et al., 2005). Total leukocyte count (TLC) and differential leukocyte count (DLC) revealed moderate decrease in lymphocyte count. (Rajak et al., 2005). In dead animals, the eyes and nose had a dirty discharge and the animal’s rear will often covered with bad smelling, watery faeces. Post mortem lesions were similar to rinderpest, with inflammatory and necrotic lesions in the oral cavity and throughout the gastro intestinal tract. In severe cases the hard palate, pharynx and upper oesophagus also have lesions. The carcass was generally emaciated.

Diagnosis was done based on clinical observation as per history and post mortem findings (OIE, 2002).

Treatment

As such no prescribed treatment is available for PPR but to give antibiotics to stop secondary bacterial infections and with symptomatic treatment helps to control this drastic disease. Therapeutic
regime was started with Cefotaxime 25 mg/kg body weight i/m bid, Chlorpheniramin maleate @ 0.5 mg/kg i/m, bid, Meloxicam along with paracetamol @ 0.5 mg/kg i/m, bid, Ambroxyl hydroxide orally, for 5 days. In some critical cases having breathing problems deriphyllin 1 ml i/m was also given. As per need hydration therapy in dehydrated animals was given like Intalyte M® 400 ml i/v. Locally antiseptic solutions like povidine iodine (gargle solution) was given on moth lesions and as mouth wash. Oxytetracycline and Chlortetracycline are recommended to prevent secondary pulmonary infections in PPR affected animals (OIE, 2000). Animals were fed with soft chopped green fodder and kept indoor. Clinical improvement started on 7th day post therapy with complete recovery after 14 days in the entire animal except in two animals which died second day post therapy. Barns, tools and other items that had been in contact with the sick animals were cleansed and disinfected with phenol as well as detergents.

References
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Animal Health divn.
LACTATION PERFORMANCE AND MILK CHARACTERISTICS OF YAKS (POEPHAGUS GRUNNIENS L) UNDER SUB-ALPINE TEMPERATE ZONE OF NORTH-EASTERN INDIA

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ABSTRACT

The present study was undertaken at Institutes’ experimental yak farm and in yak rearing tracts of West Kameng District, Arunachal Pradesh to study the lactation performance of yaks in semi intensive system and traditional migratory system of management. The lactation length in yaks reared at organized farm ranged from 270 to 330 days and the total milk yield per lactation ranged from 252.52 to 888.43 litres (459.24±42.7), while in the yaks reared under traditional system of rearing, the total milk yield per lactation ranged from 180 to 270 litres (229.9±7.64) in a lactation length of 180 to 240 days (211±5.75). In the organized farm, the peak yield was achieved at 61-90 days of lactation (1416.65±82.32 ml/day) and persisted up to 121-150 days of lactation (1377.02±86.54 ml/day), thereafter gradually decreased to 1096.82±117.41 ml/day in 301-330 days of lactation and the lactation ceased with the onset of winter season due to non availability of adequate nutrient supply, while in organized farm, the milking continued round the year. From the present study, it could be concluded that the yaks reared in semi intensive system had higher milk yield, peak yield persisting for longer period and longer lactation length as compared to the animals reared under traditional migratory system of management.

Introduction

Yak is a multipurpose (pack, milk, meat, skin, hairs, etc) bovine species of economical importance in high hill and snow bound areas on and above 3000 m mean sea level (MSL). Traditional yak husbandry system involves rearing under free-range and vertical migration of livestock in search of better pasture, going to the high altitude alpine pasture in summer and grazing at mid altitude pasture in the winter months. Scanty feed resource in long winter period (November to March) is the major constraint hampering the performance of the yaks under field conditions. In the traditional rearing system, majority of calving occurs in the months of March to July (being favourable season) and thus, the lactation is confined to summer months and ceases with the onset of winter (November-December) due to unavailability of season-based feed resources. However, Long, (1999) reported that supplementary feeding to yaks during winter helps to maintain body weight and continue the production thereby increasing the lactation length. Hence, the work was undertaken to study the lactation performance of the yak in the semi intensive system of management and compare with that of traditional migratory system of management.

Materials and Methods

A total of 22 female yaks maintained under the semi intensive system of management taken at Institute’s Experimental Farm, Nyukmadung (2750 m above MSL), Arunachal Pradesh were taken for the present investigation. The animals were fed @ 3% of body weight (250-300 kg in adults) with concentrate (crushed Maize-35 parts, Wheat bran-15 parts, Rice bran-12 parts, Soybean meal-10 parts, Groundnut cake-10 parts, Mustard oil cake-15 parts, Mineral mixture 1 part and common salt 2 parts), straw and green fodder along with locally available tree fodders in a ratio of 3:2:4. The new born calves were allowed to suckle the milk fully from the dam in our observation. Milk recording was possible only after 29.63±2.26 days of parturition. The dam was not milked prior to it, looking to the harshness of the environment and non-availability of supplementation for calves during early part of life and visual body condition of calves determined the onset of milking for commercial purpose. The milk yields were daily (morning at 7.30 AM and evening at 4.00 PM) noted till the completion of lactation. The milk composition was analysed with the help of automatic milk analyser. The milking behaviour (milk let down time, milk flow rate and total milking time) were also observed.

To study the performance of yaks in traditional system of rearing, the survey was carried out in different yak tracts of West Kameng district and the data were collected regarding the daily milk yield,
Fig. 1: Lactation yield of yak reared under semi-intensive and migratory system of rearing

![Graph showing lactation yield of yak under different systems](image)

Table 1: Milk production dynamics of yak reared under semi-intensive system

<table>
<thead>
<tr>
<th>Range of lactation days</th>
<th>Average daily milk yield (Mean and SE)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-60 days (ml)</td>
<td>1205.14±76.17</td>
<td>680.67 - 2113.37</td>
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<tr>
<td>61-90 days (ml)</td>
<td>1416.65±82.32</td>
<td>544.60 - 2306.53</td>
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<td>91-120 days (ml)</td>
<td>1369.37±75.81</td>
<td>587.53 - 2152.97</td>
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<td>121-150 days (ml)</td>
<td>1377.02±86.54</td>
<td>611.20 - 2342.00</td>
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<td>151-180 days (ml)</td>
<td>1348.95±90.55</td>
<td>553.47 - 2511.87</td>
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<td>181-210 days (ml)</td>
<td>1266.78±87.20</td>
<td>492.60 - 2350.00</td>
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<td>211-240 days (ml)</td>
<td>1219.67±77.40</td>
<td>560.50 - 2052.67</td>
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<td>241-270 days (ml)</td>
<td>1234.02±118.66</td>
<td>500.80 - 2317.13</td>
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<td>271-300 days (ml)</td>
<td>1154.40±122.93</td>
<td>220.80 - 2222.37</td>
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<tr>
<td>301-330 days (ml)</td>
<td>1096.82±117.41</td>
<td>507.83 - 2009.93</td>
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<tr>
<td>Total Lactation Yield (l)</td>
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<td>252.00-888.00</td>
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<tr>
<td>Lactation Length (days)</td>
<td>322.67±20.07</td>
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<tr>
<td>Average Daily Yield (Kg)</td>
<td>1.304±0.13</td>
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Table 2: Correlation between various milk components, milking behaviour and milk yield

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<th>Components of milk</th>
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<th>Fat</th>
<th>Protein</th>
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<th>Milk flow rate</th>
<th>Total milking time</th>
<th>Let down time</th>
<th>Yield</th>
<th>Milking behaviour</th>
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<td>-</td>
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<tr>
<td>Fat</td>
<td>-0.423**</td>
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<td>-</td>
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<td>-</td>
<td>1.000</td>
<td>-</td>
<td>Let down time</td>
</tr>
<tr>
<td>Protein</td>
<td>-0.209*</td>
<td>0.346**</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-0.397**</td>
<td>0.848**</td>
<td>Total milking time</td>
</tr>
<tr>
<td>SNF</td>
<td>-0.216*</td>
<td>0.349**</td>
<td>0.964**</td>
<td>1.000</td>
<td>-</td>
<td>1.000</td>
<td>0.170**</td>
<td>-0.397**</td>
<td>0.840**</td>
<td>Milk flow rate</td>
</tr>
<tr>
<td>TS</td>
<td>-0.427**</td>
<td>0.942**</td>
<td>0.626**</td>
<td>0.643**</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 5% level of significance, ** Significant at 1% level of significance, *NS* Non-significant
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Results and Discussion

In the present study, the lactation length in yaks reared at organized farm ranged from 270 to 330 days and the total milk yield per lactation ranged from 252.52 to 888.43 litres (459.24±42.7), while in the yaks reared under traditional system of rearing, the total milk yield per lactation ranged from 180 to 270 litres (229.9±7.64) in a lactation length of 180 to 240 days (211±5.75) which is in similar line of findings as suggested by Ramesha et al. (2008). The lactation curves of yak reared under semi-intensive system and traditional migratory system of rearing (Fig.1) shows the superiority of production performance under semi-intensive system as compared to migratory system due to inability to maintain the lactation peak under field conditions. The total milk yield is higher in organized farm than traditional system of rearing due to improved management practice like round the year feed supplementation. Zhang Rongchang (1989) studied the total milk yield of yaks reared in pastoral system and found that total yield was highest in Huanhu yak of Qinghai Province and lowest in Tibet yaks (304 vs. 280 kg). Pundir et al. (1996) reported a total milk yield of 125 kg in lactation length of 172 days in Sangla valley yak managed under traditional practice in Himachal Pradesh.

In the organized farm, the peak yield was reached by 61-90 days of lactation (1416.65 ± 82.32 ml/day) and persisted up to 121-150 days of lactation (1377.02 ± 86.54 ml/day), thereafter gradually decreased to 1096.82±117.41 ml/day in 301-330 days of lactation (Table 1); whereas in traditional yak rearing system, the peak yield persisted only for one month (61-90 days of lactation) and the lactation ceased with the onset of winter season due to non-availability of adequate nutrient supply, while in organized farm, the milking continued round the year. In the field condition, the peak yield was noticed in months of July-August when the availability of nutrients is in optimal quality and quantity from the alpine pastures and from September onwards, there is a steep decline in milk yield as the quality of the fodder resources gets deteriorated. Earlier, Pundir et al. (1996) found that average daily milk yield was 728 gm/day and peak milk yield to be 1.50 kg/day in Sangla valley yaks of Himachal Pradesh.

Also, it is estimated that the calf takes about a third of the available milk if the yak is milked twice daily and about half the milk with once a day milking (Wiener et al., 2003). Yak females produce about one third more milk, in total, if stimulated by milking twice daily compared to once a day (Lei Huanzhang et al., 1983). So, under existing system of management, it can be estimated that the total milk yield per lactation may be equal to 3/2*(lactation yield); which means around 690 litres under semi-intensive system of rearing and 340 litres under traditional migratory system of rearing. There is no universally accepted method of assessing lactational milk yield of yaks unlike the 305 day milk yield in cows and buffaloes (Nivsarkar et al., 1997). Cai Li and Wiener (1995) proposed milk production over a period of 180 days. Bat Erdene (1993) reported that in Mongolian yaks, the average lactation length was 283 days in yaks calving in March and 206 days in late calvers. The lactation length varied from 180

| Table 3: Effect of Stage of Lactation and Diurnal Variation on Yak Milk Composition |
|---------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Parameters                                  | Mid Lactation (60 days to 150 days) | Late Lactation (More than 150 days) |
|                                            | Morning | Evening | Overall | Morning | Evening | Overall |
| Milking Behaviour                            |         |         |         |         |         |         |
| Let down time (Sec.)                         | 65±7.74 | 85.83±7.79 | 76.36±6.18 | 70.29±1.91 | 87.5±2.66 | 79.80±1.96 |
| Milking Time (Sec.)                          | 198±4.36 | 110±9.22 | 150.0±14.78 | 173.23±5.87 | 94.64±3.67 | 129.80±5.62 |
| Milk Flow Rate (ml/minutes)                 | 414.23±32.09 | 393.08±41.82 | 402.69±25.98 | 370.14±14.40 | 291.38±12.48 | 326.61±10.40 |
| Milk Characteristics                         |         |         |         |         |         |         |
| Yield                                       | 1535±190.65 | 695.83±52.77 | 2230.83±180.25 | 1064.88±37.58 | 447.85±20.92 | 1512.73±51.76 |
| Fat                                         | 7.315±0.18 | 7.98±0.16 | 7.54±0.15 | 7.45±0.12 | 8.53±0.13 | 7.75±0.11 |
| Protein                                     | 4.26±0.04 | 4.24±0.15 | 4.27±0.05 | 4.25±0.02 | 4.35±0.03 | 4.27±0.01 |
| Solid-not-fat (SNF)                          | 11.18±0.13 | 11.10±0.41 | 11.17±0.17 | 11.23±0.06 | 11.47±0.06 | 11.29±0.04 |
| Total Solid (TS)                             | 18.49±0.26 | 19.09±0.43 | 18.72±0.24 | 18.69±0.15 | 20.01±0.17 | 19.05±0.14 |
| Depression in Freezing Value (F)             | 0.67±0.01 | 0.66±0.007 | 0.66±0.01 | 0.66±0.002 | 0.65±0.01 | 0.65±0.01 |
| Specific gravity                             | 1.04±0.001 | 1.03±0.001 | 1.04±0.001 | 1.036±0.001 | 1.036±0.001 | 1.036±0.001 |
to 210 days in Nepalese yaks (Joshi and Lund, 1994) and 159 to 187 days in Himachal yaks (Kaila et al., 1997).

When the correlation between various milk components, milking behaviour and milk yield was observed, it was found that except total milking time and milk flow rate, all the characteristics were significantly correlated amongst each other (Table 2). All the milk components (fat %, protein %, SNF%, total solid %) were significantly negative correlated with milk yield; however, amongst the various components of milk (fat %, protein %, SNF%, total solid %), all were significantly but positively correlated. Let down of milk time was negatively correlated; however, total milking time and milk flow rate were positively correlated with milk yield. The milk solid, fat and protein contents in milk of the yak and its hybrids are far higher than in other cattle (Zhang Rongchang, 1989). Except milk yield, milk components were higher in late lactation as compared to mid lactation which is in commensurate with findings of Zhang Rongchang et al. (1989).

Overall milking behaviour was elaborate in mid lactation, as compared to late lactation (Table 3); milking time (sec), milk flow rate (ml/min) and milk yield were higher in mid lactation as compared to late lactation. All milk components were in higher range in evening hours, due to less milk production.

In the field condition, the lactation is seasonal and milk production ceases in winters. The yak cows start giving milk after calving in March-April and milking was started only from May as full suckling was allowed to calves for initial one month and milking of the yaks is stopped at the onset of the winter (November-December). A yak does not dry off in consequence of this and continues to secrete a little milk for its calf. This happens irrespective of whether a female yak is pregnant or not. Only a cow that has lost her calf during the warm season will dry off when hand milking ceases. Other non pregnant yaks and pregnant cows with a calf still at foot will not go dry and resumes lactating through the following warm season and, normally, will be hand milked again. The "half-lactating" female yak (as it is called) will go dry at the end of her second warm season, irrespective of whether it is pregnant or not (Wiener et al., 2003).

From the present study, it could be concluded that the yaks reared in semi intensive system had higher milk yield, peak yield persisting for longer period and longer lactation length as compared to the animals reared under traditional migratory system of management.

References
USE OF ENTOMOPATHOGENIC NEMATODE STEINERNEMA CARPOCAPSAE (STSLU) FOR THE BIOLOGICAL CONTROL OF CATTLE TICKS RHIPICEPHALUS MICROPLUS

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ABSTRACT

Entomopathogenic nematodes (EPNs) have been used for biological control of certain insect pests of economically important crops. In these studies the bio-efficacy of indigenous EPNs population Steinernema carpocapsae (STSLU) was tested against important cattle tick, Rhipicephalus microplus on the basis of percent mortality under laboratory conditions for a possible biological control. The 25 cattle ticks were placed on Whatman filter paper No. 1 in each Petri plates and inoculate infective juveniles (IJs) of S. carpocapsae (STSLU) at different inoculums levels. All the treatments were replicated four times and placed at 20ºC under B.O.D. incubator condition. Results showed that cattle ticks R. microplus were found susceptible against the S. carpocapsae (STSLU) under laboratory conditions. The cattle ticks mortality ranged 03 to 100 per cent. The maximum 100 mean per cent mortality of R. microplus was observed at 500 IJs/Petri plate after 4th days and at 400 IJs IJs/Petri plate after 5th days of inoculation followed by 97.0 and 94.0 mean per cent mortality at 400 IJs/Petri plate after 4th days and at 300 IJs IJs/Petri plate after 5th days of inoculation, respectively.

Key word: Entomopathogenic nematodes, Steinernema carpocapsae, Rhipicephalus microplus, inoculation, infective juveniles (IJs)

Introduction

Entomopathogenic nematodes (EPNs) are parasites of insects. These are characterized by carrying specific symbiotic bacteria of the genus Xenorhabdus or Photorhabdus in their intestine (Boemare et al., 1993). Symbiotic bacteria play an important role in the pathogenicity of the nematodes bacteria complex to insect host and the subsequent reproduction of the nematodes in the host (Akhurst and Boemare, 1990). EPNs are currently used as biopesticides for controlling several important insect pest worlds wide (Shapiro Ilan et al., 2002). EPNs are extraordinarily lethal to many important soil insect pests.

Biological control of insect pests using EPNs has gained importance in the recent year. Because they are highly virulent, killing their host within 24 to 48 hrs. They can be cultured easily in vivo as well as in vitro on artificial diet and have a high reproductive potential, broad host range, longer storage ability and can easily be applied in soil and foliage without adverse effect on non-target organisms (Georgis et al., 1991). They are safe to the plant and animal health.

Recently, it has been demonstrated that the entomopathogenic nematode, Steinernema carpocapsae have a potential to use as a biological control agent against cattle tick, Rhipicephalus microplus (Monteiro et al., 2010), which is considered to be the most important tick parasite of livestock in the world. This hardy tick can be found on many hosts including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs and some wild animals. This tick can also transmit babesiosis (cattle fever), which is caused by the protozoal parasites, Babesia bigemina and Babesia bovis. Also, transmit anaplasmosis caused by Anaplasma marginale (Kocan et al., 1998).

The major objective of present investigation was to determine the effects of Steinernema carpocapsae on mortality of Rhipicephalus microplus at different level of inoculums under laboratory conditions for effective biocontrol of cattle ticks.

Materials and Methods

The bioeficacy test of indigenous EPNs population of Steinernema carpocapsae (STSLU) was conducted on important cattle tick, Rhipicephalus microplus under laboratory conditions.
Procedure and treatment of bioefficacy test:

Total sterilized 24 petri plates were used for this experiment. The 25 cattle ticks were placed on Whatman filter paper No. 1 in each Petri plates and inoculate infective juveniles (IJs) of *S. carpocapsae* (STSLU) at different inoculums levels. All the treatments were replicated four times and placed at 20°C under B.O.D. incubator condition. The observations were taken on per cent mortality of cattle tick after every day up to 5 days from the time of inoculation.

Treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean per cent mortality of <em>R. microplus</em> at different time interval (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>T1 = 100 IJs/Petri plate</td>
<td>00 (0.00)</td>
</tr>
<tr>
<td>T2 = 200 IJs/Petri plate</td>
<td>03 (09.83)</td>
</tr>
<tr>
<td>T3 = 300 IJs/Petri plate</td>
<td>09 (17.42)</td>
</tr>
<tr>
<td>T4 = 400 IJs/Petri plate</td>
<td>14 (21.94)</td>
</tr>
<tr>
<td>T5 = 500 IJs/Petri plate</td>
<td>19 (25.82)</td>
</tr>
<tr>
<td>T6 = Control</td>
<td>00 (0.00)</td>
</tr>
<tr>
<td>SEM±</td>
<td>0.637</td>
</tr>
<tr>
<td>CD (0.05%)</td>
<td>1.920</td>
</tr>
<tr>
<td>CV (%)</td>
<td>16.98</td>
</tr>
</tbody>
</table>

*No of cattle ticks 25 per Petri plate and each treatment were replicate 4 times.

Results and Discussion

The experiment was conducted for evaluating the potential of the entomopathogenic nematodes (EPNs) population *Steinernema carpocapsae* (STSLU) against cattle ticks *Rhipicephalus microplus* at different inoculums levels under laboratory conditions. The bio-efficacy was tested on the basis of per cent mortality of the cattle ticks. Results showed that cattle ticks *R. microplus* were found susceptible against the *S. carpocapsae* (STSLU) under laboratory conditions. The cattle ticks mortality ranged 03 to 100 per cent.

The maximum 100 mean per cent mortality of *R. microplus* was observed at 500 IJs/Petri plate after 4th days and at 400 IJs/Petri plate after 5th days of inoculation followed by 97.0 and 94.0 mean per cent mortality at 400 IJs/Petri plate after 4th days and at 300 IJs/Petri plate after 5th days of inoculation, respectively. Results showed that the mortality of *R. microplus* increased due to the increase in the inoculum level and period of exposure up to a certain level.

Similar studies in this regard were made by Kocan *et al.* (1998) who also reported that infective juveniles (IJs) of different EPNs strains (*Steinernema glaseri*, *S. riobravus*, *S. carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*) appeared to be the most effective in killing ticks and invaded and killed 30 to 100% of replete females.

Samish *et al.* (2000) reported that the mortality of *Rhipicephalus bursa*, and *Rhipicephalus sanguineus* adult ticks was recorded after 0.3 to 8.0 days their exposure in petri dishes to 5 entomopathogenic nematode strains. Samish *et al.* (1999) also reported that the Mexican strain of *Steinernema carpocapsae* was most efficient, inducing 100% tick mortality at a concentration of 50 nematodes per square centimeter.

References

Akhurst, R., and Boemare, N.E. (1990) *Biology and Taxonomy of Xenorhabdus*. In: Entomopathogenic nematodes in biological control. R. Gaugler, and
Plate 1: Dead cattle ticks infected by EPNs (S. carpocapsae STSLU)

Plate 2: EPNs (S. carpocapsae STSLU) are inside the dead cattle tick (R. microplus).


COMPARATIVE THERAPEUTIC EFFICACY OF DIFFERENT ANTHELMINTICS AGAINST GASTROINTESTINAL HELMINTHS IN HORSES

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ABSTRACT

Comparative efficacy of three anthelmintics: Fenbendazole, Ivermectin (oral) and pyrantel pamoate were evaluated against gastrointestinal helminthes of horses. Horses having faecal egg counts (irrespective of the helminth species) between 643.13±131.63 to 808.60±223.09 prior to treatment were selected and were divided into three groups of 8, 13 and 26 horses, respectively. These horses were given Ivermectin (oral) @ 0.2 mg/kg b.wt., pyrantel pamoate @ 6.6 mg/kg b.wt., and Fenbendazole @ 10 mg/kg b.wt. in group 1, 2 and 3, respectively. EPG as per the method of modified McMaster technique was calculated before and 7, 14 and 21 days after treatment. Faecal egg count reduction (FECR) % was taken as a measure for efficacy. Faecal egg count reduction % was highest at day 7, 14 and 21 in fenbendazole treated group as compared to the other two groups. Ivermectin (oral) treated group also showed good result while pyrantel treated group showed least therapeutic efficacy in terms of faecal egg count reduction (FECR) percentage.

Key words: Fenbendazole, ivermectin, pyrantel pamoate, horse.

In India, equids constitute 2.34% of total world equine population (Yadav and Mallik, 1998) and have made immense contribution in the overall economic development of the country, especially in the rural and hilly areas. Parasitic diseases due to their direct action like irritation, annoyance, intoxication, mechanical obstruction, tissue damage, reduced feed intake and anaemia, are responsible for the poor health of the equines.

Anthelmintics used for the treatment of the parasitic infections of equines includes: Benzimidazoles, Pyrantel pamoate, Piperazines and Ivermectins. The Benzimidazoles are safe and effective against most common equine parasites but many species of cyathostomes have developed resistance (Kaplan, 2002). The avermectins are new class of anthelmintic that has a broader spectrum of activity against internal as well as external parasites (Leanining, 1983). The ideal anthelmintic should be safe, easy to administer, reasonably priced and effective in eliminating common internal parasites. Majority of the conventional drugs donot fulfill these criteria. Therefore, comparative efficacy of three anthelmintics i.e. Fenbendazole, Ivermectin (oral) and Pyrantel pamoate were evaluated against gastrointestinal helminthes of horses.

Materials and Methods

To know the efficacy of different anthelmintics, horses having faecal egg counts (irrespective of the species) between 643.13±131.63 to 808.60±223.09 prior to treatment, were divided into three groups of 8, 13 and 26 horses, respectively. These horses were given Ivermectin (oral) @ 0.2 mg/kg b.wt., Pyrantel pamoate @ 6.6 mg/kg b.wt. and Fenbendazole @ 10 mg/kg b.wt. in group 1, 2 and 3, respectively. EPG was calculated by modified McMaster technique (Sloss et al., 1994) before and 7, 14 and 21 days after treatment. Faecal egg count reduction (FECR) % was taken as a measure for efficacy.

Results and Discussion

The results of the study are shown in Table 1. As evident from Table 1, faecal egg count reduction percentage was highest at day 7, 14 and 21 in Fenbendazole treated group as compared to the other two groups. Pyrantel pamoate treated group also showed good result while Ivermectin (oral) treated group showed least therapeutic efficacy in terms of faecal egg count reduction (FECR) %. Among all drugs Ivermectin showed least FECR percentage though it was 94.83, 96.17 and 96.99 per cent on day 7, 14 and 21 post treatments. This finding is not in agreement with the findings of Costa et al. (1998) who found 100% efficacy of this drug against mature and immature nematode species of horses. Comparatively lower efficacy of Ivermectin may be attributed to its lower bioavailability on oral administration (Prescott and Baggot, 1994). In addition, it is effective against adult and luminal larval stages of small strongyles in the

¹Corresponding author
mucosa but has low efficacy against inhibited stages (Eysker et al., 1992). Pyrantel pamoate showed 96.99% reduction in faecal egg count on 21 days post treatment. These findings coincide with the findings of Davies and Schwalbach (2000), who found 96.1 per cent reduction in FEC after treatment with Pyrantel pamote. Pyrantel salts are imidazole thiazole derivatives and act by mimicking the effects of acetylecoline in nematode parasite. These are not effective against inhibited stages of small strongyles though it removes sensitive stages of adults (Taylor and Kenny, 1995). Fenbendazole acts by binding to parasite tubulin (dimeric) and causes disruption of microtubules which are important for secretion of many enzymes. Fenbendazole @ 10 mg/kg b.wt., orally showed highest therapeutic efficacy on 21 days post treatment. These findings are in agreement with the findings of Lange and Thomson (1976), Duncan et al. (1976), MacBeath et al. (1981) and Malian et al. (1981) who have reported 100 per cent efficacy of Fenbendazole against gastrointestinal nematodes of horses. This drug is effective against developing larval stages and sensitive strains @ 5 mg/kg b.wt., but if used @ 10 mg/kg b.wt., inhibited stages of many cyathostomes are also killed (Steinbach et al., 2006) which may be the reason why this drug caused highest reduction in FCR percentage on day 21.

### Table 1: Efficacy of various Anthelmintics against Strongyles in Horses

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Area of Sample</th>
<th>No. of Animals</th>
<th>Treatments</th>
<th>Mean EPG SD</th>
<th>FECR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre treatment</td>
<td>Post treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 days</td>
<td>7 days</td>
</tr>
<tr>
<td>1</td>
<td>Government Organised Unit</td>
<td>8</td>
<td>Ivermectin (oral) @ 0.2 mg/kg b.wt.</td>
<td>643.13±131.63</td>
<td>32.38±16.25</td>
</tr>
<tr>
<td>2</td>
<td>Private Organised Unit</td>
<td>13</td>
<td>Pyrantel Pamoate (oral) @ 6.6 mg/kg b.wt.</td>
<td>709.23±161.96</td>
<td>36.69±24.58</td>
</tr>
<tr>
<td>3</td>
<td>Private Unorganised Unit</td>
<td>26</td>
<td>Fenbendazole (oral) @ 10.0 mg/kg b.wt.</td>
<td>808.60±223.09</td>
<td>42.12±20.76</td>
</tr>
</tbody>
</table>

### References

EFFECT OF REPLACING FISH MEAL WITH SOYBEAN MEAL WITH OR WITHOUT LYSINE AND METHIONINE SUPPLEMENTATION ON THE CARCASS TRAITS OF BROILER CHICKEN#

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Sher-e-Kashmir University of Agricultural Sciences and Technology-K, Shuhama
Alusteng-190006, Kashmir

ABSTRACT

A study was conducted to evaluate the effect of replacing fish meal with soybean meal with or without lysine and methionine supplementation on the carcass traits of broiler chicken so as to reduce the dependence on fish meal. To achieve the envisaged objectives, three hundred day-old commercial broiler chicks were procured in summer season from a reputed source reared together until 7 days of age. On 8th day, the chicks were individually weighed, distributed randomly into five groups of four replicates with fifteen chicks in each. Five experimental diets were formulated with varying levels of fish meal. The birds were reared under standard managemental conditions and on 42th day of their age, eight birds per treatment that is 40 birds in total were selected at random, starved for 12 hours to empty their crops and utilized for carcass evaluation study. There was no significant (P>0.05) difference in dressing percentage, per cent yield of feathers and giblets (liver, heart and gizzard) between various experimental groups. The percent yield of various cut up parts was not significantly (P>0.05) different between various treatments. It is concluded that fish meal can be replaced with soybean meal without having any adverse effect on the major carcass traits of commercial broiler chicken.

Key words: Fish meal, soybean meal, broiler, carcass characteristics.

Introduction

Feed represents the major item of expenditure in poultry production. There are two sources of feed protein i.e. protein of animal origin and protein of plant origin. Plant proteins are usually low in lysine and methionine and its biological value is lower. In a broiler ration, fish meal, meat and bone meal and other protein concentrates are predominantly the principal sources of animal protein. In connection with the ban on meat-and-bone meals (MBM) for feeding to farm animals including poultry, alternative sources of proteins and other substances have to be found. Fish meal, which is not banned for feeding, contains a lot of protein, amino acids and readily usable source of phosphorus, however, high price does not allow a broader use in the diets for broilers. The fish meal available in the market is not good and its quantitative supply is not steady throughout the year. The quality of fish meal is often questioned because of adulteration with other adulterating materials such as fish bones, fish scales, sand, stone, soil, fine sawdust, horns and hooves, blood meal, Animal oil, prawn, poultry by-products and wastes of tannery. Therefore, there is a need to replace animal protein with vegetable protein. Of vegetable protein feeds, soybean is the best alternative to fish meal. Soybean meal is the only common protein supplement that is included in poultry rations with no limitation as to the quantity used, as it is properly toasted to denature the trypsin inhibitors, and hence there are no anti nutritive factors to consider when formulating diets. Therefore the present study was carried out with the objective of exploring the possibility of replacing fish meal with soybean meal with or without amino-acid supplementations in broiler rations and see its effect on their carcass traits.

Materials and Methods

Three hundred day-old commercial broiler chicks were procured from a reputed source in summer season. Chicks were reared in battery cages until 7 days of age. During this period all the birds were provided with a pre-starter mash (23% crude protein and 2800 kcal/kg metabolisable energy). On 8th day, the chicks were individually weighed, distributed into 5 treatment groups, each group with 4 replicates and each replicate of 15 chicks. The body weight of chicks in different treatment groups did not differ significantly (P<0.05). Five experimental diets were formulated. Diet-1 contained 10% fish meal (control), Diet-2 contained

#Part of M.V.Sc. Thesis and Corresponding author. Email: irfanvet116@gmail.com
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Table 1: Slaughter characteristics of broiler chicken fed various experimental diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dressing (%)</td>
<td>66.02±0.70^a</td>
<td>65.22±0.56^a</td>
<td>64.37±0.64^a</td>
<td>65.15±1.06^a</td>
<td>64.75±0.478^a</td>
</tr>
<tr>
<td>Blood yield (%)*</td>
<td>4.87±0.27^a</td>
<td>4.87±0.26^a</td>
<td>4.95±0.17^a</td>
<td>3.4±0.24^a</td>
<td>3.7±0.08^a</td>
</tr>
<tr>
<td>Feather yield (%)*</td>
<td>4.45±0.09^a</td>
<td>4.42±0.20^a</td>
<td>4.40±0.18^a</td>
<td>4.42±0.26^a</td>
<td>4.70±0.51^a</td>
</tr>
<tr>
<td>Head yield (%)*</td>
<td>3.15±0.18^a</td>
<td>3.02±0.04^a</td>
<td>3.12±0.11^a</td>
<td>4.2±0.10^a</td>
<td>4.3±0.25^b</td>
</tr>
<tr>
<td>Shanks yield (%)*</td>
<td>5.72±0.36^a</td>
<td>5.85±0.21^a</td>
<td>5.50±0.20^a</td>
<td>4.22±0.11^b</td>
<td>4.60±0.31^b</td>
</tr>
<tr>
<td>Giblet yield (%)*</td>
<td>5.99±0.28^a</td>
<td>5.64±0.31^a</td>
<td>6.17±0.32^a</td>
<td>6.27±0.49^a</td>
<td>5.79±0.25^a</td>
</tr>
<tr>
<td>Giblet yield (%) **</td>
<td>9.07±0.42^a</td>
<td>8.64±0.47^a</td>
<td>9.58±0.49^a</td>
<td>9.62±0.72^a</td>
<td>8.94±0.38^a</td>
</tr>
</tbody>
</table>

*Per cent live weight basis ** Per cent dressed weight basis
Means within the same row with different superscripts are significantly different (P<0.05)

Table 2: Cutability characteristics of broiler chicken fed various experimental diets

<table>
<thead>
<tr>
<th>Cut-up part</th>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Yield (plw)*</td>
<td>21.00±0.35^a</td>
<td>20.12±0.68^a</td>
<td>20.50±0.51^a</td>
<td>19.27±0.81^a</td>
<td>20.75±0.57^a</td>
</tr>
<tr>
<td></td>
<td>Yield (pdw)**</td>
<td>32.10±0.81^a</td>
<td>31.05±0.74^a</td>
<td>31.35±0.65^a</td>
<td>29.70±0.74^a</td>
<td>31.97±0.75^a</td>
</tr>
<tr>
<td>Drum-Sticks</td>
<td>Yield (plw)*</td>
<td>10.37±0.22^a</td>
<td>10.52±0.17^a</td>
<td>10.32±0.10^a</td>
<td>9.7±0.53^a</td>
<td>9.90±0.42^a</td>
</tr>
<tr>
<td></td>
<td>Yield (pdw)**</td>
<td>15.75±0.30^a</td>
<td>16.05±0.15^a</td>
<td>16.20±0.20^a</td>
<td>14.90±0.84^a</td>
<td>15.25±0.61^a</td>
</tr>
<tr>
<td>Thighs</td>
<td>Yield (plw)*</td>
<td>10.65±0.12^a</td>
<td>10.72±0.31^a</td>
<td>10.37±0.047^a</td>
<td>10.42±0.66^a</td>
<td>10.32±0.41^a</td>
</tr>
<tr>
<td></td>
<td>Yield (pdw)**</td>
<td>16.27±0.18^a</td>
<td>16.42±0.37^a</td>
<td>16.12±0.16^a</td>
<td>15.92±0.53^a</td>
<td>16.00±0.38^a</td>
</tr>
<tr>
<td>Wings</td>
<td>Yield (plw)*</td>
<td>9.02±0.12^a</td>
<td>8.62±0.19^a</td>
<td>8.92±0.08^a</td>
<td>8.3±0.12^a</td>
<td>8.25±0.15^a</td>
</tr>
<tr>
<td></td>
<td>Yield (pdw)**</td>
<td>13.62±0.29^a</td>
<td>13.22±0.46^a</td>
<td>13.47±0.25^a</td>
<td>12.50±0.38^a</td>
<td>12.57±0.42^a</td>
</tr>
<tr>
<td>Back</td>
<td>Yield (plw)*</td>
<td>11.90±0.44^a</td>
<td>12.25±0.27^a</td>
<td>11.75±0.17^a</td>
<td>12.0±0.69^a</td>
<td>11.62±0.43^a</td>
</tr>
<tr>
<td></td>
<td>Yield (pdw)**</td>
<td>18.25±0.49^a</td>
<td>18.65±0.30^a</td>
<td>18.25±0.53^a</td>
<td>18.25±0.70^a</td>
<td>17.92±0.76^a</td>
</tr>
<tr>
<td>Neck</td>
<td>Yield (plw)*</td>
<td>3.85±0.14^a</td>
<td>3.60±0.17^a</td>
<td>3.55±0.10^a</td>
<td>3.67±0.16^a</td>
<td>4.0±0.12^a</td>
</tr>
<tr>
<td></td>
<td>Yield (pdw)**</td>
<td>6.00±0.24^a</td>
<td>5.75±0.25^a</td>
<td>5.75±0.21^a</td>
<td>6.15±0.10^a</td>
<td>5.95±0.19^a</td>
</tr>
</tbody>
</table>

*Per cent live weight basis ** Per cent dressed weight basis
Means within the same row with different superscripts are significantly different (P<0.05)

5% fish meal. Diet-3 was similar to Diet-2, but with supplementation of lysine and methionine @ 0.1% and 0.03%, respectively. Diet-4 contained no Fish meal and Diet-5 was similar to Diet-4, but with supplementation of lysine and methionine @ 0.2% and 0.06%, respectively. The lysine and methionine used was herbal preparation. Ad-libitum feeding and watering was practiced during the experimental period. Birds were reared under standard managerial conditions till the experiment got completed. On 42th day, eight birds per treatment were selected at random, starved for 12 hours to empty their crops and utilized for carcass evaluation study. Each bird was weighed immediately before severing the jugular vein at the atlantooccipital joint and then allowed to bleed. Percentage blood was determined by the difference between live weight and bled-out weight and expressed as percentage of live weight. The slaughtered birds were de-feathered and eviscerated. The weight of the feathers was calculated as the difference between the bled-out weight and the de-feathered weight and expressed as a percentage of the live weight. The carcass dressing percentage was calculated as the ratio of the eviscerated weight to the live weight and expressed as a percentage. Weight of cut-up parts (thighs, drumsticks, breast, wings, back and neck) was recorded and expressed in terms of percentage live weight and percentage dressed weight. The data obtained was statistically assessed by one-
way ANOVA using the General Linear Model procedure of Statistical Package for the Social Sciences, Base 10.0, 1999. To test the significance of difference between means Duncan’s multiple range test Duncan (1955) was used. The probability level for determining the significance was 0.05.

Results and Discussion

The dressing percentage of broiler chicken in various experimental groups ranged between 64.37±0.64 and 66.025±0.70 (Table 1). However, this difference in dressing percentage was non-significant (P>0.05). These findings are in agreement with the earlier results (Donkoh et al., 2000; Curto and Cicogna, 1968; Petricevic et al., 1988). The per cent yield of various edible by-products that is heart, liver and gizzard did not show any significant difference (P>0.05) between various experimental groups. Similarly the yield of feathers was not significant (P>0.05) between various treatment groups. Similar results were observed by (Donkoh et al., 2000; Curto and Cicogna, 1968; Aletor et al., 1989). The highest blood yield of 4.95±0.17% was recorded in T₃ group (Diet containing 5% fish meal with supplementation of lysine and methionine) which was significantly (P<0.05) higher than T₄ and T₅ groups and the lowest blood yield of 3.4±0.24% was recorded in T₄ group (fish meal free diet without supplementation of lysine and methionine) which was significantly (P<0.05) lower than other treatment groups except T₂ group (fish meal free diet with supplementation of lysine and methionine). However, there was no significant (P<0.05) difference between T₁, T₃ and T₅ groups and between T₄ and T₅ groups. The decrease in per cent yield of blood in fish meal free groups might be due to the deficiency of vitamin B₁₂ which is involved in the synthesis of red blood cells. The results of cut ability characteristics as depicted in Table 2 revealed no significant (P>0.05) difference in the per cent yield of various cut up parts that is breast, thighs, drumsticks, wings, back and neck among various treatment groups. These results are in agreement with the earlier results of (Aletor et al., 1989; Curto and Cicogna, 1968; Petricevic et al., 1988). Thus it is concluded that fish meal can be completely replaced with soybean meal in the diets of broiler chicken without having any adverse effect on the major carcass traits.

References

EFFECT OF AGE OF DONOR EWES ON EMBRYO PRODUCTION

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ABSTRACT

An experiment was conducted during summer in non-pregnant anoestrus old aged Malpura donor ewes at the institute to study multiple ovulation and embryo production. Twenty-four ewes were allocated to three groups of eight each according to their age. Group I ewes of 2-8 years of age served as control while the ewes belonging to Group II and Group III were of 2-4 and >7 years of age, respectively. Ewes were induced to oestrus using indigenous progesterone impregnated vaginal sponges and 200 IU PMSG. Oestrus response (onset and duration of oestrus) was observed at six hourly intervals by parading aproned ram. The ewes in oestrus were subjected to mating with rams of high sexual vigour and proven fertility. Seventy five per cent ewes belonging to Groups I and II while 62.5 per cent ewes representing Group III exhibited oestrus within 72 hours. The mean onset of oestrus interval was 28.0 ± 2.53, 33.6 ± 2.40 and 24.0 ± 00 hours for Group I, II and III, respectively. Ovarian response examined with the aid of laparoscope revealed that Group I ewes ovaries bore 0.75 ± 0.16 corpora luteae which significantly differed (P<0.05) from Group II (5.25 ± 1.51) and Group III (6.00 ± 2.58) with 0.13 ±0.13, 0.5 ± 0.19 and 0.88 ± 0.35 large follicles in the above groups, respectively. Influence of age was not evident on the overall superovulatory response. However, only 50 per cent aged ewes had more than 2 CL as compared to 62.5 per cent of prime aged ewes. The overall embryos recovery rate was 61.1 and 63.8 per cent in Group II and Group III, respectively. The yield of transferable embryos was relatively low in aged (n=14) compared to prime aged (n=19) animals. In conclusion, the ovarian response and embryo production in older ewes subjected to standard superovulation treatment was found to be as responsive as observed in the prime age ones.

Key words: Malpura sheep, prime age, intravaginal sponges, embryo.

Introduction

Sheep and goats are important species of livestock for India. They contribute greatly to the agrarian economy, especially in areas like Rajasthan where crop and dairy farming are not economical, and play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers. The productivity of Indian sheep and goats is low due to the nutritional limitations and physical environmental conditions under which they are reared. Major reasons for this low productivity are inadequate grazing resources, disease problems and serious lack of organized efforts for genetic improvement. In small ruminants, embryo transfer is useful not only for increasing the reproductive rate of selected donors (Cognie, 1999) and health control in germ plasm exchanges (Thibier and Guerin 2000), but also for the salvage of endangered native breeds (Solti et al., 2000). Superovulatory response of embryo donors is extremely variable and several factors such as season (Lopez et al., 1999), individual (Armstrong and Evans, 1983), breed and age of donor ((Alabart et al., 2003, Lopes et al., 2006) altered the superovulatory response. Information about embryo production in aged ewes of native breeds is scarce. Since surgical procedure of embryo collection in sheep restrict the embryo collection more than 2 or 3 times and therefore limit the total number of embryo harvest. This experiment was carried out to examine the superovulatory response and embryo production from aged ewes which have already produced lambs through natural mating during productive life.

Materials and Methods

The embryo transfer trial was carried on 24 anoestrous aged Malpura ewes during summer season. Selected 24 ewes were grouped in Group I, II and III of eight each according to age: Group I (Control group with ewes of 2-8 years; n=8) Group II (Ewes age 2-4 years; n=8) and Group III (ewes aged >7 years; n=8) .The experimental animals were maintained at the Institute’s sheep farm at Avikanagar which is located at longitude of 75° - 28°E, latitude of 26° - 26°N and altitude of 320 m above mean sea level in the semi-arid region of the country. The climate of this region is essentially tropical the highest temperature occurs from April to June when mean monthly temperature is about 42°C and may reach up to 49°C. The rainfall is erratic and mainly concentrated during July to August. The precipitation ranges from 400 to 700 mm per annum. These animals were fed and grazed as per routine practice at Farm.

Oestrus in embryo donor ewes was induced and synchronized using indigenous progesterone sponges developed at Central Sheep and Wool Research Institute, Avikanagar (Naqvi et al., 2001) Selected animals were inserted with these vaginal sponges using all aseptic measures with the help
of paraffin lubricated speculum and plunger deep into vagina near the opening of cervix (external Os). Sponge is kept in situ vagina for 12 days. After 12 days the sponge was taken out of vagina of experimental ewes. Pregnant mare serum gonadotrophin (PMSG Folligon, Intervet-Netherlands)) 200 IU was injected in all the ewes on the day of removal of sponges and follicle-stimulating hormone (FSH) of ovine origin (Ovagen, ICP, New Zealand). Superovulatory treatment was commenced four days prior to sponge removal. Each of the donor ewes received a total dose of 5.4 mg FSH (NIADDK-O FSH-17) twice a day (morning and evening) at a constant dose over a period of four days.

Oestrus in ewes was detected by parading aproned ram of high sexual vigour at 6 hourly intervals up to 72 hours from the day of sponge removal. Donor ewes exhibiting behavioural oestrus were mated twice a day (morning and evening) with a ram of proven fertility. Donor ewes were subjected to ovarian examination with the aid of laparoscope (Karl Storz, Germany) followed by laparotomy for embryo recovery (Naqvi et al., 2001, 2006a, b) between 3 to 4 days after mating. In brief, the animals were fasted for at least 24 h prior to laparoscopy and/or laparotomy. The abdominal area anterior to udder was shaved and sprayed with 70% alcohol and the ewes were sedated with Xylazine hydrochloride (Xylazine, Indian Immunologicals, India) and locally anaesthetized by infiltration of lignocaine hydrochloride (Xylocaine, Astra Zeneca, India). All the ewes treated for superovulation were examined by laparoscopy and found worth attempting for surgical embryo collection.

For embryo collection the reproductive tract was visualized through the laparotomy and the uterine horn was carefully exposed to at least the bifurcation of the two horns. The flushing media (20 ml Dulbecco’s phosphate buffered saline supplemented with 2% bovine serum albumin (Sigma chemical Co., St. Louis, MO, USA) was introduced into the base of the uterine horn using the blunt Jelco needle with syringe. The medium was flushed towards the tip where it was collected through a polythene catheter (OD 3 mm) introduced into the fallopian tube through fimbriae. The flushed media was collected in a graduated glass tube and the process was repeated for the other uterine horn. The recovered fluid was transferred into a sterile Petri dish for searching and examining the embryos under a stereo zoom microscope (Nikon, Japan) having warm (37°C) stage platform at 400X magnification. The fertilization of ova was verified by cleavage. The embryos were evaluated for quality under inverted microscope (Olympus, Japan) having warm (37°C) stage platform at 400X magnification. The quality of transferable embryos was assessed according to the morphological criteria based on symmetry of the cells, shrinkage, vacuolization or lysis (Robertson and Nelson 1998).

Statistical analysis of parameters concerning oestrus response, oestrus onset, ovulation rate, number of recovered structures and embryo quality were analysed using analysis of variance.

Results and Discussion

Data concerning oestrus response, oestrus onset, ovulation rate and number of recovered structures are presented in Table 1 and Table 2.
Seventy five per cent ewes belonging to Groups I and II while 62.5 per cent ewes representing Group III exhibited oestrus within 72 hours. The mean onset of oestrus interval was 28.0 ± 2.53, 33.6 ± 2.40 and 24.0 ± 0.00 hours for Group I, II and III, respectively. Ovarian response examined with the aid of laparoscope revealed that Group I ewes ovariates bore 0.75±0.16 corpora lutea which significantly differed from Group II (5.25±1.51) and Group III (6.00±2.58) with 0.13±0.13, 0.5±0.19 and 0.88±0.35 large follicles in the above groups, respectively. Influence of age was not evident on the overall superovulatory response. However, only 50 per cent aged ewes had more than 2 CL in compared to 62.5 per cent of prime aged ewes. The overall embryos recovery rate was 61.1 and 63.8 per cent in Group II and Group III, respectively. The yield of transferable embryos was relatively low in aged (n=14) compared to prime aged (n=19) animals. The results from the study demonstrated that the hormonal treatment was efficient in inducing oestrus and multiple ovulations in Malpura ewes, a good percentage of ewes showing oestrus and superovulation was observed in different ages. As far as oestrus behaviour is concerned it is in consistent with most of the previous studies, which employed similar treatments at this Institute (Naqvi and Gulyani 1996, 1999). Oestrus synchronization treatment was efficient since most of the ewes showed oestrus distributed over a short time period. Our results are in agreement with other studies that used MAP associated with superovulatory treatment in ewes (Boscos et al., 2002).

Data concerning superovulatory response showed that ewes were responsive to superovulatory response irrespective of age but there was variability among individuals. Superovulatory response in Malpura ewes treated with FSH is in consonance as reported for Bharat Merino (5.6±2.3) and Rambouillet (5.9±1.9) ewes maintained under semi arid conditions (Naqvi and Gulyani, 1996, 1999). In sheep, the influence of age on superovulatory response was shown by fact that natural and spontaneous ovulation rate is effected by age (Theriez et al., 1971); the best embryo production will be found at around 6 year of age (Torres et al., 1987). However, influence of age was not evident on overall superovulatory response in our study. Prime aged animals have an edge over aged animals in terms of presence of corpus luteum on laparoscopic examination but did not approach to significant difference (P<0.05). In the embryo transfer programme, the success rate not only depend on ovariatory response to exogenous gonadotropin, but also on the proportion of ova/ embryos recovered and fertilization rate (Naqvi et al., 1999). Thus, multiple ovulation and embryo transfer (MOET) could be a useful tool to utilise the aged animals for economic gains to farmers in terms of enhanced lamb crop. In conclusion, the ovarian response and embryo production in ewes subjected to standard superovulation treatment were considered satisfactory and it was found that older ewes are as responsive as the prime aged one.

References
RISK AND OCCURRENCE OF BOVINE MASTITIS IN TARAI REGION OF UTTARAKHAND

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ABSTRACT

The present study was conducted on 5698 cases (4133 cattle and 1565 buffaloes) presented to Veterinary Teaching Hospital from January 2002 to December 2009 for various disorders with a primary aim to assess the present epidemiological status of mastitis in bovines of tarai regions of Uttarakhand. The overall incidence of mastitis in bovine was (21.12%) with high incidence in cattle (23.05%) than buffaloes (15.97%). The overall incidence of mastitis was higher in case of cross bred (23.29%) than non-descriptive or indigenous bovines (13.64%). Age wise incidence of mastitis was higher in 4-6 years age group (59.39%) followed by 2-4 years age group (27.34%) and least in 6-8 years age group (13.30%). Analysis of odd ratio revealed that the risk of development of mastitis in cattle was 1.576 times more than buffaloes. Whereas breed wise analysis of data revealed that the cross bred cattle are 2.55 times more prone to disease than non descriptive breeds. In contrast to cattle the odd ratio was higher in pure or descriptive breeds of buffaloes (1.084) than non descriptive or cross breed buffaloes (0.922). Age wise higher odd ratio analysis suggested higher risk of mastitis in bovines of 4-6 years age group followed by 2-4 years and least in 6-8 years age group. Season wise incidence of mastitis was higher in monsoon as compared to other seasons.

Key words: Mastitis, bovines, risk, incidence and association.

Introduction

Today mastitis is pronounced to be one of the dreadful diseases which are limiting dairy development throughout the world despite the amount of knowledge available on the subject (Hogeveen, 2005). In addition to causing colossal economic losses to the farmers, the disease is also important from consumers and milk processors point of view. The milk from affected animal may harbour the organisms potentially zoonotic pathogenic and results in suboptimal output of substandard finished fermented products like yogurt, cheese, etc. on processing (Muhammad et al., 1995). The economic losses of mastitis due to morality rate are negligible but the production losses due to lowered milk quality or quantity, destruction of affected quarters, increased charges of treatment and culling processes are tremendous. In India, the economic losses due to mastitis are estimated at US dollar 526 million annually (Varshney and Naresh, 2004). Although various etiological factors are associated with mastitis but it is commonly caused by bacterial pathogens and is associated with almost every conceivable factor of management and the environment (Blood and Radostits, 1999). So the present study was planned with a primary aim to assess the present situation of mastitis and its association with age, breed and season in bovine of tarai region of Uttarakhand.

Materials and Methods

A total of 5698 cases (4133 cattle and 1565 buffaloes) presented to Veterinary Teaching Hospital from of tarai regions of Uttarakand from January to December 2009 formed the subject of study. The overall incidence was calculated using the formula given by Thrusfield (1995). Whereas the association and risk of disease in relation to species (cattle and buffalo), breed (cross breed and non descriptive or indigenous breed), age groups (2-4, 4-6 and 6-8 years) and seasons (Pre-monsoon, monsoon, summers and winters) was analyzed by calculating the odd ratio at 95% confidence interval using online programme (http://statpages.org/ctab2x2.html).

Results and Discussion

The overall incidence of mastitis in bovine was (21.12%) during the study period. The incidence of mastitis was higher in case of cattle (23.05%) than buffaloes (15.97%). Our observations are in line with Joshi and Gokhale (2006) who reported an occurrence of mastitis in India varying from 10-50% in cows and 5-20% in buffaloes. Almaw et al. (2009) also reported an overall prevalence of 25.22% in bovines. Whereas in contrast to the present study Almaw et al. (2008), Bishi (1998) and Workineh et al. (2002), reported higher prevalence of 34.30, 38.2% and 34.4%, respectively, in bovines. The difference in occurrence of mastitis may be due to

¹Teaching Personnel
mastitis in crossbreds compared to the local zebu cattle. The lower occurrence of mastitis in local non-descriptive breeds than that of cross breed bovines may be attributable to genetic factors. (Table 1 and Fig. 1)

Age wise incidence of mastitis was higher in 4-6 years age group (59.39%), than 2-4 years age group (27.34%) and least in 6-8 years age group (13.30%) as depicted in figure. 2.1. Further analysis of data revealed that the risk of mastitis is more in 4-6 years age group bovines as suggested by odd ratio in Table 2. The higher risk of mastitis in this particular age group may be due to the fact that in this age group animals are generally in 3rd or 4th lactation and the milk yield in this particular period is higher as compared to other lactations and thus increases the susceptibility of an animal for mammary gland infection. Our findings are in agreement with Lakshmi et al. (2009) and Aarestrup and Jensen (1997) who observed that age and stage of lactation are the main factors which influence the risk of mastitis in bovines.

Table 1: Risk of mastitis in cross bred and non-descriptive breeds of bovines

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Cattle</th>
<th></th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
<th>Bovines (Total)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odd ratio 95% CI</td>
<td>Per cent positive</td>
<td>Odd ratio 95% CI</td>
<td>Per cent positive</td>
<td>Odd ratio 95% CI</td>
<td>Per cent positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.B</td>
<td>2.550 2.049-3.175</td>
<td>25.94%</td>
<td>0.922 0.684-1.243</td>
<td>15.66%</td>
<td>1.923 1.616-2.287</td>
<td>23.29%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.D</td>
<td>0.392 0.312-0.448</td>
<td>12.07%</td>
<td>1.084 0.804-1.402</td>
<td>16.78%</td>
<td>0.520 0.437-0.677</td>
<td>13.64%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

95% confidence interval

Table 2: Risk of mastitis in different age groups of bovines

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>2-4 years</th>
<th>4-6 years</th>
<th>6-8 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4 years</td>
<td>0.256 (0.216-0.304)</td>
<td>2.437 (1.727-2.429)</td>
<td></td>
</tr>
<tr>
<td>4-6 years</td>
<td>3.905 (3.291-4.034)</td>
<td>-</td>
<td>9.518 (3.865-5.167)</td>
</tr>
<tr>
<td>6-8 years</td>
<td>0.410 (0.333-0.506)</td>
<td>0.105 (0.086-0.129)</td>
<td>-</td>
</tr>
</tbody>
</table>

In parenthesis: confidence interval at 95%

Table 3: Risk of mastitis in different seasons

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Pre-monsoon</th>
<th>Monsoon</th>
<th>Summers</th>
<th>Winters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-monsoon</td>
<td>-</td>
<td>0.534 (0.446-0.639)</td>
<td>0.640 (0.531-0.772)</td>
<td>1.117 (0.911-1.369)</td>
</tr>
<tr>
<td>Monsoon</td>
<td>1.873 (1.564-2.244)</td>
<td>-</td>
<td>1.199 (1.016-1.415)</td>
<td>2.092 (1.741-2.515)</td>
</tr>
<tr>
<td>Summers</td>
<td>1.563 (1.296-1.883)</td>
<td>0.834 (0.707-0.984)</td>
<td>-</td>
<td>1.745 (1.442-2.112)</td>
</tr>
<tr>
<td>Winters</td>
<td>0.895 (0.730-1.098)</td>
<td>0.478 (0.398-0.574)</td>
<td>0.573 (0.473-0.694)</td>
<td>-</td>
</tr>
</tbody>
</table>

In parenthesis: confidence interval at 95%
Fig. 1: Overall occurrence of mastitis in bovines of tarai region of Uttarakhand

Fig. 2: Age wise risk of mastitis in bovines
Season wise analysis of data revealed higher incidence of mastitis in monsoon (27.44%) followed by summers (23.98%), pre-monsoon (16.80%) and least in winters (15.31%) as depicted in figure 3.1. Joshi and Gokhale (2006) also observed higher prevalence of mastitis in monsoon than summers and winters. Further analysis of data revealed risk of occurrence of mastitis is higher in monsoon as compared to other seasons as suggested by the odd ratio in Table 3.

Year wise analysis of data revealed higher incidence of mastitis in the year 2004 (23.87%), followed by 2003 (23.66%), 2009 (22.05%), 2008 (21.50%), 2006 (21.29%), 2002 (20.99%), 2007 (18.70%) and least in the year 2005 (17.06%).

It can be concluded that the overall incidence of mastitis is relatively higher in bovines of tarai region of Uttarakhand which can pose severe threat to the growing dairy industry of the state. Therefore, different epidemiological factors that interplay in mastitis occurrence should be studied to reduce its occurrence.

References
PREVALENCE AND CLINICAL OBSERVATIONS OF MANGE IN DOGS

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ABSTRACT

The overall prevalence of mange and its types (demodectic and sarcoptic mange) was calculated including their month, age and sex wise prevalence. The prevalence of mange was 15.71% and that of demodectic and sarcoptic mange was 8.72% and 6.99%, respectively. Maximum prevalence was observed in month of December (48.64%) and minimum in June (2.70%). Among the two types of mange, maximum prevalence of demodectic mange was observed in month of December (43.24%) and nil in April and June. Highest prevalence of sarcoptic mange was observed in month of August (28.20%) and nil in month of March and May. While demodectic mange was observed to be more prevalent in dogs below six months of age (45.71%), sarcoptic mange was higher in dogs above one year of age (53.57%). Sex wise, sarcoptic mange was observed more in males (64.28%) whereas demodectic mange was more prevalent in females (65.71%). On clinical evaluation alopecia, erythema, pruritus and crust formation were observed in demodectic mange while as in sarcoptic mange apart from alopecia and erythema, scaling was a consistent finding. Hyper pigmentation was characteristic in chronic cases of demodectic mange. The mean value of skin fold thickness was significantly increased in demodectic (6.1±0.05 mm) as well as in sarcoptic mange (7.1±0.12 mm) as compared to control group (4.52±0.01 mm). On histopathological examination epidermal hyperplasia, folliculitis, perifolliculitis and distension of hair follicles were observed in demodectic mange while as in sarcoptic mange fibrous tissue proliferation and eosinophilic infiltration were observed. Cutaneous cytology revealed Gram positive cocci in both types of mange (demodectic and sarcoptic) were exudative skin lesions were present.

Key words: Prevalence, mange, alopecia, erythema.

Introduction

Throughout the centuries pets have been kept for companionship, security, working and hunting. Many dog owners live in very close contact with their canine companions and it is common for dog to nuzzle and lick their owners. Given the high frequency of very close contact with their owner’s dogs have the potential to play a significant role in disease transmission and public health. A large number of diseases have been reported of pet-related zoonotic importance (Stehr-Green and Schantz, 1987). Among such diseases skin affections of dogs are a source of infection to humans.

Skin is the most sensitive part of dog’s body and has tremendous aesthetic value. In small animal practice, a majority of ailments are constituted by dermatological abnormalities as the concurrent or chief owner’s complaint (Nesbitt, 1983; Scott and Paradis, 1990 and Feizo et al., 1998). In dogs mange caused by Demodex canis (demodectic/}

# Corresponding author: Part of M.V.Sc. Thesis.

red mange) and Sarcoptic Scabiei var canis (Sarcoptic mange/scabies) have serious health implications besides zoonotic and aesthetic importance. The primary effects of mange in dogs are severe pruritis, alopecia, scaling, hyper pigmentation and thickening of skin. Complications are usually associated with traumatic dermatitis and secondary bacterial infections. Present study was undertaken to investigate the prevalence and clinical assessment of mange in dogs.

Materials and Methods

A total of 401 dogs bought to Veterinary Clinical Complex, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya Palampur were undertaken for the study from March, 2009 to February, 2010. These dogs were screened for mange. The overall prevalence of mange and its types (demodectic and sarcoptic) was calculated including their month, age and sex wise prevalence.
The entire skin and hair coat of all the dogs was examined thoroughly in day light for type and location of skin lesions, if any. The skin fold thickness was recorded with the help of a vernier calliper. For histopathological examination a 1 sq cm area of skin was taken from margin of lesion with the help of scalpel and kept in 10% formalin. The impression smears were taken from animals with exudative skin lesions for studying cutaneous cytology. Further 10 apparently healthy dogs with no skin lesions were chosen to serve as control.

Results and Discussion

Out of total 401 dogs 63 were found to be positive for mange (35 for demodectic mange and 28 for sarcoptic mange), indicating 15.71% prevalence of mange in dog population of Palampur and nearby villages of (H.P). Maximum prevalence was observed in month of December (48.64%) and minimum in June (2.70%). Among the two types of mange, prevalence of demodectic mange was 8.72% while that of sarcoptic mange was 6.99%. Maximum prevalence of demodectic mange was observed in month of December (43.24%) and nil in April and June. Similar findings were reported by Gupta (1999). Highest prevalence of sarcoptic mange was observed in month of August (28.20%) and nil in month of March and May. In August the maximum prevalence could be because of the hot and humid weather that favours the growth of mites (Gupta, 1999).

<table>
<thead>
<tr>
<th>Type of Mange</th>
<th>No. of dogs positive</th>
<th>Per cent prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demodectic mange</td>
<td>35</td>
<td>8.72</td>
</tr>
<tr>
<td>Sarcoptic mange</td>
<td>28</td>
<td>6.99</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>15.71</td>
</tr>
</tbody>
</table>

Table 1: Prevalence of demodectic and sarcoptic mange

While demodectic mange was observed to be more prevalent in dogs below six months of age (45.71%), sarcoptic mange was higher in dogs above one year of age (53.57%). Sex wise, sarcoptic mange was observed more in males (64.28%) whereas demodectic mange was more prevalent in females (65.71%) (Table 1 and 3). This is in concordance with earlier observations (Gupta and Prasad, 2001; Kumar et al., 2006).

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Demodectic mange</th>
<th>Sarcoptic mange</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6</td>
<td>16 (45.71)</td>
<td>2 (7.14)</td>
</tr>
<tr>
<td>7-12</td>
<td>12 (34.28)</td>
<td>11 (39.28)</td>
</tr>
<tr>
<td>&gt; 12</td>
<td>7 (20)</td>
<td>15 (53.57)</td>
</tr>
</tbody>
</table>

Table 2: Age wise prevalence of mange

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>Demodectic mange</th>
<th>Sarcoptic mange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>30</td>
<td>12 (34.28)</td>
<td>18 (64.28)</td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>23 (65.71)</td>
<td>10 (35.71)</td>
</tr>
</tbody>
</table>

Table 3: Sex wise prevalence of mange

*Figure is out of 401

Figures in parentheses are per cent of cases suffering from mange.

* Figure is out of 35  **Figure is out of 28
Clinically alopecia, erythema, pruritus and crust formation were observed in demodectic mange while as in sarcoptic mange apart from alopecia and erythema, scaling was consistent finding. Hyperpigmentation was characteristic in chronic cases of demodectic mange. In demodectic mange lesions were observed on face (77.14%), peri orbital region (71.42%) and head (57.14%) while as in sarcoptic mange ears (89.28%) and tails (85.7%) were mostly affected. Similar observations were reported by Gupta (1999).

The mean value of skin fold thickness was significantly increased in demodectic (6.1±0.05 mm) as well as in sarcoptic mange (7.1±0.12 mm) as compared to control group (4.5±0.01 mm) (Table 4). This increase in the value of skin fold thickness might be due to inflammation and hyperkeratosis as well as proliferation of prickle cell layer as also mentioned by Muller et al. (1989) and Baker and Thomsett (1990).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Demodectic mange</th>
<th>Sarcoptic mange</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skinfold thickness (mm)</td>
<td>6.1± 0.05 a</td>
<td>7.1± 0.12 b</td>
<td>4.52 ± 0.01c</td>
</tr>
</tbody>
</table>

Values with different superscripts between columns differ significantly (P<0.05)

Histopathological examination revealed epidermal hyperplasia, folliculitis, perifolliculitis and distension of hair follicles in demodectic mange while as in sarcoptic mange fibrous tissue proliferation and eosinophilic infiltration were observed. This reaction was in accordance with the observations of Gupta (1999) and Aujla (2000). Cutaneous cytology revealed Gram positive cocci in both types of mange (demodectic and sarcoptic) were exudative skin lesions were present.

References

Helminthic infection causes great economic losses to dairy industry by way of retarded growth, low productivity and increased susceptibility of animals to other diseases. In spite of significant production losses, which may run into millions of rupees (Jithendran and Bhat, 1999), the problem is persisting because of chronic and insidious nature. The hot and humid tropical climate is very favourable for the development and survival of pre-parasitic stages particularly nematodes and it is likely that throughout the year infective larvae are available on pasture for grazing animals. To combat these infections, there is a need for regional surveys of gastrointestinal parasites of dairy animals confined to a particular locality. Incidence of parasites in cattle and buffalo has been reported from different parts of India (Singh et al., 2005; Gupta et al., 2008). The information so gathered can be utilized for devising effective control measures against the parasitic infections to improve the productivity of our livestock. The present work was designed to gather information in respect of gastrointestinal parasites in cattle and buffaloes of LSF, Adhartal, Jabalpur.

Materials and Methods

Total of 163 faecal samples of cattle (91) and buffalo (72) were included in the study. Samples were collected either directly from the rectum or when freshly passed and subjected to coprological examination by standard qualitative and quantitative techniques. (Sloss et al., 1994).

Results and Discussion

Results of the studies are presented in Table 1 and 2. Out of 163 animals, 113 were found positive

### Table 1: Incidence of helminth infection in cattle and buffaloes

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Animal</th>
<th>No. of Samples</th>
<th>Total infected</th>
<th>S</th>
<th>C</th>
<th>T</th>
<th>M</th>
<th>A</th>
<th>F</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cattle</td>
<td>91</td>
<td>61 (67.03)</td>
<td>15</td>
<td>4</td>
<td>--</td>
<td>--</td>
<td>12</td>
<td>2</td>
<td>28 (30.77)</td>
</tr>
<tr>
<td>2</td>
<td>Buffalo</td>
<td>72</td>
<td>52 (72.2)</td>
<td>3</td>
<td>12</td>
<td>1 (1.39)</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>14 (19.44)</td>
</tr>
<tr>
<td>3</td>
<td>Total</td>
<td>163</td>
<td>113 (69.32)</td>
<td>18</td>
<td>16</td>
<td>1 (0.61)</td>
<td>1</td>
<td>32</td>
<td>3</td>
<td>42 (25.77)</td>
</tr>
</tbody>
</table>

Per cent is given in parenthesis

S: Strongyle, T: Trichus, M: Moniezia, C: Coccidia, A: Anthelmintic, F: Fasciola

### Table 2: Incidence of mixed helminth infection in cattle and buffaloes

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Animals</th>
<th>S/C</th>
<th>S/C/A</th>
<th>S/A</th>
<th>C/A</th>
<th>A/F</th>
<th>S/A/F</th>
<th>S/C/F/A</th>
<th>C/A/F</th>
<th>S/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cattle</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Buffalo</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Per cent is given in parenthesis

S: Strongyle, T: Trichus, M: Moniezia, C: Coccidia, A: Anthelmintic, F: Fasciola
for single or mixed infection. Among various infections maximum incidence was of amphistomes (19.63%) followed by strongyle (11.04%) and coccidia (9.82%). Other helminthic infections observed were Fasciola gigantica (1.84%), Trichuris sp. (0.61%) and Moniezia sp. (0.61%). As many as 25.77% samples were found positive for mixed infection (Table 2). In cattle, 30.77% mixed infection were reported whereas 19.44% mixed infection was observed in buffaloes. It was also observed that out of 30.77% mixed infections in cattle, highest infection was of strongyle and coccidia (28.57%) followed by strongyle, coccidia and amphistomes (25.0%).

Strongyle along with amphistome also showed a considerable infection (21.42%) in cattle. Buffaloes showed an infection of 46.15% of amphistomes along with coccidia and Fasciola, respectively. Higher rate of Fasciola and amphistome mixed infection in buffaloes was similar to the findings of Gupta et al. (2008). It was reported that most of the animals examined during the present study had low to moderate strongyle eggs and coccidia oocyst counts, suggesting that the infections were usually sub-clinical. In contrast, trematodes egg counts were relatively high. The amphistome infection in the present study was higher in case of buffaloes (27.78%) as compared to cattle (13.91%). It is similar to the findings of Gupta et al. (2008) and Garg et al. (2009). It was also observed that amphistome infection was higher than Fasciola in both cattle and buffaloes which was also reported by Singh et al. (2009). Keyyu et al. (2005) also reported that the proportion of animals shedding the amphistome egg was always higher than the animals shedding Fasciola eggs in all zones, management systems, farms and age groups.

Acknowledgements

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References

INFLUENCE OF SEASON AND AGE ON DNA INTEGRITY OF BARBARI BUCK SPERMATOZOA

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ABSTRACT

The structural architecture and intactness of the sperm DNA regulates the outcome of fertilization followed by the process of embryonic development. The integrity of sperm DNA is affected by biological, physical, and environmental factors. The present investigation was designed to elucidate the influence of different seasons and age of the animal on the intactness of the sperm DNA in bucks. The semen was collected round the year and the period was divided into three seasons: S1 (July to October), S2 (November to February) and S3 (March to June). 18 adult bucks were selected and were divided into three different age groups: G1 (7 months to less than 2 years of age), G2 (2 years to less than 4 years of age) and G3 (4 years to 7.5 years of age). 18 ejaculates were collected from each buck in each season and DNA integrity was assessed by comet assay with slight modifications. The results of present study revealed significant (P<0.01) effect of age and season on DNA integrity of the buck spermatozoa. The comet positive spermatozoa were observed highest in S3 season as compared to S2 and S1 season where as in age groups, G3 group exhibited highest DNA damage than G2 and G1 groups. It can be concluded from the present study that summer season has most deleterious effect on sperm DNA as compared to other season while DNA integrity of spermatozoa reduces with increase in age of Barbari bucks.

Key words: Age, season, sperm DNA, comet assay

Introduction

Sperm DNA integrity is an important criterion for the success of natural or assisted fertilization including normal development of the embryo, foetus, transmission of genetic information and therefore, the maintenance of good health in future generations (Watson, 2000). However, a variety of factors, including season, storage temperature and storage time have been reported to affect the quality of extended buck semen (Barkawi et al., 2006) but effect of these factors on sperm DNA integrity has not been adequately assessed in bucks. Goat semen cryopreservation still yet not standardized like that of cattle. From the very ancient time, the goat is considered as a seasonal breeder and hence extensive studies have been conducted for the assessment of the semen quality in various seasons in different breeds of goats in different geographical areas of the world (Chemineau and Xande, 1982; Restall, 1991; Barkawi et al., 2006). Seasonality in goat reproduction and semen quality are extensively studied and established that with the rise in the photoperiod, the quality of semen increases (Talebi et al., 2009; Srinivas et al., 2002). Studies in the buffalo have also shown the reduction in the concentration of spermatozoa as well as a steady rise in the number of dead spermatozoa with the enhancement of age (Nandre et al., 2007). With ageing the degree of DNA damage gradually increases in the sperms which instead increase the percentage of the dead sperms leading to a poor outcome to fertility in the aged man (Singh et al., 2003). The higher the proportion of DNA damage, the higher will be the possibility of infertility (Shen et al., 1999). The comet assay is a sensitive assay to elucidate the integrity of DNA in terms of intactness or fragmentation as degree of DNA damage which is directly correlated with the sperm survival (Singh et al., 2003).

Scanty literature is available regarding the effect of seasons and age of animal on the DNA intactness of the sperms in Indian breeds of goats. Therefore, the present investigation was designed to elucidate the influence of different seasons and age of the animal on the intactness of the sperm DNA in Barbari bucks.

Material and Methods

The present investigation was carried out in the Hi-Tech laboratory of Department of Physiology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh which (Mathura) is located in semi-arid zone of northern India at 27° N latitude and 78° E longitudes, 176m above the sea level. Eighteen clinically healthy Barbari bucks were selected from the goat farm of the college and divided into three age groups viz. G1 (7 months to less than 2 years of age), G2 (2 years to less than 4 years of age) and G3 (4 years to 7.5 years of age) containing six animals in each group. The duration of the study was 12 months which was divided into three seasons, S1 (July to October), S2 (November to February) and S3 (March to June). Semen was collected using artificial vagina (length=20cm and diameter=4.5cm) in morning hours and immediately after

1Department of Biochemistry
Table 1: Effects of age and season on DNA damaged per cent spermatozoa (Mean±SE) of Barbari buck neat semen.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SEASON</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
</tr>
<tr>
<td>G1</td>
<td>11.66±0.37</td>
<td>10.63±0.38</td>
<td>15.55±1.25</td>
</tr>
<tr>
<td>G2</td>
<td>8.23±0.23</td>
<td>8.62±0.26</td>
<td>9.22±0.26</td>
</tr>
<tr>
<td>G3</td>
<td>31.38±0.53</td>
<td>30.53±0.64</td>
<td>31.24±0.74</td>
</tr>
</tbody>
</table>

Significant level = 1% (P<0.01)

a, b, c = Superscript shows the significant difference between season (S1, S2, S3),
A, B, C = superscript shows the significant difference between age groups (G1, G2, G3),
NS = Non Significant.

collection transported to the laboratory for evaluation of semen characteristics. Eighteen ejaculates were collected from each buck in each age group in each season. This was followed by pooling of the semen samples within the same age group.

Protocol for comet assay

The single cell gel electrophoresis (Comet assay) was performed according to the method of Singh et al., (2003) with minor modifications. The comet assay was performed in neat semen in three seasons and in the three different age groups of the bucks. In brief, 10 µl of sperm suspension presenting more than 85% live cells was mixed with 125 µl of low melting point agarose (0.75%) and applied as a coat on normal agarose precoated frosted slides. After setting of gel, normal melting point agarose (1%) was applied as third coat. Slides were immersed in a pre-warmed (37°C) lysing solution-I (1.25 M NaCl, 50 mM EDTA, 100 mM Tris base, 2 mg/ml reduced glutathione and 0.05 mg/ml DNAse free proteinase K, pH 10.0) for 3 hours at 37 °C followed by lysis with lysing solution-II (1.25 M NaCl, 50 mM EDTA, 100 mM Tris base, 1% α- mercaptoethanol, 1% Triton X-100, pH 10.0) for 5 hours at 4°C. After lysis the slides were kept 1 hour in chilled electrode buffer (500mM NaCl, 0.1M Tris-Base, 1mM EDTA, 0.2% DMSO, pH 9.0) for chromatin decondensation followed by electrophoresis at 24 V for 1
hour. The slides were treated with neutralization buffer (20 mM Tris, pH 7.4) and dried in incubator at 37°C.

The slides were then incubated in PBS buffer containing ethidium bromide (0.01%) for 10 minutes. After wiping the stain, the slides were observed under upright microscope (Nikon) at 400x magnification and images were captured using the integrated camera. Three slides were prepared from each age groups of buck and at least 100 comets were observed.

The obtained results were analyzed by using the SPSS software version 14 and the means were compared by computing ANOVA and DMRT on the DNA integrity of the sperms.

Results and Discussion

The mean comet per cent of buck spermatozoa of different groups has been presented in Table 1. The results of the present study revealed a significant effect (P<0.01) of season and age of bucks on DNA integrity. The highest numbers of comets were observed in summer season (S1) as compared to the other seasons (S2 and S3) of the year in G1 and G2 age groups. The effect of season was reported to be non-significant in age group G3. These results obtained in the present study were corroborated with that of Nandre et al. (2007) in buffaloes. They also reported significantly high percentage of DNA damage (comet) in summer season as compared to the other seasons (S2 and S3) of the year in G1 and G2 age groups. The effect of season was reported as non-significant in age group G3. These results obtained in the present study were corroborated with that of Nandre et al. (2007) in buffaloes. They also reported significantly high percentage of DNA damage (comet) in summer season as compared to winter season. This rise in comets in summer season may be due to increase in ambient temperature that might be responsible for causing heat stress and production of reactive oxygen species (ROS). This increase in ROS may result into rise in DNA damage (comet assay). The study reported a negative correlation between ageing and percentage of apoptotic sperm. The apoptosis resulted into increase in comet percentage and hence reduces fertility in aged human subjects (Singh et al., 2003). Trisini et al. (2004) studied relationship between age and deoxyribonucleic acid damage in human and observed statistically significant increase in the number of cells with high DNA damage as compared with younger men.

It can be concluded from the present study that both season and age significantly affected the sperm DNA integrity in neat semen of Barbari bucks. The rise in DNA damage in aged animals and in summer season may be due to high level of oxidative damage of the DNA which is due to ageing and higher temperature of summer season. Further studies are required to evaluate the probable causes behind these findings.

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References

MOLLUSCICIDAL EFFECTS OF ACETONE EXTRACT OF AZADIRACHTA INDICA (NEEM) ON SNAILS LYMNAEA AURICULARIA AND INDOPLANORBIS EXUSTUS#

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ABSTRACT

The molluscicidal effect of acetone extract of different components of Neem (Azadirachta indica) plant (leaf, seed, bark and whole plant) was evaluated against the laboratory reared snails Lymnaea auricularia and Indoplanorbis exustus. The extracts were prepared by cold extraction method by standard protocols. In acetone extract of Neem leaf 100 per cent mortality was recorded in 1:10 within 72 h against L. auricularia and I. exustus but the higher dilution caused higher mortality in I. exustus as compared to L. auricularia. Control group also show variable percentage of mortality but it never exceeded 46.67 per cent. Compared to leaf, the acetone extract of Neem seed showed higher percentage of mortality and 100 per cent mortality was recorded within 48 h in 1:10 and 1:20 in both snails. In whole plant extract of Neem seed 100 per cent mortality was recorded within 72 h in 1:10 against L. auricularia and similar effect was shown within 48 h against I. exustus. Cent percentage mortality was recorded up to 1:15 concentration in 96 h in case of L. auricularia and up to 1:20 concentration is case of I. exustus, respectively. The acetone extract of Neem bark showed variable results and highest mortality recorded in 1:10 concentration was 86.67 per cent within 96 h against L. auricularia and in I. exustus 100 per cent mortality was recorded within 96 hours.

Key words: Aceton, Azadirachta indica, L. exustus, L. auricularia extract.

Introduction

There are numerous methods being employed for the control of snails for controlling the snail borne diseases. The most important of all methods is chemical method. Increased use of chemicals result in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Baskaran et al., 1989; Kalavathy et al., 2001). The aquatic flora and fauna are affected by the toxic substances which eventually enter into their systems or bring about external damages (Pant and Singh, 1983; Hodson, 1988; Johl and Dua, 1995). Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stress (Areechon and Plump, 1990). Recent emphasis is on the use of natural pesticides, which are usually of plant origin. Azadirachtin derived from Neem (Azadirachta indica) is a very effective and extensively used pesticide. Thus the present study was conducted to evaluate the molluscicidal effects of acetone extract of Azadirachta indica (Neem) on snails Lymnaea auricularia and Indoplanorbis exustus.

Materials and Methods

Set-up of aquaria

Biologically balanced aquaria were set up in the Malacology laboratory of the Department of Veterinary Parasitology, GADVASU, Ludhiana. Rectangular aquaria (50×25×25) cm, rectangular jars (24×11×25) cm and round jars (25×20) cm were filled up to 5 cm depth with mud collected from dry ponds after sterilization in hot air oven at 180 °C. Tap water was added in the aquaria leaving 5 cm of the top empty. Unicellular algae and copepods (Daphnia and Cyclops) were added to check protozoan and bacterial growth. Hydrilla verticillata was planted in the aquaria for the aeration of water. Piece of chalk was added in the aquarium for providing calcium for the growth of snails. Water in aquaria was changed twice in a week in the winter and thrice in a week in the summer.

Collection of snails

Fresh water snails Lymnaea auricularia and Indoplanorbis exustus were collected from different ponds, ditches, paddy fields, banks of river Sutlej and villages in and around district Ludhiana.

Collection of plants

Leaf, bark and fruit of Neem (Azadirachta indica) were collected locally from natural habitat and identified by Department of Botany, Punjab Agricultural University, Ludhiana. They were sun dried and later kept in hot air oven at 45°C for 45 min followed by pulverization in an electric grinder to form powder. Fruits were broken to extract seeds which were later on dried and pulverized to form fine powder and kept in refrigerator till further use.

Preparation of extracts

Ten grammes of the fine powder of each component was taken in conical flask and added 50 ml of acetone and

#Part of M.V.Sc. Thesis of the first author submitted to GADVASU, Ludhiana and Corresponding author. Email: manzer07@gmail.com
kept in refrigerator for maceration for 48 h with intermittent shaking and then filtered through muslin cloth. The filtrate (acetone) obtained were filtered through Whatman No. 1 filter paper and were used as stock.

**Preparation of testing solutions**

Six different concentrations of the acetone extracts of leaf, bark, seed and whole plant were prepared as 1:10, 1:15, 1:120, 1:25, 1:30 and 1:35 in distilled water. Adult snails (L. auricularia and I. exustus) were tested in each test and control solutions side by side.

**Treatment of snail**

Forty five live snails were divided in three batches of 15 each and subjected to each concentration of acetone extracts of leaves, seeds, bark and whole plant of Neem along with control. The effect of the different concentration of the extracts on the snail was studied at exposure time of 6, 12, 24, 48, 72 and 96 h at 25°C.

**Statistical analysis**

The data was analyzed by CRD using minitab software and Mean, SE and CD were calculated.

**Results and Discussion**

**Acetone Extracts**

**Effect of acetone extracts of A. indica (Neem) leaves on snails**

Among the different concentrations cent per cent mortality was recorded in 1:10 within 72 h and in 1:15 within 96 h in L. auricularia. The other dilutions recorded variable mortality with none reaching 100 per cent mortality. In control groups the mortality recorded was far less than that of test group with maximum mortality of 44.44 per cent shown by 1:10 h after 96 h. The mortality is control group may be due to toxic effect of the solvent itself while in test group it may be attributed to active component of extract. I. exustus showed higher mortality than L. auricularia. In I. exustus 100 per cent mortality was recorded in 1:10 within 72 h and in 1:15 and 1:20 concentrations, within 96 h (Table 1). The effect was both time and concentration dependant. Jaiswal and Singh (2008) reported the similar molluscidical effects of acetone extract of Carica papaya and Areca catechu. The time dependant toxic effect of Neem may due to active moiety of Neem extract which increase the active component in the snail body with the increase in exposure of time.

**Effect of acetone extracts of A. indica (Neem) bark on snails**

Higher concentration of the acetone bark caused higher mortality with none of them reaching 100 per cent mortality in L. auricularia. However, in I. exustus 1:10 concentration showed 100 per cent mortality after 96 h. The mortality in other dilutions showed variations with none reaching cent per cent (Table 3). Jaiswal and Singh (2008) reported the similar results while evaluating molluscidical effect of acetone extract of Carica papaya and Areca catechu. The time dependant toxic effect of Neem may due to active moiety of Neem extract which increase the active component in the snail body with the increase in exposure of time.

**Effect of acetone extracts of A. indica (Neem) whole plant on snails**

The different concentrations of the acetone extracts of whole plant showed variable effects on the adult stages of L. auricularia. Cent per cent mortality was evident up to maximum dilution of 1:15 with in 96 h and on increasing the dilution to 1:35 the mortality percentage decreased with the increase in the dilution but increased with the increase in time period (Table 4).

Similar results of acetone extracts of Neem were observed against I. exustus. Cent per cent mortality was recorded in 1:10 concentration within while 72 h and 96 h were required for 100 per cent mortality in 1:15 and 1:20 concentrations, respectively. Jaiswal and Singh (2008) reported the similar molluscical effects of acetone extract of Carica papaya and Areca catechu. The time dependant toxic effect of Neem may due to active moiety of Neem extract which increase the active component in the snail body with the increase in exposure of time.

**Acknowledgements**

Authors are thankful to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for providing facility to carry out the research work.

**References**

### Table 1: Effect of acetone extracts of *A. indica* (*Neem*) leaves on snails

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### Table 2: Effect of acetone extracts of *A. indica* (*Neem*) seed on snails

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<th>Exposure time (h)</th>
<th>% Mortality (Mean ± SE) at different concentrations, Total No. of snails (N=45)</th>
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T: Test; C: Control
Table 3: Effect of acetone extracts of *A. indica* (*Neem*) bark on snails

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Table 1: Effect of acetone extracts of *A. indica* (*Neem*) whole plant on snails

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<td>% Mortality (Mean ± SE) at different concentration. Total No. of snails (N=45)</td>
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T: Test C: Control

CD_{95%}(05) = 8.55, 7.23, 8.55, 6.99, 6.47, 6.47

CD_{95%}(05) = 8.15, 6.72, 8.96, 6.20, 7.23, 8.77

T: Test C: Control
EFFICACY OF LEVOFLOXACIN-ORNIDAZOLE AND α-TOCOPHEROL COMBINATION IN THE TREATMENT AS WELL AS PREVENTION OF POST-PARTUM AFFECTIONS IN BUFFALOES (BUBALUS BUBALIS)

N. M. Markandeya, V. D. Muley, S.U. Digraskar and J. Bhattacharyya1

Department of Veterinary Gynaecology and Obstetrics
College of Veterinary and Animal Sciences, MAFSU, Parbhani-431401

ABSTRACT

Thirty six (36) buffaloes referred to the Department of Veterinary Gynaecology and Obstetrics with complaint of postpartum affections were utilized and divided into three main groups (Group A, B and C) and each further into two subgroups (Group A1, B1, B2 and C1, C2) to evaluate efficacy of intrauterine Levofloxacin-Ornidazole with and without α-tocopherol combination therapy. The group A (n=12) comprised of Clinical cases of most common postpartum infections as retention of placenta (ROP)/metritis, while group B (n=12) composed of buffaloes with similar managerial practices and due for parturition, whereas the clinical cases of Infectious repeat breeding ailment formed the group C (n=12) of the present therapeutic trial. The affected buffaloes of each sub-treatment groups (n=6) were subjected to intrauterine Levofloxacin-Ornidazole (Groups A1, B1, and C1) and Levofloxacin-Ornidazole plus α-tocopherol (Wokadine™-IUXp)* (Groups A2, B2, and C2) antioxidant therapy for comparative evaluation. The buffaloes of group A were treated for 5 day of post-partum, group B for 2 days after calving, while that of group C on the day of oestrus. All the treated buffaloes were assessed for clearance of uterine infection, period required for uterine involution and overall clinical and reproductive recovery. The present trial, therefore, indicated that intrauterine therapy of Levofloxacin-Ornidazole was effective in treating postpartum affections in buffaloes. However, synergism of Levofloxacin-Ornidazole and α-tocopherol (Wokadine™-IUXp)* showed superior efficacy in the treatment as well as prevention of postpartum affections and had favorable effect on the productivity in buffaloes.

Key words: Postpartum infections, buffaloes, intrauterine preparation.

Introduction

Postpartum uterine infections result from uterine contamination with bacteria during parturition. Uterine infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium and release of bacterial toxins that lead to establishment of uterine disease (Azawi, 2010). Post partum fertility has a profound impact on the economic viability of dairy farming. Post-calving period is the most crucial transitory phase in bovine life where various physiological, gynaecological, biochemical and immunological changes are occurring. During this phase, cattle are exposed to increased risk of infection of uterus, as during parturition the anatomical barriers are broached and remain open for several days (Goff and Horst, 1997). Infection of the uterus is largely influenced by the balance between bacterial contamination, the local and systemic immune status during pregnancy and parturition. Infectious diseases are more prevalent during this period because of an impaired immune status before and immediately after parturition (LeBlanc et al., 2002).

As reproductive performance is critical for viable dairy unit, uterine health achieves greatest importance in the post-partum period and essentially needs suitable therapeutic management and veterinary attention. Bovine uterine disorders remain a complex entity and its diagnosis and treatment is an increasing veterinary challenge. There is thus a greater need for farmers, researchers and veterinarians to adapt new medicinal strategies with aim of reducing the incidence of post-partum affections contributing greatly in declining fertility in dairy herds. The present study was undertaken to evaluate therapeutic efficacy of intrauterine Levofloxacin-Ornidizole in comparison with intrauterine Levofloxacin-Ornidizole+ α-tocopherol combination in treatment and prevention of post-partum affections in buffaloes.

Materials and Methods

Thirty six (36) buffaloes, referred to the Department of Veterinary Gynaecology and Obstetrics with complaint of postpartum affections were utilized and divided into three main groups (Group A, B and C) and each further into two subgroups (Group A1, A2, B1, B2, and C1, C2) to evaluate efficacy of intrauterine Levofloxacin-Ornidazole with (Group A1, A2) and without (Group B1, B2) antioxidant combination therapy. The group A (n=12) comprised of clinical cases of most common postpartum infections as retention of placenta (ROP)/metritis, while group B (n=12) composed of buffaloes with similar managerial practices and due for parturition, whereas the clinical cases of Infectious repeat breeding ailment formed the group C (n=12) of the present therapeutic trial. The affected buffaloes of each sub-treatment groups (n=6) were subjected to intrauterine Levofloxacin-Ornidazole (Groups A1, B1, and C1) and Levofloxacin-
The time required for placental expulsion was 4.66±0.24 hours in A. The period required for placental expulsion was also took place within normal period. The period required in A group for expulsion of placenta and for uterine involution was 6.33±0.40 hours and 32.66±0.88 days respectively. However, the response of Levofloxacin-Ornidazoloe and α-tocopherol (A2) was superior in treating post partum uterine infections as compared to Levofloxacin-Ornidazole intrauterine chemotherapy. The time required for placental expulsion was 4.66±0.24 hours in A2 group as compared to 6.33±0.40 hours in A1 group. The period required for uterine involution was 26.00±0.36 days in A1 group as against 32.66±0.88 days in A1 group. The use of selenium and Vit. E combination is very common in veterinary clinical practice under advanced animal management system but use of antibiotic and antioxidant is a recent approach. The efficacy of α-tocoferol was recorded in terms of slight increment in milk yield as compared to non-treated animals.

The use of Levofloxacin-Ornidazoloe with and without α-tocopherol combination as a preventive strategy in avoiding post partum infections and enhancing reproductive efficiency was evaluated in buffaloes of B and C groups. Levofloxacin-Ornidazole 60 ml intrauterine therapy (B2) for 2 days postpartum was helpful in causing placental expulsion in 5.58±0.23 hours and uterine involution 28.50±0.42 days. The performance of Levofloxacin-Ornidazole and α-tocopherol combination therapy (B2) was also more efficacious as a preventive strategy as placental expulsion and uterine involution were observed in 4.57±0.22 hrs over 5.58±0.23 hours in B2 treated group. The period required for uterine involution B2 was 27.00±0.36 days over 28.50±0.42 days in B1 treated groups, respectively.

Infectious endometritis in buffaloes also indicated better response to Levofloxacin-Ornidazole and antioxidant combination therapy (C2) as 4 out of 6 (66.66%) buffaloes conceived after single intrauterine therapy on the day of oestrus. In the present trial, however, Levofloxacin-Ornidazole intrauterine therapy (C2) could result in conception of 3 out of 6 (50%) buffaloes after single intrauterine therapy on the day of oestrus. However, non responded endometritic buffaloes of both C1 and C2 group after therapy showed clear mucus discharge.

Use of α-tocopherol through intrauterine route along with new generation fluoroquinolones is a recent concept in veterinary practice. For avoiding drug resistance combination therapies against gram+ve, gram-ve, aerobic and anaerobic infections with supportive drug for endometrial mucosa is considered as recent effective line of treatment. Metronidazole is a most commonly used intrauterine chemotherapeutic agent against anaerobic organisms from genital tract. However, use of Ornidazole is still more efficacious with advance versions of quinolones derivatives.

Markandeya et al. (2010) reported efficacy of intrauterine antibiotic therapy of Levofloxacin with Vitamin E combination in resolving infectious repeat breeding problem in cows and buffaloes. They further discussed that antibiotic plus vitamin (antioxidant) therapy is an innovative approach and additionally the same can be administered by intrauterine route of administration. Levofloxacin, new synergetic agent from fluoroquinolone class of antibacterial agent for intrauterine treatment is active against most strains of the microorganisms both in vitro and in clinical infections. Where as, alpha-tocopherol acts as cellular antioxidants favoring natural defense mechanism.

Table 1: Details of the technical protocol of the clinical trial

<table>
<thead>
<tr>
<th>Group</th>
<th>Indications</th>
<th>No of buffaloes</th>
<th>No of Animals</th>
<th>Drug</th>
<th>Dosage &amp; route</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Retained placenta (ROP)/metritis cases</td>
<td>12</td>
<td>6</td>
<td>Subgroup A1</td>
<td>60 ml IU</td>
<td>5 days post-partum</td>
</tr>
<tr>
<td></td>
<td>Prevention of post-partum infections</td>
<td>12</td>
<td>6</td>
<td>Subgroup A2</td>
<td>60 ml IU</td>
<td>2 days</td>
</tr>
<tr>
<td>C</td>
<td>Infectious/Repeat breeder post-partum</td>
<td>12</td>
<td>6</td>
<td>Subgroup C1</td>
<td>30ml IU</td>
<td>Once at the day of Oestrus</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Subgroup C2</td>
<td></td>
<td></td>
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</table>

Note: N=6: total no of animals six
Subgroup A1, B1, C1: Levofloxacin-Ornidazole and Alpha-tocopherol (Wokadine IU-XP*) intrauterine treatment
All treatment groups were treated as per standard dosage with specific therapy according to need
* Vetoquinol, India Animal Health Pvt. Ltd., Mumbai- 400 076.
Table 2: Comparative clinical efficacy of two intrauterine preparations against post-partum Uterine infections (ROP/metritis) in buffaloes

<table>
<thead>
<tr>
<th>Group A₁ (Levofloxacin-Ornidazole IU)</th>
<th>Group A₂ (Levofloxacin-Ornidazole and alpha-Tocopherol IU)</th>
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<tr>
<td>Buffalo number</td>
<td>Placental expulsion hrs</td>
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<tr>
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<td>------------------------</td>
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<tr>
<td>1</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
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<tr>
<td>3</td>
<td>6.0</td>
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<td>4</td>
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<td>5</td>
<td>7.5</td>
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<tr>
<td>6</td>
<td>7.0</td>
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<tr>
<td>Mean ± SE</td>
<td>6.33 ± 0.40</td>
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Table 3: Comparative clinical efficacy of two intrauterine preparations as a preventive strategy in avoiding post-partum affections in buffaloes

<table>
<thead>
<tr>
<th>Group B₁ (Levofloxacin-Ornidazole IU)</th>
<th>Group B₂ (Levofloxacin-Ornidazole and alpha-Tocopherol IU)</th>
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<td>Buffalo number</td>
<td>Placental expulsion hrs</td>
</tr>
<tr>
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<td>------------------------</td>
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<td>1</td>
<td>6.0</td>
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<td>5</td>
<td>5.0</td>
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<tr>
<td>6</td>
<td>5.5</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>5.58 ± 0.23</td>
</tr>
</tbody>
</table>

Table 4: Mean Comparative clinical efficacy between two intrauterine preparations in management of post partum problems in buffaloes

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>No of animals</th>
<th>Placental expulsion hrs after intrauterine therapy</th>
<th>Uterine involution (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-Clinical cases (ROP)</td>
<td>A₁</td>
<td>06</td>
<td>6.33 ± 0.40</td>
<td>32.66 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>A₂</td>
<td>06</td>
<td>4.66 ± 0.24</td>
<td>26.00 ± 0.36</td>
</tr>
<tr>
<td>II-Preventive strategy</td>
<td>B₁</td>
<td>06</td>
<td>5.58 ± 0.23</td>
<td>28.50 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>B₂</td>
<td>06</td>
<td>4.57 ± 0.22</td>
<td>27.00 ± 0.36</td>
</tr>
</tbody>
</table>

Sub group A₁, B₁: Levofloxacin and ornidazole intrauterine preparation
Sub group A₂, B₂: Levofloxacin and ornidazole and alpha-tocopherol intrauterine preparation
Aerobic as well as anaerobic infections need to be considered before selecting antibiotics for treatment of endometritis and hence combination therapies are necessary to combat both type of infections. Prasad et al. (2006) reported that 25 per cent cross bred cows with endometritis were recovered after enrofloxacin treatment. Purohit et al. (2003) reported that subclinical uterine infection was most effectively treated with ciprofloxacin @ 2000 mg by intrauterine route for 3 days in cows and 85 per cent animals conceived on the first service.

Conclusion
The present clinical trial in buffaloes revealed that Levofloxacin-ornidazole intrauterine therapy was effective in the treatment of postpartum infections due to retention of placenta or metritis. The treatment was also effective for speedy uterine involution in treated cases. The use of the drug combination with preventive strategy in parturated buffaloes was observed to be efficacious in terms of prompt expulsion of placenta and physiological and speedy uterine involution. Addition of α-tocopherol in clinical cases of infections in post-partum buffaloes was found to be superior in terms of increment in production performance of buffaloes.

Repeat breeding is the most serious and challenging infertility problem and it is more closely related with endometritis and infectious cause of the reproductive tract. Mixed infections are common and there is need to treat aerobic and anaerobic infections simultaneously with view to reestablish functional normalcy of endometrium.

The present trial, therefore, indicated that intrauterine therapy of Levofloxacin-Ornidazole was effective in treating postpartum affections in buffaloes. However, synergism of Levofloxacin-Ornidazole and α-tocopherol (Wokadine™-IUxp)* showed superior efficacy in the treatment as well as prevention of postpartum affections and had favorable effect on the productivity in buffaloes.

References

X-PERTS ANSWER

All the practicing veterinarians are requested to send their problems/queries on any clinical aspects faced by them. The solution to the problem will be obtained from national level experts of the concerned field. The solution suggested by the expert will be sent by post and the problem as well as the solution will be published in the next issue of this Journal for the benefit of other readers. This will be a permanent feature of this Journal in forth coming issues.
EFFICACY ASSESSMENT OF SYNTHETIC PENICILLINS IN URI CASES IN LAMB AND ADULT SHEEP

S.K. Dixit, B.N. Tripathi, G.G. Sonawane, Fateh Singh, Jyoti Kumar and F. A. Khan

Central Sheep and Wool Research Institute
Avikanagar-304501 (Rajasthan)

ABSTRACT

Two groups of twelve diseased animals (six lambs and six adults) recorded clinical signs viz. high rise in temperature, polypnoea with dyspnoea, watery nasal discharge, lacrimation with or without ocular discharge inappetance and in some cases reduced ruminal movements. Nasal swabs recorded presence of both kind of organisms mainly E. coli, Staphylococcus spp., Proteus spp., along with unidentified isolates. These animals were treated with Ampicillin plus cloxacillin (AC-VET Forte, Intas) and Acetaminophen (Fibrinil) @ 10 mg/kg intramuscularly 8 hourly for 3-5 days without appreciable recovery in lambs but usable in adults with supportive drugs.

Key words: Synthetic penicillins, upper respiratory tract, sheep

Introduction

Upper respiratory tract infections (URI) among new born and adult sheep are very common in animal husbandry sector and their management through antibiotics are also a very regular phenomenon. Some of the limiting factors in the way of therapeutic management of disease may be enlisted as unavailability of locally studied scientific information pertaining to efficacy of drugs in clinical cases and to some extent nature, type, pathogenicity status and resistance pattern etc of invading organism; in vitro sensitivity to drugs and their in vivo behaviour with concerned individual and species. In present study an attempt was made to assess the therapeutic efficacy of synthetic penicillins particularly in URI cases in lambs and adults both under common influencing factors such as age, season, immune status and managemental condition.

Materials and Methods

A group of 12 animals was randomly selected from a herd maintained at Central Sheep and Wool Research Institute, Avikanagar, during their regular health screening. Six adults and six lambs of both sex and weight varying around 30-40 kg and 8-10 kg, respectively, were named as G-I and G-II. They were maintained under similar feeding and managemental condition. Faecal examination of all the animals was carried out to rule out the possibilities of parasitic infestation. Nasal swabs/tissue samples collected from these animals were processed for bacterial isolation, identification and characterisation of microorganisms using standard techniques. The bacterial isolates were characterized by morphological, cultural and biochemical characteristics after their inoculation on primary, differential and/or selective media. The tissue homogenates were enriched in brain heart infusion broth (Hi Media) at 37°C for 48 hr followed by streaking on blood agar, Mac-Conkey agar, deoxycholate citrate agar, Lactose fermenting colonies on eosin methylene blue agar. Gram's staining was carried out to differentiate contamination from the isolated pathogens based on morphological characteristics (Merchant and Packer, 1983). IMVIC pattern i.e. Indole, MR, VP and citrate utilization tests, and catalase and oxidase tests (Cruickshank et al., 1975) were used for further characterization alongwith serotyping of E. coli at National Salmonella and Escherichia Centre, Kasauli. Histopathology of lung tissue samples was carried out using routine method with haematoxylin and eosin staining (Lillie, 1965). Treatment consisted of a combination Ampicillin plus cloxacillin (AC-VET FORTE, Intas) and Acetaminophen (Fibrinil). Both the drugs were used @ 10 mg/kg intramuscularly 8 hourly in both the groups for 3-5 days. Assessment of efficacy was judged on the basis of trend of clinical symptoms and improvement of health status of sick animals.

Results and Discussion

The clinical examination of affected animals recorded rectal temperature from 101-105.6°F. Pulse rates range from 68-96 and resting respiratory rates range from 12-20 per minute. These are easily elevated in the anxious small ruminant. Normal
rectal temperatures range from 101.5-104.0°F and may reach up to 104.5°F in a full fleeced animal on a hot day. In case of sheep no such well defined criteria seem to have been formulated by any renowned internationally accepted agency but primarily efforts were made to rule out the possibilities of involvement of other than respiratory tract infection. Mucous membrane colour assessed at the conjunctiva, gums, or vulvas were not suggestive of any critical condition in most of the cases. Pale to white colour observed in a few cases were suggestive of mild anaemia and may be correlated with reduced intake (Radostits et al., 2007) in diseased condition. Auscultation of the lungs revealed no detectable abnormality in bronchovesicular sounds which can be indicative of any alarming respiratory disease. Presence of risen rectal temperature 105 to 105.5°F triggers the thought of many infections that cause fever by inducing production of several cytokines, of which interleukin-6 and tumour necrosis factor are most likely involved and probably act on the organum vasculosum of the lamina terminalis, a circumventricular organ outside the brain, which results in activation of the preoptic area of the hypothalamus to induce the production of prostaglandins (Aiello, 1998; Dixit et al., 2011). In a few cases, the depth of respiration was a bit short and shallow but not associated with obvious abnormal lung sounds. Eliciting a cough by tracheal palpation was not a detectable parameter in any of the case ruling the possibility of severe lung involvement. Assessment of abdominal contour did not show any detectable abnormality of immediate clinical significance. Rumen contractions occur at the rate of 1-2/minute recorded in the left paralumbar area but in some cases some reduced frequency with doughy touch was recorded and may be attributed to internal secretions such as histamine and operation of adjunctive compensatory mechanism. Laboratory investigations of collected samples represent presence of both (Gram positive and Gram negative) kinds of organisms on bacterial cultural examination and characterization. Identified isolates were E. coli, Staphylococcus sp, Proteus sp. along with unidentified isolates. On sheep blood agar E. coli produced medium sized, round, smooth, and whitish-grey colonies along with partial haemolysis of RBCs in some cases. Twelve E. coli isolates (O66-4, O60-2, O95-2, O71-1, O84-1, O102-1, O147-1) were serotyped at National Salmonella and Escherichia Centre, Kasauli in consonance with the findings of various authors (Saulam et al., 1997; Raji et al., 2000; Yener et al., 2001; Oruc, 2006). Mycoplasma sp. was, however, isolated from one lung tissue sample.

Nearly all the treated animals started showing improvement in their clinical parameters from next 12-18 hours of treatment. Various symptoms such as dyspnoea, nasal discharge, and high rise in temperature, lachrymation and abdominal respiration either reduced or adopted reducing trend. Presence of dyspnoea may be a compensatory process of gas exchanges through lung parenchyma (Koneko, 1980) and high rectal temperature 105 to 105.5°F may be produced by bacterial, viral or chemical pyrogens. It is the result of a “resetting” of the thermoregulatory mechanism to function above the normal level. In many animals prostaglandins are responsible for the readjustment as evident by use of prostaglandin inhibitors as antipyretic agents (Aiello, 1998). Symptoms such as dullness, depression, inappetance reduced ruminal motility and scanty faeces persisted for longer period of time and marginal recovery could be noticed after 24-36 hours of onset of therapy. Auscultation of the lungs did not reveal any abnormal bronchovesicular sounds or rales in treated cases during the course. Recovery noticed at the time of withdrawal of treatment can not be rated good as two lambs died after 5 days of treatment and pneumonia was confirmed on post-mortem examination and histopathology. Grossly, the lungs revealed severe consolidation (grey hepatisation) in diaphragmatic lobes. Rest of the lobes were congested. Affected lungs were collected immediately in 10% neutral buffer formalin for further processing. Following fixation in 10% neutral buffer formalin, sections of pneumatic lung tissues were trimmed, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin-eosin (HE) for histopathologic examination (Culling, 1968). Microscopically, bronchopneumonia was characterised by severe infiltration of mononuclear cells in the bronchi and bronchioles. Bronchi and bronchiolar epithelium and peribronchial lymphoid tissue revealed marked hyperplasia. The lumen of the alveoli was filled with fibrinous exudates containing mononuclear cells and neutrophils (Fig. 1). One adult animal required support of bronchodilators for two days. A complex interaction of environmental factors producing stress, a variety of micro organisms working synergistically to damage the cells lining the respiratory tract allowing colonization and invasion of other organisms and a compensated host response may result in onset of nasal discharge, lachrymation dullness, depression, inappetance and dyspnoea etc. (Aiello, 1998). The presence of bacterial pathogens in the environment or as a normal flora of upper respiratory tract could invade the lungs and blood stream in the event of reduction in immunological resistance of the susceptible animals (Raji et al., 2000) or enteroinvasive E. coli can invade the intestine and colonise into the other body organs including lungs through blood circulation (Quinn et al., 1998). It is known to be a
genetically heterogeneous group of bacteria, which is typically nonpathogenic and inhabitates as a normal microflora of the intestinal tract of animals and humans (Gyles, 2007) and produce virulence factors by acquiring the genes that enable them to cause intestinal or extra-intestinal diseases to act as a systemic pathogen (Kaper et al., 2004). Absence of Pasteurella sp. or Mannheimia sp. does not rule out the possibilities their presence due to indiscriminate use of different antibiotics. Varying degree of broncho-pneumonia and interstitial pneumonia with congestion and haemorrhagic changes was observed on histopathological examination. Interstitial pneumonia was characterized by an increase in mononuclear cells in inter-alveolar septa, peribronchial or peribronchiolar proliferation of lymphocytes, and by the presence of a varying number of neutrophils and/or macrophages within the alveolar lumina in consonance with results of Oruc (2006).

The present therapeutic regimen used in treatment has not shown a good recovery probably due to predominance of Gram negative pathogens. Ampicillin and cloxacillin are broad-spectrum bactericidal antibiotic and they are well known to act synergistically against a variety of bacteria (Bornside, 1968; Sutherland and Batchlor, 1964; Riviere and Papich, 2009) by inhibiting the synthesis of bacterial cell walls. It inhibits cross linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram positive and Gram negative bacteria. But here we failed to get very appreciable response in lambs. However, in adults it can be used with support of bronchodilators (Dixit et al., 2011).

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