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DETECTION OF EQUINE HERPESVIRUS-1 AND EQUINE HERPESVIRUS-4 IN MULES AND DONKEYS BY REAL TIME PCR

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ABSTRACT

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The equine herpesviruses (EHVs) are important pathogens in all members of Equidae family world wide. Because of the causative agents of several diseases and latency makes major challenges to equine industry. In the present study blood samples of 108 mules and donkeys from different rural areas of North-East of Iran, as a region with most equine population, were examined for presence of Equine herpesvirus -1 and 4 using real time PCR as a rapid molecular diagnostic test that has high sensitivity and specificity. Based on results, it was conclude that EHV-1 DNA was not detected in any blood samples whereas EHV-4 was present in 14.8% of donkeys and mules. Which can have important role as a source of infection for other equids population.

Key words: Equine herpesvirus-1 and 4, donkeys and mules, respiratory disease, real time PCR, Iran

Introduction

Equine herpes virus are DNA virus with linear double-strand and belong to Herpesviridae family. The two most common types major importance are Equine herpes virus type 1 (EHV-1), which causes abortion, respiratory disease and neurologic disease; and Equine herpes virus type 4 (EHV-4), which causes only respiratory disease but also occasionally causes abortion. EHV-1 and EHV-4 are the members of varicello virus genus in Alpha herpes virinae subfamily and are endemic in equine populations worldwide (Reed and Toribio, 2004; Fortier et al., 2010; MacLachlan and Dubovi, 2011). These viruses have 145 to 150 kb genomic DNA and glycoproteins that are important for cell attachment, entry and cell-to-cell spreading and induction of immune responses (Reed and Toribio, 2004; Azab et al., 2010). The common characteristics between all herpes virus infections are latency and persistent infection with periodic or continuous shedding (Sellon, 2007; Ataseven et al., 2009; MacLachlan and Dubovi, 2011).

EHV-1 and EHV-4 can establish latent infections in trigeminal ganglia and T-lymphocytes. Latently infected animals are the major reservoirs of virus and frequent shedding of the virus from these asymptomatic carriers cause spread of virus to susceptible population (Slater et al., 1994; Chesters et al., 1997; Pusterla et al., 2009; Fortier et al., 2010).

Epidemiological studies, suggest that the infection occurs during the few early weeks or months after birth occurs, generally before or after weaning, from adult mares that asymptotically shed virus (Nicola Pusterla et al., 2005; Sellon, 2007).

Techniques that are used for the diagnosis of EHV infection include: virus isolation as “gold standard”, serological tests and nucleic acid detection techniques (PCR). Several PCR-based methods have been developed for detection and identification of EHV-1 and EHV-4 DNA in aborted foetuses or nasal swabs (Sellon, 2007).

qPCR technology offers a flexible and rapid method as compared to virus isolation. This is sensitive, specific and quantitative method which provides a very useful diagnostic tool for infectious diseases studies, so also a valuable aid for screening large numbers of samples (Diallo et al., 2006; Perkins et al., 2008; Dzieciatkowski et al., 2009; Hoffmann et al., 2009). The main target of this study is to survey circulation of virus and possible source of infection in equine population in North-East district of Iran that has the highest equine population with high prevalence of EHV-4.

Materials and Methods

A total 108 blood samples from donkeys and mules in different rural areas in North-East of Iran were collected during 2011 to 2012 and examined in this study. These regions are having the highest equine population in Iran. From each case, 2 ml of blood was collected from jugular vein by venipuncture into tubes containing EDTA (Vacumed® K3 EDTA. FL medical. Italy).

DNA was extracted from 180 µl of each whole blood sample using a commercial DNA extraction kit (DNA Extraction kit, MBST Inc., Iran) according to manufacturer’s instructions. Quality of extracted DNAs was confirmed by the agarose gel electrophoresis and spectrophotometrical analysis.

Among members of Alpha herpes virinae glycoprotein B is highly conserved and contains very specific sequences that can be used for differentiation between closely related equid herpes viruses (Wagner et al., 1992; Diallo et al., 2007). The conserved and variable regions of glycoprotein B gene of each equine herpesvirus (EHV-1 and EHV-4) were determined by alignment of sequences available in Gene Bank and the type specific primers for EHV-1 and EHV-4 were designed by Primer software, (PREMIER Biosoft International, Inc). Primer sequence specificity was checked BLASTn in the NCBI data base and the sequences have 100% homology to those of EHV-1 and EHV-4 strains deposited in the Gene Bank (http://www.ncbi.nlm.nih.gov/BLAST).

The primer sets were synthesized by Bioneer (Bioneer Inc. Korea) and Macro Gene Corporation (Macro Gen Inc. South Korea). The resulting amplicons for EHV-1 and EHV-4 were 113bp and 100 bp in long, respectively. Primers are listed in

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To determine the efficiency of nucleic acid extraction and verify the absence of PCR inhibitors in the DNA templates, all samples were tested for the presence of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (an equine housekeeping gene) as an internal control (IC) by specific primers (forward: 5′-ATCTGACCTGCCGCTGGAG-3′, Reverse: 5′-CGATGGCCTGTCTCAACCCTTC-3′) (Katia Cappelli, 2008; Hoffmann et al., 2009).

### Reference virus strains

Purified DNA of EHV-1 strain 89C25 and EHV-4 strain TH20p, the Japanese prototype of EHV-4 (Kawakami et al., 1962) (provided by Dr. Matsumura, Japan Racing Association, Tochigi, Japan) were used as controls at real-time PCR assay. The DNAs had been purified from foetal horse kidney cells infected with EHV-1 (89C25p strain) or EHV-4 (TH20p strain) using QIAamp DNA Blood Mini Kit (QIAGEN).

### qPCR assay

Samples were tested for the presence of EHV-4 by a SYBR Green-based real-time PCR assay. The amplification carried out in a 25 µl reaction volume containing 12.5 µl of the Fermentas Maxima®SYBR Green/ROX qPCR mix (2X) (Thermo Fisher Scientific Inc. USA), 100 nM of each primer, 2 µl of template DNA (Fig. 1). The EHV-1 real-time PCR was performed in 20 µl reaction volume containing 4 µl of the Solis BioDyne HOFFIREpo® Eva Green® qPCR Mix Plus (ROX) (Solis BioDyne Inc., Estonia), 5X, 100 nM of each primer and 2 µl of template DNA. qPCR was performed in a Bio-Rad CFX 96 qPCR Detection System. Thermal cycler conditions for EHV-1 were: 95°C for 15 min, followed by 40 cycles of 10 sec 95°C denaturation, 30 sec 64.1°C annealing, 30 sec 72°C extension and for EHV-4: 95°C for 10 min, followed by 40 cycles of 10 sec 95°C denaturation, 30 sec 59.3°C annealing, 30 sec 72°C extension.

All samples were tested in triplicate and sample threshold and baseline values were calculated by the CFX manager software (Bio-Rad) (Fig. 2a and b). All reactions included no-template controls (NTC). EHV-1 and EHV-4 DNAs were used as positive and negative control respectively to determine the specificity of the assays. The sensitivity of the qPCR reaction was determined using standard curve that were run for both EHV-1 and EHV-4 assays and amplification efficiency of the gB gene was calculated from the coefficient of determination and the slope value of a standard curve generated on log10 dilutions as described by Hussey et al. (2006).

Data processing was based on standard curve method and efficiencies of each reaction was calculated as Efficiency = 10 (-1/Slope) -1[12] that amplification of the log dilution series showed linearity with slope = -3.1 and R2 = 0.998 for EHV-4 (Fig. 3).

No fluorescence signal was detected in the tubes that contained negative (no template control) samples. However in the tubes with positive reference template high fluorescence signal was detected, confirming that the assay was highly specific for the detection of EHV-1 and EHV-4 in the samples. The analytical specificity of the assay was determined by testing cross-reaction to EHV-1 and EHV-4 reference strains. No cross-reaction was observed with any of the herpesviruses tested.

The species specificity of the PCR products was confirmed by sequencing. The purified PCR products were then sequenced using the respective sequencing primers; sequence of the EHV-4 PCR products was 100% identical to the sequence of EHV-4 strains available in the Gene bank data base (Fig. 4).

### Results and Discussion

Results indicated that EHV-1 DNA was not present in any samples while EHV-4 DNA in 14.8% of donkeys and mules blood samples was detectable (Fig. 4). The Internal Control (GAPDH) was detected in all samples; therefore, DNA losses did not occur during nucleic acid extraction and DNA polymerase inhibition was not observed during real-time PCR amplification.

On equine in Iran population was in Chaharmahal and Bakhtiyari province which showed the prevalence rate of EHV-1 and EHV-4 to be 39.08 and 68.96%, respectively. However, these studies were based on indirect recognition of virus by ELISA (Momtaz and Hematzadeh, 2003). In another serologic investigation 3 EHV 1 and 15 EHV 4 of the 600 sera were found positive. Thus suggested that infection was associated with sex, age and respiratory disease status. EHV 1 and EHV 4 showed extensive antigenic cross reactivity and serological determinations of the infection caused by either of the two virus types have been difficult (Pusterla et al., 2009).

Pervious study in equine population of north east of Iran by real time PCR showed the high prevalence of equine herpes virus-4 (88%) in equine population of this region (Karan, 2013). These types of equids are traditionally used as transport in Iran. Due to traditional housing of horses in nearby of donkeys and mules and dispatch of horses for competition and breeding in North-East of Iran, spread of EHVs between populations is easier resulting into a greater incidence of infection and subsequent latency as compared to other populations. Prevalence of this virus in donkeys and mules has also been reported in nearby countries of Iran (Tekelioglu et al., 2005; Ataseven et al., 2009). Unlike of serologic investigation in Iran by all samples were negative for EHV-

<table>
<thead>
<tr>
<th>Virus</th>
<th>Target Gen</th>
<th>Genome Position</th>
<th>Primers sequence 5’-3’ (Forward, Reverse)</th>
<th>Amplicon Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHV-4gB (Accession#:M26171.1)</td>
<td>2629 - 2610</td>
<td>2530 - 2549</td>
<td>TACCCCTGGAGGTTTACACG</td>
<td>100</td>
</tr>
<tr>
<td>EHV-1gB (Accession#:M36298)</td>
<td>1102 - 1084</td>
<td>990 - 1007</td>
<td>ATACTCGCTGAGGAGTGG GTGAAGTTTCTCCCAAGGT</td>
<td>113</td>
</tr>
</tbody>
</table>

*Glyco protein B*
Fig. 1: Amplification plots of EHV-4 (This figure was generated automatically by the CFX manager software (Bio-Rad) version 2.1)

Fig. 2a: Post amplification melting peak analysis

Fig. 2b: Post amplification melting curve analysis
1. The negative results are not due to absence of sensitivity in used method in this study, because the accuracy of our quantitative test was 6 copy numbers of the target gene per reaction. In clinical aspect, symptoms such as neurological signs and history of abortion did not observed in, donkeys and mules of these districts either. This finding is in according to our earlier studies in these regions (Sarani et al., 2013). As same as other studies although our test showed presence of EHV-4 in donkeys and mules; greater per cent of these animals did not show any clinical sign; which might be due to establishment of latency in these populations. Moreover, the qPCR can only determine number of target DNA in sample and not infective elements, this value does not show infectious potential of the viral shedding and spread in equine population (Perkins et al., 2008).

This study demonstrates that working mules and donkeys represent a likely reservoir of EHV-1 and EHV-4 infection and can have role in epidemic event of syndromes of EHV, and must be included in hygienic programmes for eradication of disease.

Acknowledgements
The author is grateful to Dr. Tomio Matsumura, Japan Racing Association, Tochigi, Japan for providing the reference strains for this study and Dr. A. Ghiadi, Head of Directors, Council of Turkmen Horse Breeding and Consulting Co. (THBC) for his help.

References
Katia Cappelli, M. F. et al. (2008) BMC Molecular Biology. 9(49).

Fig. 3: Standard curve of the EHV-4 real-time PCR assay obtained from triplicates of 10-fold dilutions of standard DNA

Fig. 4: Sequencing result of real time PCR products
SEROPREVALENCE OF NEWCASTLE DISEASE INFECTION IN POULTRY BY
ENZYME LINKED IMMUNOSORBENT ASSAY AND AGAR GEL
IMMUNODIFFUSION IN RANCHI (JHARKHAND) INDIA

Anuradha Kumari* and Arun Prasad†
Department of Microbiology, Ranchi Veterinary College, Kanke, Ranchi, 834 006, India

ABSTRACT

The objective of this study was to study ND seroprevalence from suspected cases using enzyme linked immunosorbent assay (ELISA) and agar gel immunodiffusion test (AGID) in poultry in and around Ranchi and also to evaluate AGID with ELISA. In the present study, ELISA and AGID based ND seroprevalence study of 92 suspected sera samples in the year 2014-15 had revealed 63.04% and 51.09%, respectively. A total of 92 suspected poultry samples of central and western plateau agro-climatic zone in and around Ranchi district, Jharkhand during 2014-2015 were tested for the estimation of diagnostic sensitivity (Dsn) and diagnostic specificity (Dsp). The Dsn and Dsp were found to be 51.72% and 50.00%, respectively.

Key words: Newcastle disease, AGID, seroprevalence, ELISA, Dsn, Dsp

Introduction

Newcastle Disease (ND) is an acute infectious viral disease of domestic poultry which belongs to order Mononegavirales, family Paramyxoviridae and subfamily Paramyxovirinae (de Leeuw and Peeters, 1999). The most susceptible avian species are chickens with respiratory signs, nervous system impairment, gastrointestinal and reproductive problems (Nanthakumar et al., 2000).

For ND diagnosis, virus neutralization (VN) (Rubin and Franklin, 1957), single-phase complex plaque technique (Khadzhiev, 1984), haemagglutination (HA) and haemagglutination inhibition (HI) test (Haque et al., 2010), ELISA (Madsen et al., 2013), duplex-RT-PCR (Shirvan and Mardani, 2014) are commonly used frequently.

The present work has been planned to monitor ND seroprevalence using ELISA and AGID in poultry sera in Ranchi, Jharkhand and to get a comparative assessment of efficacy of the test.

Materials and Methods

The blood samples were collected from 92 chickens aged 2-6 wk from suspected cases in and around Ranchi between January 2014 and April 2015.

(A) ELISA: ELISA was carried out by Newcastle disease virus antibody test kit, (Affinitech Ltd.). Test was considered negative or positive if produced NDV ELISA unit was less than 15 or more than 15, respectively.

(B) AGID: This technique was based on the ability of antibodies to form precipitation lines specifically with the antigen. Free diffusion of both the antigen and antibody takes place in agarose gel resulting in precipitation lines, which were visible to the naked eye. The procedure was followed as per Ouchterlony (1948) with some modification. Sensitivity and specificity of AGID were calculated, considering ELISA as reference test as per Lalkhen and McCluskey (2008). Statistical analysis between ELISA and AGID was done as per Snedecor and Cochran (2004).

Results and Discussion

ND seroprevalence was reported on the basis of ELISA and AGID as 63.04% and 51.09%, respectively (Table 1, Fig. 1 and Fig. 2). However, it was also reported by several workers from India and abroad even in the apparently healthy birds (Mai et al., 2014 and Geetha, 2014). In the present survey, ND seroprevalence has been reported on the basis of ELISA as 63.04% while the prevalence rate of disease was also reported through ELISA by Musako and Abolnik (2012) and by Sharma et al. (2015) as 73.3% in Nigeria and 66.3% in West Indies, respectively. Seroprevalence by AGID was recorded as 51.09% in the present work which was in confirmation with 52.94% by Roy and Venugopalan (1997), 64.10% by Mazengia et al. (2010) in Ethiopia and 41.8% by Egbal et al. (2012).

The relative sensitivity and specificity of AGID compared to ELISA were calculated as 51.72% and 50.00%. Taking ELISA as a Gold Standard test, the sensitivity and specificity of the AGID assay were found to be low. Detail of true positive, false negative, true negative and false positive are depicted in Table 2. On the basis of Chi-square (X²) test, ELISA and AGID were found non significant (Table 3).

Acknowledgements

The authors are thankful to the Vice Chancellor and Dean, Ranchi Veterinary College, B.A.U., Kanke, Ranchi, Jharkhand for providing necessary facilities to carry out the proposed work.

References


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Table 1: Overall place-wise seroprevalence of ND infection in poultry using ELISA and AGID

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Place</th>
<th>SAMPLES</th>
<th>ELISA (%)</th>
<th>AGID (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>RVC, Kanke</td>
<td>10</td>
<td>8(80)</td>
<td>6(60)</td>
</tr>
<tr>
<td>2.</td>
<td>Hochar</td>
<td>5</td>
<td>2(40)</td>
<td>4(80)</td>
</tr>
<tr>
<td>3.</td>
<td>Husir</td>
<td>7</td>
<td>4(57.14)</td>
<td>4(57.14)</td>
</tr>
<tr>
<td>4.</td>
<td>Patratoli</td>
<td>5</td>
<td>4(80)</td>
<td>4(80)</td>
</tr>
<tr>
<td>5.</td>
<td>Chirondi</td>
<td>5</td>
<td>2(40)</td>
<td>2(40)</td>
</tr>
<tr>
<td>6.</td>
<td>Simartoli</td>
<td>4</td>
<td>3(75)</td>
<td>3(75)</td>
</tr>
<tr>
<td>7.</td>
<td>Boria</td>
<td>7</td>
<td>4(57.14)</td>
<td>3(42.86)</td>
</tr>
<tr>
<td>8.</td>
<td>Sangrampur</td>
<td>5</td>
<td>4(80)</td>
<td>2(40)</td>
</tr>
<tr>
<td>9.</td>
<td>Arsanday</td>
<td>7</td>
<td>3(42.86)</td>
<td>3(42.86)</td>
</tr>
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<td>10.</td>
<td>Miatcolony</td>
<td>4</td>
<td>2(50)</td>
<td>0(0)</td>
</tr>
<tr>
<td>11.</td>
<td>Chuditola</td>
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<td>2(50)</td>
<td>3(75)</td>
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<tr>
<td>12.</td>
<td>Bliha</td>
<td>2</td>
<td>1(50)</td>
<td>0(0)</td>
</tr>
<tr>
<td>13.</td>
<td>Mahuatoi</td>
<td>3</td>
<td>2(66.67)</td>
<td>1(33.33)</td>
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<td>14.</td>
<td>Chandave</td>
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<td>4(80)</td>
<td>1(20)</td>
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<td>15.</td>
<td>Kumharia</td>
<td>7</td>
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<td>16.</td>
<td>Banatu</td>
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<td>3(75)</td>
<td>1(25)</td>
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<td>17.</td>
<td>Kokar</td>
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<td>2(66.67)</td>
<td>1(33.33)</td>
</tr>
<tr>
<td>18.</td>
<td>Chutia</td>
<td>5</td>
<td>3(60)</td>
<td>2(40)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>92</td>
<td>58(63.04)</td>
<td>47(51.09)</td>
</tr>
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Table 2: Relative performance of AGID to ELISA for ND

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<tr>
<th>Test</th>
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<th>AGID</th>
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<tr>
<td></td>
<td>Results</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>AGID</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>30(TP)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>28(FN)</td>
</tr>
<tr>
<td></td>
<td>Total results</td>
<td>58(ELISA +ve sample)</td>
</tr>
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</table>

Table 3: Statistics for ELISA and AGID for ND

<table>
<thead>
<tr>
<th>Result/Technique</th>
<th>ELISA</th>
<th>AGID</th>
<th>TOTAL</th>
<th>X^2</th>
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<tr>
<td>POSITIVE</td>
<td>58</td>
<td>47</td>
<td>105</td>
<td>2.88**</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>34</td>
<td>45</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>92</td>
<td>92</td>
<td>184</td>
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</table>


ISOLATION, IDENTIFICATION AND MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDICES OF COMMENSAL ESCHERICHIA COLI ISOLATED FROM HEALTHY CHICKS

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ABSTRACT

In the present study, 26 commensal Escherichia coli isolates were identified by using various phenotypic and genotypic methods. The antimicrobial susceptibility patterns were demonstrated that highest level of resistance to erythromycin (100%), doxycycline (97.3%) and trimethoprim (63%); there was a low level of resistance to aminoglycosides and cephalosporins. MAR index was more than 0.2 for all multi drug resistant E. coli isolates.

Key words: Chicks, E. coli, antimicrobial susceptibility, multiple antibiotic resistance index

Introduction

Antimicrobials are an integral part of animal farming to treat clinical diseases and to increase productivity through the use of these agents as essential feed additives to maintain healthy flock (Witte, 1998). Use of antimicrobials for therapeutic purpose has resulted in antimicrobial resistance and consequently, loss of efficacy of this antimicrobial agent (Miranda et al., 2008). However, with the very wide use of antibiotics, resistance becomes much more of problem as susceptible microbes were eliminated and numbers of resistant microorganisms increased (Okeke et al., 2005). Escherichia coli are one of the most important bacteria against which many antimicrobials have been tried and a variable degree of resistance has been recorded in both humans and animals (Amara et al., 1995). Multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (Daini et al., 2005). Multiple antibiotic resistances (MAR) indexing has been shown to be a cost effective and valid method of bacteria source tracking (Mthembu, 2008).

Materials and Methods

In the present investigation 26 cloacal swab samples from healthy chicks were used. All faecal samples were mixed thoroughly with distilled water and centrifuged at the rate of 4000 rpm for 5 minutes to clarify the samples of any debris. One ml of clear supernatant from centrifuged samples was processed and yielded 26 positive isolates of Escherichia coli (Fig. 1). 16S rRNA gene is well conserved (Krumperman, 1983). The MAR index was calculated as a standard target for the identification of species of bacteria (Amann et al., 1995). The MAR index is defined as a/b, where “a” represents the number of antibiotics to which the isolate was resistant, and “b” represents the number of antibiotics to which the isolate was exposed.

The sequence of the primer pair used was as follows:
Forward primer: 5’ GCTTGACACTGACATTGAG 3’
Reverse Primer: 5’ GCACATTCTCTCGGATT 3’
The expected product size was 662 bp. The annealing temperature was used 57°C for 45 sec.
Antibiotic susceptibility testing was done as per the disc diffusion method (Bauer et al., 1966).
All Multidrug resistant isolates were evaluated for their Multiple Antibiotic Resistance (MAR) index. In an effort for risk assessment of MDR isolates this index was given by Krumperman, 1983. The MAR index, is defined as a/b, where “a” represents the number of antibiotics to which the isolate was resistant and “b” represents the number of antibiotics to which the isolate was exposed.

Results and Discussion

A total of 26 faecal samples of healthy poultry were processed and yielded 26 positive isolates of Escherichia coli with a prevalence of 100%. All the E. coli isolates showed typical primary and secondary biochemical reactions characteristic to E. coli.

All the isolates were subjected to PCR amplification targeting 16S rRNA gene fragments using species specific primers. All the 26 isolates produced an amplicon of 662 bp confirming them to be E. coli (Fig. 1). 16S rRNA gene is well established as a standard target for the identification of species of bacteria (Amann et al., 1995). The 16S rRNA acts as a critical component of bacterial cell function and the gene coding for 16S rRNA has been found to be highly conserved.

In the present investigation, 26 E. coli isolates were subjected to resistotyping with 16 different class antibiotics. The resistance pattern against cephalosporin group showed variable results with 26%, 22.2%, 22.2%, 11.1% resistance against cefotaxime, cefipime, cefazolin and cefuroxime respectively. The resistance pattern of ticarcillin and pipracillin

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Table 1: List of antibiotics used for antibiogram study against *E. coli*

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Class of antibiotics</th>
<th>Antibiotics</th>
<th>Concentration (mg or unit/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall synthesis inhibitor</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; gen. cephalosporin</td>
<td>Cefazolin(CZ)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; gen. cephalosporin</td>
<td>Cefuroxime(CXM)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; gen. cephalosporin</td>
<td>Cefotaxime(CTX)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>4&lt;sup&gt;th&lt;/sup&gt; gen. cephalosporin</td>
<td>Cefipime(CPM)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Aminopenicillin</td>
<td>Ampicillin(AMP)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Carboxopenicillin</td>
<td>Ticarcillin(Tl)</td>
<td>10</td>
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<tr>
<td></td>
<td>Ureidopenicillin</td>
<td>Pipacillin(P1)</td>
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<td></td>
<td>Polypeptide</td>
<td>Polymyxin-B(PB)</td>
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<tr>
<td>Protein synthesis inhibitor (30-S)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; gen.aminoglycoside</td>
<td>Kanamycin(K)</td>
<td>30</td>
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<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; gen.aminoglycoside</td>
<td>Gentamicin(GEN)</td>
<td>10</td>
</tr>
<tr>
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<td>3&lt;sup&gt;rd&lt;/sup&gt; gen.aminoglycoside</td>
<td>Amikacin(Ak)</td>
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<td></td>
<td>Macrolides</td>
<td>Streptomyacin(S)</td>
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<tr>
<td></td>
<td>Semisynthetic tetracycline</td>
<td>Doxycycline(DO)</td>
<td>30</td>
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<tr>
<td>Protein synthesis inhibitor (50-S)</td>
<td>Macrolides</td>
<td>Erythromycin(E)</td>
<td>15</td>
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<tr>
<td></td>
<td>Phenicoles</td>
<td>Chloramphenicol(C)</td>
<td>30</td>
</tr>
<tr>
<td>Anti metabolite</td>
<td>Sulphonamide</td>
<td>Trimethoprim(TR)</td>
<td>5</td>
</tr>
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</table>

Table 2: Antibiogram of different isolates of *Escherichia coli* from chicks

<table>
<thead>
<tr>
<th>Isolate</th>
<th>CZ</th>
<th>CXM</th>
<th>CTX</th>
<th>CPM</th>
<th>AMP</th>
<th>Ti</th>
<th>PI</th>
<th>PB</th>
<th>K</th>
<th>GEN</th>
<th>AK</th>
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Fig.1: Gel picture showing 16-S rRNA based confirmation of *E. coli*

L1, L2, L3, L4 showing 662 bp product, M-1kb DNA ladder
was 37.1 and 51.8, respectively. The isolates were 0, 7.4% and 7.4% resistant against amikacin, gentamicin, and kanamycin, respectively. The amikacin was found to be the most effective to all isolates. Resistance to doxycycline and trimethoprim was 96.3% and 63% obtained, respectively to all isolates. In the present investigation, all of the isolates were resistant against erythromycin.

All multidrug resistant isolates were evaluated for their MAR index. This index is an epidemiological tool which is used for risk analysis of environment through bacterial contamination and now a days it is used to assess whether the isolate has originated from an environment where several antibiotics have been used or not. In the present study, all the isolates had an MAR index of more than 0.2 (Table 3). This is indicative of the fact that lots of different antibiotics are being used in the poultry environment from where the samples had been collected. High prevalence of multidrug resistance indicates serious need for antibiotics surveillance program. Even the isolates from the samples which had been collected from healthy chicks also had an MAR index value of 0.25 to 0.56. These results suggest that *E. coli* colonizing the chick at initial stage is multi drug resistant and such isolates are persisting in the poultry environment. Thus, such environments can be potential nidus for the spread of resistance.

**References**

Mthembu, M.S. (2008) *The usefulness of multiple antibiotic resistances (MAR) indexing technique in differentiating faecal coliform bacteria from different sources*. Thesis (M. Sc.) University of Zululand.
CHARACTERIZATION OF ESCHERICHIA COLI ISOLED FROM FAECES OF SPOTTED DEER (AXIS AXIS)

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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT

In the present paper, the phenotypic characterization of E. coli isolates from 20 faecal samples of spotted deer is reported. Out of 20 isolates, two isolates lost ability to produce metallic sheen consistently on EMB agar on repeated subculture and were discarded. Thus, 18 (90%) isolates obtained were further subjected to genotypic confirmation by polymerase chain reaction with species specific primer 16S rRNA gene segment. Out of total 18 samples 16 isolates were confirmed as E. coli.

Key words: E. coli, phenotypic and genotypic confirmed by polymerase chain reaction

Introduction

Escherichia coli a major facultative gram negative organism comprises the normal flora of gastrointestinal tract belong to family Enterobacteriaceae, and are non-spore forming, peritrichous and fimbriate bacillus. Wild animals and birds are traditionally referred as non-domesticated animal species. Most E. coli strains are harmless, but some pathogenic strain can cause bloody diarrhoea, anaemia, stomach cramps gastroenteritis, urinary tract infections, dysentery, haemorrhagic uraemia, extra intestinal infections, septicaemia, pneumonia and meningitis in humans and animals (Levine, 1987). The infection is chronic and difficult to treat medically depending on among others factors, the pathogenic quantities of the E. coli strain present. This disease reduced the health of wild animal species. Widespread infection over the body strain present. This disease reduced the health of wild animal species. Widespread infection over the body which is difficult to manage and even the antibiotic therapy does not work satisfactory. The present study reports phenotypic characterization of genotypically confirmed E. coli strains from spotted deer (Axis axis) from Bikaner zoo.

Materials and Methods

Sample collection

The faecal samples from diarrhoeic and non-diarrhoeic spotted deer from Bikaner zoo were collected aseptically with the help of sterile cotton swabs soaked in nutrient broth and placed in sterile test tubes, taking all precautions to avoid contamination. The samples were transported to the laboratory as soon as possible for further processing.

Phenotype characterization

The procedure for isolation and identification of bacterial cultures was adopted in the study as per technique of Cowan and Steel (1975). Faecal samples were inoculated in nutrient broth and incubated for 24 hours at 37°C. A loopful of material from nutrient broth was streaked upon MacConkey Agar (MCA) plates and the plates were incubated at 37°C for 24 hours. After 24 hours incubation lactose fermenter and non-lactose fermenter colonies were processed as per technique of Edward and Ewing (1986) and cultures showing typical metallic sheen on EMB agar were subjected to the biochemical reactions for further confirmation. These purified cultures were inoculated on nutrient agar slants and incubated at 37°C for 24 hours. These were sealed with wax and kept at 4°C till further use. The monthly subculture was also done to maintain the viability of these cultures. Gram-staining properties were determined following the procedure described by Merchant and Packer (1967), and. E. coli like colonies were subjected to different biochemical tests, including sugar fermentation test, Indole production test, Methyl-Red and Voges-Proskauer (IMIVC) tests, following the standard methods as described by Cowan and Steel (1975).

Genotype characterization

Identification of E. coli was carried out with a PCR based method involving specific primer targeted against 16S rRNA gene (Khaled et al., 2010). The sequence of the primer pair used was as follows:

Forward Primer - 5'GCTTGAACATGATTTGAG3'
Reverse Primer - 5'GCTATTCTCTTCTTCCGAA3'

Result and Discussion

In the present investigation out of 20 faecal samples from spotted deer 18 isolates of E. coli were observed based on phenotypic characterization and biochemical reactions. However, when they were subjected to ribotyping targeting 16S rRNA gene with species specific primer, only 16 were confirmed as E. coli which produced an amplicon of 662bp size. Most of the E. coli isolates followed typical biochemical pattern that has been reported by earlier researchers (Edward and Ewing, 1986) and Barrow and Feltham (1993). Yellow slant, yellow butt but no H₂S on TSI agar showing enzymatic attack on sugars lactose and or sucrose to produce acid and followed typical IMVic pattern with tryptophan splitted to indole (indole positive), mixed acid fermentation (MR positive), no acetyl methyl carbinol production (VP negative) and no utilization of citrate as sole source of carbon (citrate negative) in order to observe the cultural properties of these isolates. The citrate utilizing E.coli had been reported by Kulshrestha and Kumar (1977), Chakraborty and Nag (1997), Chachra et al. (1999), who incriminated citrate utilizing E.coli strains with a frequency of 4.36%, 6.02% and 27.02%, respectively.

Similar findings of citrate utilization have been also reported by Ishuguro et al. (1978), Ishiguro and Sato (1979), Lee and Choi 169
(1983), Dubey et al. (2001) and Arya (2002). After primary and secondary biochemical test 2 isolates lost ability to produce metallic sheen consistently on EMB agar on repeated subculture and thus 18 (90%) isolates were further subjected to genotypically confirmed by polymerase chain reaction with species specific primer 16S rRNA gene segment.

Out of total 18 samples 16 isolates of E. coli were obtained with a recovery of (80%). The present observation is similar to those of Shahrani et al. (2014) who obtained 630 E. coli isolates from 824 samples (76.45%) collected from diarrhoeic calves in Iran. However, recovery of this organism was lower (20%) by Ammar et al. (2015) who carried out a study on bacterial isolation from different source in Sharkia province during all year seasons.

Table 1: Identification and characterization of E. coli isolates from spotted deer from Bikaner zoo

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<thead>
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<th>Isolate No.</th>
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<tr>
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<td>SD4</td>
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<td>SD5</td>
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<td>SD20</td>
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(+)= Positive, (-)= Negative, ve = Gram negative rods, S.D = spotted deer (YY-)= Yellow slant and yellow butt but no H₂S production, F= Fermentative

References
SEROPREVELANCE OF PARATUBERCULOSIS IN AN ORGANISED SHEEP FARM

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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001, Rajasthan, India

ABSTRACT

The present investigation involved detection of antibodies to M. paratuberculosis in sera sample of Magra and Marwari sheep (total 404), of CSWRI, ARC, Bikaner farm, by ELISA. Humoral immune response against M. paratuberculosis was shown by 16.58% of animals tested. In Marwari flock only one animal was positive for presence of antibodies to M. paratuberculosis and in Magra flock 66 animals were positive. The antibodies could be detected in all the age groups of the animals. Percentage of animals showing presence of the antibodies was maximum in 1-2 years of age group and decreased gradually in older animals. The range of antibody titre of animals positive for JD by ELISA was between 71.12 to 255.20 (S/P values).

Key words: Mycobacterium paratuberculosis, ELISA and sheep

Introduction

Johne’s disease or paratuberculosis is recognized as one of the most serious and widespread chronic infections of ruminants. It is a chronic progressive enteric disease caused by the infection with Mycobacterium paratuberculosis, a small (0.5 x 1.5 microns), facultative intracellular, acid-fast bacillus (Chiodini et al., 1984 and Stabel, 2000). The clinical disease is characterised by chronic or intermittent diarrhea, emaciation and death. In the end stages of the disease, large number of M. paratuberculosis organisms may be shed in the faeces, thereby contaminating the pasture and providing opportunity for transmission of infection to other hosts (Chiodini Van Kruiningen, 1986; Perez et al., 1997 and Goodger et al., 1996).

Johne’s disease of sheep is of considerable economic importance but relatively little attention has been paid to its investigation. It is particularly prevalent in temperate climate and also occurs in wet, humid areas of tropics. The disease has been reported world wide and also in India (Kumar et al., 1981; and Clarke, 1997).

Diagnosis of paratuberculosis is difficult because of the fastidious growth pattern of the microorganism and the paradoxical immune response of the host animal to infection with strong cell mediated immune responses during the early subclinical stages of infection and strong humoral responses latter on (Stabel, 1998).

There are basically three types of diagnostic tests currently in use for the detection of Mycobacterium paratuberculosis infection: bacteriological examination, immunological assays and genetic probe tests.

Faecal smear examination have been found efficient for field screening (Kumar et al., 1982). Culture of Mycobacterium paratuberculosis is considered the most accurate method of diagnosing paratuberculosis in cattle but the same procedure have often been found to be unreliable for detecting Johne’s disease in sheep (Collins and Sockett, 1993).

Intradermal Jonhin test for diagnosis of Johne’s disease is of little value. Gamma interferon in vitro cellular assays have been developed for diagnosis of Johne’s disease. There are several serological tests used most for detecting antibodies to Mycobacterium paratuberculosis, the most common are Agar Gel Immuno Diffusion Test (AGID), Complement Fixation Test (CFT) and Enzyme Linked Immuno Sorbent Assay (ELISA) (Burnside and Rowley, 1994 and Dubash et al., 1996) and either for milk or serum samples. Since the first use of ELISA for detection of antibodies to M. paratuberculosis (Collins, 1996; Cox et al., 1991 and Kreeger, 1991). It can be used for cattle, sheep and goat to M. paratuberculosis, many advancement have been made in ELISA to increase its sensitivity.

Materials and Methods

The present study was conducted on sheep of Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner farm. At the farm sheep were kept in two different sectors, located at distance of around one kilometre, one for Magra breed and another for Marwari breed. On Magra sector previous history of Johne’s (JD) was recorded. A total of 404 sera samples were collected from Magra sector. Although sheep of both sectors were kept separately there were chances of getting infection in sheep of Marwari breed from Magra breed due to sharing of common pasture for grazing, hence 25 sheep from Marwari breed were also taken under the study, age of animals were recorded.

Collection of blood samples

Total 404 sheep were tested for presence of antibody to Mycobacterium paratuberculosis in the serum. Blood was collected aseptically directly from the jugular vein and the serum was separated.

Serological investigation

The antibodies to Mycobacterium paratuberculosis in the serum samples were detected by using Enzyme Linked Immuno Sorbent Assay (ELISA) using a commercial ELISA kit acquired from Institute Pourquere, France.

The Mycobacterium paratuberculosis antigen was
supplied coated to the wells of the 96 wells polystyrene microplate. In order to eliminate the cross reaction samples to be tested were diluted and incubated in a dilution buffer containing a Mycobacterium phlei extract. After incubation, samples were placed in the wells of microplates. If antibodies specific to Mycobacterium paratuberculosis were present in the sample they formed Mycobacterium paratuberculosis-antibody complexes by which the antibodies of ruminant were fixed to the microplate wells. After washing an anti-ruminant immunoglobulin antibody coupled to an enzyme, was added in the wells and incubated. This conjugate bound to the immune-complex. After another washing step, the enzyme substrate (TMB) was added to the enzyme forming a blue compound becoming yellow after blocking. The intensity to the colour was indicative of the level of antibodies in the sample.

The optical density were read at 450 nm. The microplate reader was blanked on air.

Validation criteria

The results were considered reliable if:

1. The positive control had a minimal mean OD_{450} value of 0.350.
2. The ratio between the mean OD_{450} value of the positive control and OD_{450} value of the negative control was greater than or equal to 3.
3. Interpretation

For each sample the ratio S/P was calculated:

\[
S/P = \frac{\text{Mean OD}_{450} \text{value of the positive control} - \text{OD}_{450} \text{of the negative control}}{\text{OD}_{450} \text{value of the sample} - \text{OD}_{450} \text{of the negative control}}
\]

Any sample with a S/P equal or lower than 60 was considered to be from an animal, which had not been infected by Mycobacterium paratuberculosis. Any sample with a S/P was between 60 and 70 was considered to be doubtful. Any sample with a S/P equal or greater than 70 was considered from an animal, which has been infected by Mycobacterium paratuberculosis.

Results and Discussion

The antibodies to Mycobacterium paratuberculosis in the serum samples were detected by using Enzyme Linked Immuno Sorbent Assay (ELISA). Total 404 sera samples of sheep were tested by ELISA for the presence of antibodies to Mycobacterium paratuberculosis, out of which 379 animals were of Magra breed and 25 animals were of Marwari breed. The results show that 67 (16.58%) animals were found positive for JD, 66 (17.41%) of Magra breed and 1 (4%) of Marwari breed. The animals found positive for JD by ELISA in different age groups are presented in Table 2.

Humoral immune response against M. paratuberculosis in serum samples of two sheep flocks detected by absorbed

### Table 1: Number of animals found positive of JD by ELISA

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<th>Breed</th>
<th>No. of animals tested</th>
<th>No. of positive animals (%)</th>
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<tr>
<td>Magra</td>
<td>379</td>
<td>66 (17.41)</td>
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<tr>
<td>Marwari</td>
<td>25</td>
<td>1 (4.00)</td>
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<tr>
<td>Total</td>
<td>404</td>
<td>67 (16.58)</td>
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</tbody>
</table>

### Table 2: Age wise distribution of animals found positive for JD by ELISA

<table>
<thead>
<tr>
<th>Age of animals (in years)</th>
<th>Total animals screened for presence of antibody to M. paratuberculosis</th>
<th>No. of positive animals (%)</th>
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<td>2 (8.69)</td>
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<tr>
<td>Total</td>
<td>404</td>
<td>67 (16.58)</td>
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</tbody>
</table>

### Table 3: Antibody titre in terms of S/P values of animals found positive for JD by ELISA

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Age (in years)</th>
<th>ELISA S/P value</th>
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ELIZA in terms of S/P value has revealed (Table 1) significant antibody titre in 16.58% animals. The antibodies could be detected in all age groups of the animals. The proportion of the animals showing antibody titre is maximum in 1-2 years of age group and decline gradually in older animals. The range of antibody titre in JD positive animals was between 71.12 to 255.20 (S/P values). The maximum titre showing animals fell into age group around 2 and 3 years of age. Antibody titre could be detected in only in
Magra farm. Only one animal in Marwari flock showed antibody titre. Our results on detection of JD in sheep by ELISA test are comparable to the results of Perez and coworkers (1997). Kurade et al. (2004) found 28.23% of sheep positive for paratuberculosis and also in conformity to findings of Hope and coworkers (2001). Burnside and Rowley (1994), who reported 9.54% and 11.65% of animals positive by ELISA, respectively. Similar findings were reported by Kumthekar and coworkers (2013), who find 2.3% of sheep positive for paratuberculosis by ELISA.

Detection of antibodies against *M. paratuberculosis* have been described as effective tools in the establishment of the prevalence of MAP infection in a flock, and also to screen and confirm the diagnosis of paratuberculosis in animals that present compatible clinical symptoms. A successful control programme requires detection of infection at an early stage and in animals which are subclinical carriers. The test selected for this current study is quick and relatively easy to perform in contrast to the culturing method which is laborious and requires an incubation period of about 8-16 weeks or more. Our results clearly show that detection of subclinical carriers by absorbed ELISA is more effective and specific than detection of AFB shedders. Moreover, it also enables to detect the infection in animals below one year of age.

We recommend strict biosecurity measures within the farms for elimination of the infection. Proper management practices viz. overall cleanliness of the farm, manure handling, newborn-calf care, and restriction of contact between calves and mature animals. Also, sufficient housing space should be created in the large farms to prevent the animal-to-animal closeness and reduce transmission of contagious infections. We also strongly recommended the implementation of a combination of both proper management practices and test-and-cull methods in subclinically infected ovine flocks without overt disease as control strategy for eliminating the pathogen in the ovine farms and preventing their spread to other farms.

**References**


EFFECTS OF KETOSIS ON PLASMA METABOLITES IN BUFFALOES*

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ABSTRACT

The aim of present investigation was to determine the variations in concentration of plasma metabolites (viz. glucose, creatinine, urea and BUN) in ketotic buffaloes as compared to healthy buffaloes. The animals were divided into two groups: in the first group included ketotic buffaloes (n=18) and the second group included clinically healthy buffaloes (n=10). Biochemical examination of blood serum showed a highly significant (P< 0.01) reduction in blood glucose whereas serum creatinine, blood urea and BUN remains unaltered in ketotic buffaloes as compared to healthy buffaloes.

Key words: Ketosis, buffaloes, plasma metabolites

Introduction

The production disease in livestock occupy an important place as they greatly affect high yielding dairy animal. Amongst these diseases, ketosis is a multifactorial metabolic disorder of energy metabolism which is caused by negative energy balance and characterized by relatively high concentration of ketone bodies with concurrent decrease of blood glucose level. It occurs in high producing dairy animal during early lactation in both industrialized and developing countries. The present study will be beneficial for early diagnosis of ketosis in buffaloes particular in and semiarid climatic condition of Rajasthan, which may help to reduce the economical losses of dairy industry because of impaired milk production, decreased productive efficiency, increased voluntary culling and increased treatment cost.

Materials and Methods

In this study, the blood samples were collected from Murrah buffaloes of 4-8 years of age, belonging to Kuchaman city, Nagaur, Rajasthan.

In this study animals were divided into two groups:

(1) Controlled group: comprising of ten clinically healthy buffaloes.

(2) Ketotic group: Comprising of eighteen diseased buffaloes clinically diagnosed with ketosis in recently parturated stage.

The average weight of these buffaloes were 400-480 kg and their age was 4-8 years. Clinically diagnosed ketotic buffaloes having 3rd to 6th lactation number and recently parturated about 15-20 days back and having history of inappetence, pica and sudden fall in milk production. Further the urine sample of suspected animal were analyzed by means of Rothera’s test and strip test for confirmation of ketosis. Serum samples were analyzed for total serum glucose, creatinine, urea and BUN by using commercially available diagnostic kits on spectrophotometer-169 of Systronics. The data obtained were statistically analyzed as per explained by Snedecor and Cochran (2004).

Results and Discussion

In the present study, mean serum glucose level in ketogenic buffaloes (34.91±1.64 mg/dl) was highly significantly (P<0.01) decreasing than mean serum glucose level of healthy buffaloes (63.14±3.74 mg/dl). The observed trend was in close agreement with the findings of Hagawane et al. (2009), Ghanem and El-Deeb (2010), Youssef et al. (2010), Farag and Metwally (2012) in different breeds of buffaloes. The justified reasons for the development of hypoglycaemia in post-parturient ketogenic animals may be attributed to the negative energy balance reflecting greater demand of glucose in the mammary gland (Anantwar and Singh, 1993). The lowered means of blood glucose concentration in early stage of lactation may due to large amount of blood glucose withdrawal by the mammary gland for the synthesis of milk lactose.

The mean±SE value of serum creatinine in ketotic buffaloes (0.88±0.21 mg%) and in healthy buffaloes (0.86±0.02 mg%). The mean value statistically increased nonsignificantly (P>0.05) in ketotic buffaloes as compared to control. This finding is partially supported by Sevinc et al. (1997) in ketotic cows in which they recorded significant increase in serum creatinine level in ketotic cows. In present study, ketogenic animals were in the early stage of ketosis so nonsignificant difference observed in serum creatinine level in ketotic buffaloes as compared to healthy ones. This variation may be attributed to the different species, different feeding and managemental condition, climatic condition, experimental planning, duration of the experiment.

The mean±S.E. value of blood urea and blood urea nitrogen in ketotic and healthy buffaloes were (36.65±1.645), (17.137±1.584) and (28.09±1.022), (13.134±1.938), respectively. The mean value statistically increased nonsignificantly (P>0.05) in ketotic buffaloes as compared to control. The observed trend in blood urea and BUN in present study is supported by Rezapos and Taghinejad-Roudbaneh (2011) and Anoushepour et al. (2014) in ketotic ewe and is partially supported in ketotic cows in which they reported significant increase in blood urea and BUN as a result of ketosis. Although, in our study nonsignificant increase in blood urea and BUN in ketotic buffaloes was observed. This variation may be attributed to the different species, composition of ration, different managemental condition.

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climatic condition, experimental planning and duration of experiment.

References

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NS = Non Significant (P>0.05), *=Significant at 5% level (P≤0.05), **=Significant at 1% level (P≤0.01)
Note: Mean comparison have been made within two phases.
OCCURRENCE AND PATHOLOGY OF UPPER GASTROINTESTINAL PARASITES IN CAMEL (CAMELUS DROMEDARIES)†

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ABSTRACT

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Samples of tongue, oesophagus and stomach compartments of 246 camels (Camelus dromedaries) from western Rajasthan were examined between February 2014 to January 2015. By gross and histopathological examination 21.78% of suspected samples were found positive for parasites. Sarcocystis in tongue (5.12%), in oesophagus (10.25%) and Haemonchus species worms in abomasum (6.41%) were found in the upper gastrointestinal tract of camel. In sarcosporidiosis, different sized dark stained sarcocysts in between muscle bundles of oesophagus and tongue were seen with the mild cellular infiltration mainly of eosinophilic granulocytes surrounding the lesions. Haemonchus longistipes infected abomasum showed thickened wall and oedematous folds with focal areas of haemorrhage with reddish thread like Haemonchus spp. worms between mucosal folds. Histologically, abomasum showed marked haemorrhages and congestion between gastric glands and hyperplasia of gastric glands with infiltration of eosinophils, lymphocytes and macrophages. These lesions could reduce the productivity of the infested dromedary. In conclusion, strategic deworming of camel using broad-spectrum anthelmintics is necessary to increase the productivity of camels.

Key words: Camel, parasites, occurrence, histopathology, Rajasthan

Introduction

The camel (Camelus dromedarius) has an important place in the desert ecosystem. This species is often adapted to hot and desert travel, that’s why it is known as “Ship of the desert”. The camel tolerates high temperature, solar radiation and water deprivation. The temperature of skin remain cool due to coarse and well ventilated hairs on its back which allow evaporation to take place on the surface of the skin (Mathur et al., 2013). In the present context, the camel is not only a draught species but also used for racing, desert safari, milk, meat, leather and it’s hair is also useful. Pathogenic diseases, poor nutrition and traditional management system have restricted their full utilization (Bekele, 2002). Gastrointestinal parasites injure their hosts by a wide variety of mechanisms, mainly reduction in voluntary food intake and loss of productivity. However, the clinical manifestation is subclinical or asymptomatic in which animals appear normal but are performing at below their full potential. Sarcocyst and Haemonchus longistipes are common parasites of upper gastrointestinal tract of camel. Haemonchus longistipes is the most pathogenic nematode of camel that may be associated with clinical disease and can be fatal. Anaemia is one of the pathogenic effects of gastrointestional parasites. Moreover, few studies have been conducted on GI helminths of camels (El Bihari, 1985; Abdul-Mogod, 2001; Bekele, 2002). Hence, the present study was designed to provide preliminary information on the occurrence along with describing both gross and microscopic changes caused by these parasites of camels in Western Rajasthan.

Materials and Methods

Sampling and study area

For the present study, 246 samples of the upper gastro-intestinal tract were collected during February 2014 to January 2015 from carcasses of camels subjected to post-mortem examination to veterinary clinics of various districts of western Rajasthan and the upper gastro-intestinal tract samples of dead camels from Municipal Corporation. The tissue samples were also collected from the carcasses of camel submitted to the Department of Veterinary Pathology, College of Veterinary and Animal Science, Bikaner for routine post-mortem examinations. The samples received from the field veterinarians and Border Security Force (BSF), in the Department of Veterinary Pathology were also included in this study. All the samples were properly preserved in 10 per cent formal saline after cutting in to Individual parts.

Histopathological examination

For histopathological examination, processing of tissues done by paraffin embedding using acetone and benzene technique (Lillie, 1965). The tissue sections of 4-6 micron were cut and stained with haematoxylin and eosin staining method as a routine. As far as possible the results were recorded by gross and microphotography.

Results and Discussion

In the present investigation, a total number of 246 specimens of upper gastro-intestinal tract of camel were examined irrespective of age, breeds and sex. Out of these, specimens suspected for abnormalities were further processed for histopathological examination in which 21.78% cases were positive for parasites. Sarcosporidiosis was observed in 5.12% cases in tongue and 10.25% cases in oesophagus. Nematodiasis was found in the 6.41% cases in abomasum of upper gastrointestinal tract of camel. In sarcosporidiosis, different sized dark stained sarcocysts in

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between muscle bundles of oesophagus were seen with the mild cellular infiltration mainly of eosinophilic granulocytes surrounding the lesions (Fig. 1). Each sarcocyst was surrounded by thin layer of muscle fibres which contain numerous bradyzoites.

In nematodiasis *Haemonchus* spp. infested abomasum showed thickened wall and oedematous folds with focal areas of haemorrhage. Abomasum showed varying degrees of ulceration and congestion of the mucosa with reddish thread like *Haemonchus* spp. worms between mucosal folds. Histologically, abomasums showed marked haemorrhages, congestion (Fig. 2) and hyperplasia of gastric glands with infiltration of eosinophils, lymphocytes and macrophages (Fig. 3).

Few studies have been conducted on the occurrence and pathological lesions of gastrointestinal parasites of dromedary (*Camelus dromedarius*) in Rajasthan. By gross and histopathological examination 21.78% of suspected specimens were found positive for parasites, in which sarcosporidiosis was 15.37% and nematodiasis was 6.41%. These findings are lower than those reported by Shekarforoush (2006), Borji et al. (2010) and Rajneesh et al. (2011). The difference in the incidence might be due to difference in environmental conditions and managemental conditions. There is paucity of literature as helminths infections of camels are generally regarded as less of a problem than those in other ruminants. However, gastrointestinal nematodes are known to undermine the overall health and productivity of camels. *Haemonchus longistipes* is the most pathogenic strongyle nematode of camels that may be associated with clinical disease and can be fatal. The damage caused by these nematodes included abundant mucus secreting gastric cells, flattening of the mucosa, villous atrophy, haemorrhages and cellular infiltration, mainly of eosinophile. *Haemonchus* spp. worms are voracious blood sucking abomasal nematode and its infestation most importantly reduces voluntary feed intake and increases endogenous losses of protein via excreta (Pathak and Tiwari, 2012). These lesions could reduce the productivity of the infected dromedary (McGavin and Zackary, 2007). In conclusion, the results of this study showed that strategic deworming of camel using broad-spectrum anthelmintics is necessary to increase the productivity of camels. Moreover, further epidemiological studies should be conducted in different seasons and regions of the state to provide more information about the seasonal dynamics of the gastrointestinal parasites of dromedary in Rajasthan.

**References**


OCCURRENCE AND PATHOLOGY OF COLIBACILLOSIS IN INTESTINE OF CATTLE (BOS INDICUS)

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ABSTRACT

Colibacillosis continues to remain one of the most important disease entities of cattle/cattle calves. The present study was undertaken to elucidate occurrence and pathology of colibacillosis in cattle. This study revealed that occurrence of colibacillosis in intestine was 41.02 per cent in cattle in Rajasthan state. The gross changes in intestine were congestion and haemorrhages in intestine. Histopathological changes revealed haemorrhagic enteritis, hyperplasia of goblet cells, degeneration and desquamation of lining epithelium of villi in intestine.

Key words: Cattle, E. coli, intestine and histopathology

Introduction

In India cattle population is an integral part of the agriculture. The cattle biodiversity in India constitutes 33 well defined breeds of cattle. India is highest milk producing country of the world and total milk contribution of cows is 40% in total milk production of 90.7 million metric tones. Cattle is one of the key animal in agriculture economy contributing substantially to the gross national products by the way of good quality milk, export quality leather, physical power etc. Colibacillosis is one of the most important bacterial disease and is a major cause of morbidity and mortality in ruminant, particularly during first few weeks of their life. Prevalence of colibacillosis has increased in recent years due to several reasons, which include size of herd, improper feeding, poor livestock rearing system and increased population density. E. coli infection is worldwide problem in new born animals and serious outbreaks occur mostly in first fortnight of life (Acres, 1983).

Materials and Methods

For the present study, a total of 345 cattle (mainly 0-5 years of age) irrespective of sex and breeds were examined. Out of these 78 cattle/calves showing frank macroscopic lesions were used for further study.

For this, tissue samples of intestine and liver were collected aseptically and confirmed by streaking on MacConkey agar petriplates eosine and methelylene blue. For histopathological examination, All samples were promptly preserved in 10% formal saline and processing of tissues were carried out in paraffin embedding using acetone and benzene technique. The tissue sections of 4-6 micron thickness were cut and stained with haematoxylin and eosin staining method as a routine.

Results and Discussion

Colibacilli incidence in this study of intestine was 41.02%. Grossly, the affected intestines revealed haemorrhagic foci and patechial haemorrhages. Yellowish coloured material present in lumen of intestine at few places which is appear as mucinous exudates. All these findings were also reported by Singh and Singh (1983) and Janke et al. (1989). On microscopic examination, intestine showed histopathological alteration which included petechial haemorrhages, congestion and hyperplasia of goblet cells (Fig 1). It also showed marked infiltration of neutrophils, macrophages and lymphocytes in mucosa and submucosa, desquamation of the epithelial cells which covers upper part of villi. These findings were also observed by Wales et al. (2001) and Sharma et al. (2003).

Fig. 1: Microphotograph of intestine showing marked haemorrhages in submucosa and marked mononuclear infiltration (H&E, 100X).

References

OCCURRENCE AND PATHOLOGY OF AMPHISTOMIASIS IN RUMEN OF SHEEP (OVIS ARIES)

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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT

The present study was conducted to investigate the occurrence and pathology of amphistomiasis in rumen of sheep (Ovis aries). A total of 210 (two hundred ten) ruminal tissue samples were collected from sheep. Out of these, 102 ruminal samples showing frank macroscopical lesions were used for further study. The amphistomiasis was found in 29.41 per cent (30 out of 102) cases. Grossly adult flukes were found in rumen. Many of them were attached with ruminal mucosa. Histopathological changes revealed that oral sucker of flukes plugged on ruminal papillae’s, excessive keratinization, shortening and blunting of ruminal papillae. In some cases marked eosinophilic infiltration in submucosa also reported.

Key words: Sheep, amphistomiasis, rumen, histopathology

Introduction

Sheep (Ovis aries) are one of the earliest domesticated hoofed animals. Due to improper care, unhygienic environment, extreme climate and close contact with infected animals they get infected with a variety of parasites. Parasitism in goats and sheep is a substantial problem. Amphistomes were the predominant gastro-intestinal helminths affecting sheep (Singh et al., 2013). Amphistomiasis caused by ruminal flukes, is an important parasitic disease of goats and sheep, causes blunting and shedding of papillae, excessive keratinisation, rough body coat, emaciation and anorexia. Sometimes diarrhoea as well as mud coloured foul smelling faeces (Singh et al., 2009).

Materials and Methods

A total of 210 ruminal tissue samples were collected in plastic bag from slaughter house Bikaner and necropsies reported at CVAS, Bikaner, during the period from November 2011 to December 2012. Out of these, 102 samples showing frank macroscopic lesions were processed by histological techniques. For this, tissue samples showing frank macroscopic lesions were preserved in 10 per cent formal saline and processed mechanically by paraffin embedding by acetone and benzene technique (Lillie, 1965). The tissue sections of 4-6 micron thickness were cut and stained with haematoxylin and eosin method of staining.

Results and Discussion

In the present study, amphistomiasis was found in 29.41 per cent cases (30 out of 102). Grossly, presence of live reddish flukes are plugged on papillae (Fig. 1). Similar finding also be reported by (Chishti, 2011 and Balachandran et al., 2010).

Microscopic features such as marked eosinophils infiltration in sub mucosa and excess cornification of stratum corium without ruminal ulceration. Some flukes were attached with ruminal papillae’s by their oral sucker (Fig. 2). Similar findings also described by (Kumari et al., 2015; Singh et al.,

Fig.1: Gross photograph of rumen showing live reddish ruminal fluke, plugged on ruminal papillae’s

Fig.2: Microphotograph of rumen amphistomiasis, showing amphistome plug on ruminal papillae along with infiltration of eosinophil in submucosa(H&E, 45x).

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1984; Vasilev et al., 1985; Tariq et al., 2011 and Uddin et al., 2010).

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References
OCCURRENCE OF CIRCULATORY DISTURBANCES - A PATHOLOGICAL CONDITION OF INTESTINE IN DROMEDARY CAMEL

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ABSTRACT

The present study was undertaken during period of September 2013 to January 2014 to study occurrence and histopathology of various circulatory disturbances of intestine in camel. During study period a total of 172 samples of camel intestine, irrespective of age, sex and breeds were examined. Out of these samples, 103 (59.88%) showing frank gross lesions were collected and tissue sections from these samples were subjected to histopathological examination. Various circulatory disturbances of intestine were recorded in 37.86 per cent (39 out of 103). Gross examination showed distended venous network on serosal surface of intestine in congestion, brownish-red coloured streaks and petechie on the mucosal surface of intestine in haemorrhagic condition and thickened submucous tissues than normal tissues were observed in oedema. Microscopic examinations revealed predominance of engorged blood vessels in congestion, focal haemorrhagic infiltration of intestinal tissue in haemorrhagic condition and showed thickening and expansion of submucosa in oedematous changes.

Key words: Camel, circulatory disturbances, histopathology

Introduction

The camel (Camelus dromedarius) forms an integral part of the culture and agriculture of the fragile desert eco-system. With its unique biophysiological characteristics, the camel has become an icon of adaptation to challenging ways of living in arid and semi-arid regions. The camel is used as a beast of burden for transporting goods as well as people of desert area besides agricultural works. Milk is often the only regular food source for its owners. It is also used by border security forces for guarding the frontiers of our country. The camel has played a significant role in civil law and order, defence and battles from the ancient times till date (NRCC. VISION 2030). About 85% of the camel population inhabits mainly eastern and northern Africa and rest in Indian subcontinent and Middle East countries (NRCC, VISION 2030). The majority of world’s camel population is of dromedary type except small population of Bactrian camels in central Asia. 81.37% population of Indian dromedary camels is possessed by only Rajasthan (0.323 million) state and rest camel population is found in Gujarat, Haryana, Bihar and Uttar Pradesh (19th Indian livestock census, 2012). In Rajasthan, camel population has decreased by 35.23% over the previous two census and the total 0.323 million camels were left in 2012 (19th Livestock Census 2012) which were 0.498 million in 2003 (17th Livestock Census, 2003).

Although, camel is a well adapted animal for arid and semi-arid area but some time it also suffered from some gastro-intestinal disturbances and diseases of gastro-intestinal system affects performance of the animal and it is also a challenge to the veterinarian to arrive at a definite diagnosis of a particular problem. The major etiological agents which are responsible for the cellular and vascular damage are physical, chemical, bacterial, viral, fungal and parasitic agents, the severity varying with nature of their lesions. Certain lesions that affect the intestine comprises of some frequently encountered diseases in clinical practice for instance infarcts, congestion, haemorrhage and oedema etc. In addition to circulatory disturbances, intestinal coccidiosis (Kinne et al., 2002), multicentric fibromyxoid peripheral nerve sheath tumour (Khodakaram-Tafti and Khordadmehr, 2011), para-tuberculosis (Alharbi et al., 2012), tuberculosis (Kasaye et al, 2013), haemorrhagic enteritis (Kumar et al., 2013), gastrointestinal parasitic infestation (Radfar and Aminzadeh Gowhari 2013; Duguma et al. 2014) and intestinal obstruction (Kumar et al., 2014), were also reported. Systematic studies on the histopathology of circulatory disturbances in camel were few and the published literature consists almost entirely on case reports hence, present research work was planned to study the occurrence and histopathology of circulatory disturbances in camels of western Rajasthan.

Materials and Methods

For the present study 172 samples of the intestinal tract were collected from camels of either sex, irrespective of age groups and breeds during the period of study from September, 2013 to January, 2014. Out of these, 103 samples showing gross lesions were used for further study. The tissue specimens for proposed investigation were collected from carcasses of camels subjected to post-mortem examination to various veterinary clinics of various districts of western Rajasthan (Bikaner, Jaisalmer, Jodhpur and Barmer) because majority of camel population 47.91 per cent (0.1560 million out of 0.3257 million) resides in these four districts (19th Livestock Census, 2012). The intestinal samples of dead camels from Municipal Corporation Bikaner and samples of camels submitted to the Department of Veterinary Pathology from Teaching Veterinary Clinical Complex, field veterinarians and...
Border Security Force (BSF) for routine post-mortem examinations were also included in this study. All the samples were collected in 10 per cent formal saline for histopathological examination. The tissues were processed mechanically for paraffin embedding by acetone and benzene technique (Lillie, 1965). The tissue sections of 4-6 micron thickness were cut and stained with H and E stain.

Results and Discussion

An overall incidence of circulatory disturbances conditions of intestinal tract was observed as 37.86 per cent (39 out of 103) corresponded well with the findings of Bhati (2008), recorded 34.39 per cent in buffaloes. These conditions were recorded as follows:

Circulatory disturbances

I. Congestion

This condition was recorded in 27.18 per cent cases while Bhati (2008) reported 19.57 per cent case in buffaloes. In congestion grossly, serosal surface of intestine was distended, showing brownish red colour and distended venous network. These blood vessels were tortuous and filled with blood. The gross findings of congestion were, in close approximation to the findings recorded by Cohrs (1967) and Runnells et al. (1965). Microscopically, there was predominance of engorged blood vessels (Fig. 3) especially in the villi and submucosa and mucosa of intestine. Degenerative changes were seen in the epithelial lining of villi particularly near the tips. Cellular infiltration comprised mainly neutrophils and mononuclear leukocytes (Fig. 3). The microscopic findings were showing engorged blood vessels in mucosa and submucosa corresponded well with the reports of Cohrs (1967), Runnells et al. (1965), Sastry and Rao (2005).

II. Haemorrhage

This condition was recorded in 8.74 per cent cases in present study which is contrast to findings of Musken et al. (2007) who recorded 14 per cent incidence. The gross findings were brownish-red coloured streaks on the mucosa (Fig. 2), haemorrhagic mesenteric lymph nodes, presence of intraluminal blood and petechiae over entire mucosa which were similar to the findings recorded by Nillo et al. (1974).
Bekele et al. (2002) and Costa et al. (2009). The microscopic findings of haemorrhage such as congested blood vessels and focal haemorrhagic infiltration (Fig. 4) of tissue correspond well with the findings of Abutarbush and Radostits (2005).

III. Oedema

This condition was recorded in 1.94 per cent cases. Gross findings of oedema like thickened submucous tissue than normal submucous tissue (Fig. 1). These oedematous changes were in well agreement with findings recorded by Cohrs (1967), Tafti et al. (2001) and McGavin and Zachary (2007). Microscopic findings were pronounced oedematous changes in submucosa which thickens and expands submucosa with pink staining fluid correspond well with the reports of Konishi et al. (1975), Cheryl et al. (2001), Jubb et al. (2007) and McGavin and Zachary (2007).

Acknowledgements

The authors are highly thankful to Dean College of Veterinary and Animal Science, Bikaner for providing necessary facilities.

References


OCCURRENCE AND PATHOLOGY OF VARIOUS CONDITIONS OF OVARY IN FEMALE GENITAL TRACT OF CATTLE

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ABSTRACT

The present investigation was carried out from May 2009 to October 2009. During this period a total number of 390 samples of female genital tract were examined from cattle of different age and breeds. Out of these 55 samples showing gross lesions were collected and tissue sections from these were subjected to histopathological examination. The overall occurrence of various pathological conditions affecting the female genital tract of cattle was observed as 40 per cent. Ovary revealed pathological conditions in 35.25% cases as: oophoritis 3.20%, follicular cyst 9.61%, cystic corpus luteum 0.64%, granulosa cell tumour 1.92%, persistent corpus luteum 3.84%, parovarian cyst 1.28%, hypoplasia 0.64%, ovarobursal adhesion 3.20%, subactive ovary 2.56%, sclerosed ovary 3.20%, follicular atresia 2.56%, anovular cords 1.28%, folliculoids 0.64%, haemorrhage 0.64% cases.

Key words: Cattle, ovary, cyst, pathological conditions

Introduction

India is an agriculture dependent country and rearing of livestock is subsidiary to agriculture. Animals are the backbone of agriculture economy of our country. The cattle husbandry and dairying is an important thrust to rural economy in this arid region of Rajasthan. The population of cattle in Rajasthan is 12.41 million according to 18th Indian Livestock census, 2007. Cattle are prone to various infectious and non-infectious diseases which leads to drop in production and economy. Histomorphological lesions in genital organs with or without apparent gross lesions lead to disturbances in reproductive cycle and disturbed cycle results in a large proportion of failure of conception and are an important cause of sterility. The conditions of ovary include oophoritis, cystic ovarian disease, neoplasm and other conditions like atretic follicles, hypoplasia, folliculoids, granulosa cell tumour etc. Cystic ovarian disease is the most important reproductive disorder responsible for abnormal oestrus behaviour and infertility. In Rajasthan, so far very little efforts have been made to study the etiology, occurrence and pathology of various lesions in genital tract of cattle. Therefore, it becomes pertinent to study the genital tract affections in cattle.

Materials and Methods

The specimens of organs of female genital tract of cattle for the proposed investigation were collected from the carcasses of cattle irrespective of age and breeds. For the present investigation, a total number of 390 specimens of female genital tract of cattle were collected from various municipal areas of Bikaner, Jodhpur, Kota districts irrespective of age and breeds. The samples were also collected from the carcasses submitted to the Department of Veterinary Pathology, College of Veterinary and Animal Science, Bikaner for routine post-mortem examination. During post-mortem examination, the samples were thoroughly examined visually and manually for various pathological abnormalities such as colour, consistency, shape and size, presence of tumours etc. The study was conducted from May to October 2009. For histopathology the fixed tissues were processed mechanically for paraffin embedding by acetone and benzene technique (Lillie, 1965). The sections of 4-6 micron thickness were cut and stained with routine hematoxylin and eosin staining method. As far as possible, results were recorded by gross observations and photomicrographs.

Results and Discussion

Out of these 390 specimens, 55 specimens suspected for abnormalities were further processed for histopathological examination which revealed several overlapping conditions. These genital tracts were examined grossly and 55 selected organs were preserved in 10% formalin and finely submitted to histopathology. All organs revealed several overlapping conditions, may be of hormonal as well as infectious origin.

In the present study, various kinds of affections were recorded in ovary to the extent of 35.25 per cent. The present incidence was slightly higher than the observations of Mukherjee (1980) as 30.50 per cent. However, in available literature this incidence varied from 4.41 per cent (Rao and Rajya, 1976b) to 41 per cent (Narnaware et al., 2009). The percentage incidence of ovarian abnormalities was towards the higher due to diseases, poor nutrition, inadequate herd management, anatomical deformity of the genital tract, hereditary, hormonal disturbances or environmental changes, makes the animal infertile.

In present study 3.20 per cent cases of oophoritis were recorded, while Mukherjee (1980) reported 3.80 per cent cases of necrotic oophoritis and Sharma et al. (1993) reported 3.33 per cent incidence of oophoritis. Oophoritis was identified...
histologically, as diffuse infiltration of lymphocytes in the medulla and perivascular lymphocytic infiltration in the hilar region. Similar observations were described by Summer and Campbell (1974) and Azab et al. (2006).

Cystic ovaries might be due to substances, such as phytoestrogens, that may reduce fertility. Feeding of cows for prolonged periods on clover, lucerne (alfalfa) or other plants rich in phytoestrogens may lead to cystic ovaries.

A follicular cyst was recorded in 9.61 per cent cases. Similar observations were recorded by Mukherjee (1980) as 9.60 per cent. The most widely accepted theory of the origin of cystic ovarian disease is that, there is aberration of the preovulatory surge of luteinizing hormone, either the absence of the surge or mistiming of the surge, which cause the disease. There is clearly a genetic predisposition also to the disease in certain families (Jubb et al., 2007).

Cystic corpus luteum was observed in 0.64 per cent cases. Slightly higher incidence of 0.80 per cent was recorded by Nair and Raja (1974). However in available literature this incidence varied from 0.17 per cent (Shalash, 1958) to 11.11 per cent (Wahid et al., 1991). These cysts represent an abnormal accumulation of fluid at the centre of corpus luteum, where a small fluid containing cavity is normal. Nothing can be said about their cause although this could be conceivably being some accidental insufficiency of blood supply (Jones et al., 1997).

Granulosa cell tumor was recorded in 1.92 per cent cases. Higher incidence was recorded by Mukherjee (1980) as 5.70 per cent. Two cases of granulosa cell tumor was reported by Wahid et al. (1991). A small spherical eosinophilic mass, so called call-exner body, lies in the center of the rosette. Tumor was lined by granulosa cells, which were surrounded by thecal cells. Similar findings were recorded by Jones et al. (1997) and Jubb et al. (2007).

Persistent corpus luteum was observed in 3.84 per cent cases, which was slightly lower than the incidence reported by Sujata (2000) in 4.00 per cent cases. Lower incidence was reported by Rao and Rajya (1976b) in 0.16 per cent. These findings are same as described by Dwivedi and Singh (1971). Present findings indicated that persistence of corpora lutea in these cases might be due to lack of diminished secretion of luteolytic substance by the uterus as suggested by McDonald (1975).

Parovarian cyst was recorded in 1.28 per cent cases, almost same incidence was reported by Rao and Rajya (1976b) as 1.09 per cent cases. However, in available literature incidence of parovarian cyst varied from 1.09 per cent (Rao and Rajya, 1976b) to 6.5 per cent (Narnaware et al., 2009).

Hypoplasia was seen in 0.64 per cent case, which was closely related to the incidence reported by Khan (1970) as 0.66 per cent cases. However, reported literature about incidence of hypoplasia of ovary varied from 0.08 per cent (Rao and Rajya, 1976b) to 1.50 per cent (Sujata, 2000).

Ovarobursal adhesion was seen in 3.20 per cent cases. Higher incidence recorded by Fathalla et al. (2000) as 8 per cent. Ovarobursal adhesions were probably due to breeding and postpartum complications. Many factors contribute to the predisposition of the reproductive tracts to infections during these periods (Fathalla et al., 2000).

Subactive ovary was seen in 2.56 per cent cases which was similar to Shalash (1958) as 2.64 per cent cases. Higher incidence was recorded by Khan (1970) as 12.34 per cent. The subactive ovaries were due to the low level of thyrotropic hormone in blood, diminished release of gonadotrophin and malnutrition specially the deficiency of vitamin A, mineral and trace elements in the ration (Dwivedi and Singh, 1971).

Sclerosed ovary was recorded in 3.20 per cent cases, which was slightly lower than those reported by Mukherjee (1980) as 3.80 per cent cases. This condition could be due to hypothalamic dysfunction by low blood levels of thyrotropic hormone. Nutritional error might have a significant role in production of disease (Sastry and Rao, 2005).

Follicular atresia was observed in 2.56 per cent cases, which was closely related to the incidence reported by Sujata (2000) as 2.50 per cent cases. Formation of atretic follicles may be due to malnutrition and possibly iodine deficiency were the causes of follicular atresia in an estrous animals (Rahman et al., 1977).

Anovular cords was recorded in 1.28 per cent cases, which was slightly higher than the incidence reported by Rao and Rajya (1976b) as 1.05 per cent cases. These anovular cords and follicles were associated with hypoplastic ovaries. Similar correlation was suggested by Sastry and Rao (2005).

Folliculoids was observed in 0.64 per cent case, which was slightly higher than those reported by Rao and Rajya (1976b) as 0.15 per cent cases. These folliculoids were associated with anovular cords.

Haemorrhage was recorded in 0.64 per cent case, which was higher than those reported by Rao and Rajya (1976b) as 0.09 per cent cases. It may be due to bacterial infection, such as Salmonella pullorum, mechanical and toxic conditions (Cohrs, 1967).

References
Census (2007) 18th Indian Live Stock Census. www. FAO. org. in
Table 1: Incidence of various pathological conditions of ovary in female genital tract in cattle

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of condition</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>OVARY</td>
<td>55</td>
<td>35.25</td>
</tr>
<tr>
<td>1.1</td>
<td>Inflammatory condition</td>
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<td>3.20</td>
</tr>
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<td>Oophoritis</td>
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<td>3.20</td>
</tr>
<tr>
<td>1.2</td>
<td>Cystic ovaries</td>
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<td>10.25</td>
</tr>
<tr>
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<td>Follicular cyst</td>
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<td>9.61</td>
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<td>Cystic corpus luteum</td>
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<td>0.64</td>
</tr>
<tr>
<td>1.3</td>
<td>Neoplasm</td>
<td>3</td>
<td>1.92</td>
</tr>
<tr>
<td>1.3.1</td>
<td>Granulosa cell tumour</td>
<td>3</td>
<td>1.92</td>
</tr>
<tr>
<td>1.4</td>
<td>Miscellaneous conditions</td>
<td>31</td>
<td>19.87</td>
</tr>
<tr>
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<td>Persistent corpus luteum</td>
<td>6</td>
<td>3.84</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Parovarian cyst</td>
<td>2</td>
<td>1.28</td>
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<td>Hypoplasia</td>
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<td>Sclerosed ovary</td>
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<td>Anovular cords</td>
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OCCURRENCE AND PATHOLOGY OF COCCIDIOSIS IN SMALL INTESTINE OF CATTLE IN RAJASTHAN

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ABSTRACT

A total number of 518 samples of small intestine were examined from cattle of different age groups, breeds and either sex. Out of these 151 samples showing gross lesions were collected and tissue sections from these were subjected to histopathological examination with objective to find out the occurrence, etiology, type, pattern and morphology of various pathological conditions of small intestine of cattle in Rajasthan.

Key word: Coccidiosis, small intestine, cattle, Rajasthan

Introduction

Bovine coccidiosis occurs in all parts of the world and serious outbreaks may occur in dairy herds (Soulsby, 2005) via affected or carrier animals (Sastry and Rao, 2005) because when animals are in crowded conditions associated with poor sanitation, faecal-oral transmission of large numbers of organisms can occur (McGavin and Zachary , 2007).

Materials and Methods

Collection of samples

A total of 518 samples were procured and examined from small intestines between May to October 2010 from various municipal areas of Bikaner, Jodhpur and Kota districts from the carcasses of cattle irrespective of sex, age and breeds. The samples were also collected from the carcasses submitted to the Department of Veterinary Pathology for routine post-mortem examination. The samples were thoroughly examined visually and manually for various pathological abnormalities such as colour, consistency, shape and size, presence of tumours and ulcers. Out of 518 samples, 151 showing frank macroscopic lesions were collected for further histopathological examination. The fixed tissues were processed mechanically for paraffin embedding by acetone and benzene technique (Lillie, 1965) and the sections of 4-6 microns thickness were cut and stained with routine haematoxylin and eosin staining method. The duplicate sections were stained with special stain Periodic acid Schiff for parasites (Culling, 1974) wherever necessary. The results were recorded by gross and photomicrographs.

Results and Discussion

Occurrence

In the present study, overall occurrence of coccidiosis in small intestine of cattle was recorded as 3.31 per cent. This condition was noted in 5 (3.31%) cases. Grossly, variable hyperaemia and haemorrhages were seen in the intestine. Microscopically, the small intestine showed hyperemia, destruction of epithelium, leukocytic infiltration and edema (Fig. 1). Coccidia were visible in the epithelial cells of crypts of Liberkuhn (Fig. 2).

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haemorrhages in the intestine are in close approximation with earlier reports of Thomson (1989) and Chauhan (2003).

The Microscopic findings showing coccidia in the epithelial cells of crypts of lieberkühn in terminal part of ileum along with destruction of epithelium, leukocytic infiltration and oedema are in accordance with the findings of Soulsby (2005) and Jubb et al. (2007).

References


NOTICE

Due to increasing cost of publication, it has been decided to levy following charges from author(s) w.e.f. January, 2017 issue:

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CHIEF EDITOR
PATHOMORPHOLOGICAL AND HISTOPATHOLOGICAL OBSERVATIONS OF BILE DUCT CARCINOMA IN DOGS (CANIS FAMILARIS)

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ABSTRACT

A total of 761 carcasses of dogs were examined for liver lesions irrespective of age, sex and breeds. Out of these, 294 liver samples showing frank macroscopic lesions were collected in 10 per cent formal saline for histopathological examination. Bile duct carcinoma was recorded in three cases (1.02%) which were characterized by massive solitary growth or multiple nodules of variable size. The gross and histopathological characteristics have been described.

Key words: Dogs, bile duct carcinoma, histopathology

Introduction

Liver is known to be as largest gland of the body (Ford, 1965) and important vital organ because it is essential for the life. The liver represents the most important organ of the body in respect to metabolism. Liver plays a major role in detoxification, phagocytosis and nutrition. It also imparts a great role in metabolism of protein, carbohydrate, fat and haemoglobin. Various pathogenic organisms, parasites and toxins entering the blood stream have to pass through the liver because liver has rich venous and arterial supply, so the chances of blood born infections and toxic conditions of liver are numerous. Bile duct carcinoma is one of the very important neoplastic conditions in dogs.

Materials and Methods

In the present study, a total of 761 carcasses of dogs were examined for liver lesions. Out of these, 294 liver samples showing frank macroscopic lesions were collected in 10 per cent formal saline for histopathological examination. The preserved tissues were processed mechanically for paraffin embedding by acetone and benzene technique (Lillie, 1965). The tissue sections of 4-6 micron thickness were cut and stained with haematoxylin and eosin method of staining.

Results and Discussion

Bile duct carcinoma was recorded in three cases (1.02 per cent). The gross findings of bile duct carcinoma observed as massive solitary growth sometimes multiple nodules of variable size. The affected liver showed tumor mass in cut surface (Fig. 1). These findings were in close approximation to the findings recorded by Moulton (1978).

Microscopically, there were formation of ductules and acini and sometimes papillary formations were also seen (Fig. 2). These structures were lined by anaplastic cuboidal or columnar cells. The tubules did not contain bile but in well differentiated specimens might contain mucin. These findings are in concurrence with the observations of Patnaik et al. (1981) and Ranganath et al. (2001).

References


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HISTOLOGICAL STUDIES ON THE SPLEEN OF MARWARI GOAT
(CAPRA HIRCUS)

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ABSTRACT

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The microscopic studies of 30 spleens showed that the capsule was composed of smooth muscle, collagenous and elastic fibres. Trabeculae emerged from the capsule and entered into parenchyma and subdivided it into smaller compartments by forming a net like framework. Splenic parenchyma was composed of white and red pulps. White pulp was lymphoreticular tissue consisted of lymphatic nodule and peri-arterial lymphatic sheath. Peri-arterial lymphatic sheath was less abundant. Red pulp was consisted of pulp arterioles, sheathed capillaries, terminal capillaries, splenic sinusoids and splenic cords. The splenic sinusoids were less abundant and poorly developed.

Key words: Spleen, Marwari goat, histology

Introduction

In the developing countries, a number of large and small farmers rear goat for their livelihood. It is sometimes referred to as ‘Poor man’s cow’. Research on this species has been largely neglected, specially its histology. So the detailed study on the different aspects of goat anatomy is required. Selection of the species is based on the local inhabitance and meagre availability of literature on this quadruped. Goat is well known for its production qualities and resistance to adverse climatic conditions prevailing in the arid and semiarid zone.

Materials and Methods

For the histological examination the small pieces of tissues (2 mm size) were collected from 30 spleens. From each spleen, the tissues were collected from fifteen fixed anatomical regions to explore the regional differences if any. The tissues were preserved in 10% formal saline, Bouin’s fluid and Zenker’s fluid for 48 hrs, 15 hrs and 18 hrs, respectively and processed for light microscopy by using paraffin of melting point of 58-60°C. The paraffin blocks were sectioned to obtain 5-6 µm thick sections which were stained with the following routine histological stains to demonstrate different components of spleen.

1. Ehrlich’s haematoxylin and eosine stain for routine observation (Luna,1968).
3. Verhoeff’s elastin stain for connective tissue fibres (Drury and Wallington, 1967).

Results and Discussion

The spleen was covered by thick fibro-elastic and muscular capsule (Fig. 1 and 2) as also reported by McLeod et al. (1964) in bovine, Bajpai (1992) in goat. The capsule was invested by serous peritoneal covering, which was in accordance with the observation of Trautmann and Fiebig (1957) in domestic animals. Simple squamous mesothelial cells of peritoneal covering were irregular in shape with centrally placed spherical nucleus and attenuated strands of cytoplasm (Fig.1) in present study. Similar finding was observed by Miller et al. (1965) in dog, Awal et al. (1992) in indigenous cattle, Bajpai (1992) in goat. The smooth muscle fibres were arranged in three layers; outer, middle and inner (Fig. 2). The fibres were parallel to the surface in outer and inner layer and oblique in middle layer. These findings are consonance with the finding of Bajpai (1992) in goat. Branching trabeculae emerged from capsule and entered in to the interior of the splenic parenchyma, which concurred with the findings of McLeod et al.
lymphatic sheath (Fig. 2), findings are similar to Banks (1981) and Nicander et al. (1993). The peri-arterial lymphatic sheaths were less abundant in the spleen of goat, which was in agreement with the findings of Bajpai (1992) in goat.

In the present study, splenic nodules of various sizes were observed as an ovoid mass. The nodule was composed of aggregations of the lymphatic tissue along the course of small pulp arteries as was previously described by Nickel et al. (1979) in domestic animals. It was also confirmed by the findings of Awal et al. (1992) in indigenous cattle that the splenic corpuscles were ovoid mass of compact lymphatic tissue and had a fine meshwork of reticular connective tissue consisting mainly lymphocytes of various sizes. While describing the spleen of goat, Bajpai (1992) reported that lymphatic nodules generally occurred singly and occasionally aggregations of two to three nodules were observed.

 Eccentrically situated arteries, the nodular artery or central artery were observed in the nodule (Fig. 2). This concurred with the findings of Trautmann and Fiebiger (1957) in domestic animals that each splenic corpuscle contained a small artery of variable position, falsely called as central artery. Trautmann and Fiebiger (1957) in domestic animals that the space between the white pulp and trabeculae were occupied by the red pulp. Similar findings were observed in the present study. Red pulp consisted of pulp arterioles, sheathed capillaries, terminal capillaries, splenic nodules and splenic cords which was concurred with the findings of Greep (1965). The splenic sinusoids were less abundant and poorly developed, similar observations were evidenced by Snook (1950) in horse.

References
MORPHOLOGICAL STUDIES OF THE LIVER IN MARWARI GOAT (CAPRA HIRCUS)

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ABSTRACT

Present study was conducted on the livers of 50 adult Marwari goats. The liver was situated entirely in the right abdominal cavity except a small portion of the ventral lobe directed obliquely downward and forward from 13th rib to 6th intercostal space. It was reddish brown in colour and somewhat rectangular in shape with two surface and four borders. The parietal surface was convex and adopted to the diaphragm. The dorsal border was thick and it was related to the right kidney. The ventral border was thin and curved. The medial border presented the oesophageal notch. The lateral border presented an umbilical fissure. The liver exhibited three distinct lobes as to dorsal, ventral and caudate. The common bile duct joined the pancreatic duct before its termination into the duodenum.

Key words: Marwari goat, liver, parietal, visceral, oesophageal notch and pancreatic duct

Introduction

Goat is well known for its production qualities and resistance to adverse climatic conditions prevailing in the arid and semiarid zone (Chahar and Barhat, 2002). In the developing countries, a number of large and small farmers rear goat for their livelihood. It is sometimes referred to as “Poor man’s cow”. Research on this species has been largely neglected, especially its anatomy. So the detailed study of the different aspects of goat anatomy is required. Selection of the species is based on the local inhabitance and meagre availability of literature on this quadruped. In present study, detailed topography of liver of Marwari goat has been enlightened.

Materials and Methods

The present study was conducted on fifty apparently healthy adult Marwari goat (Capra hircus) of either sex. The livers were procured from the freshly slaughtered animals at Municipal Slaughter House, Bikaner. After dissecting the abdominal viscera relations of the liver with other visceral organs and its topography were studied.

Results and Discussion

The liver was somewhat rectangular, exhibiting two faces and four borders (Fig. 2 and 3). It was distinctly lobated and strongly curved and accurately adopted to the abdominal face of the diaphragm. It’s colour was brown to reddish-brown in the fresh state. In this study, the goat liver was somewhat rectangular, exhibiting two surfaces and four borders and was distinctly lobated.

Position

In the present study, the liver of Marwari goat was situated entirely to the right of the median plane in the abdominal cavity except a small portion of the ventral lobe. Its long axis was directed obliquely downward and forward from the 13th rib to the 6th intercostal space (Fig.1). In sheep May (1955) and Pareek (2000) found similar position of liver. While in ruminants Sisson and Grossman (1958), Raghavan (1964), Prasad and Sinha (1980) and Dyce et al., (1996) found similar position of liver.

Borders

The medial border presented oesophageal notch below its middle, above which the posterior venacava was deeply embedded in this border. It was in agreement with the findings of May (1955), Getty (1977) and Pareek (2000) in sheep, while Raghavan (1964) and Nickel et al., (1979) have reported that in ruminants the posterior venacava was partly embedded in the medial border above the oesophageal notch. The lateral border of the liver in Marwari goat, presented a deep umbilical fissure as reported by May (1955), Raghavan (1964) and Getty (1977) in domestic animals. Dyce et al. (1996) described the much deeper notch in sheep and goat as a differentiating point between liver of large and small ruminants. The dorsal border in Marwari goat was noted to be very thick. It presented a deep impression formed by the right kidney and a smooth area related with caecum. These findings resembled with the finding of May (1955) and Pareek (2000) in sheep and Raghavan (1964) in ox. It was compared with the findings of Grossman (1960) in camel, Getty (1977) in ox and Prasad and Sinha (1980) in buffalo, who observed adrenal impression on the dorsal border, in addition with impression of right kidney. The ventral border was smooth and curved which was agreed by Raghawan (1964) in ruminant liver, with an exception in the finding of Smuts and Bezuidenhout (1987) who stated that the ventral border was thick and marked by numerous fissures in camel, whereas Grossman (1960) mentioned the thin ventral border in camel.

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Surfaces

The parietal surface was convex, which for the most part, adopted to the right half concavity of the diaphragm; remaining surface was related to the 10th to 13th ribs and the intercostal muscle and impressions of respective ribs were seen. The falciform ligament was attached to this surface, extending from the oesophageal notch to the umbilical fissure. A small area at the dorso-medial part was adherent to the diaphragm (Fig. 2).

The visceral surface was concave and very irregular, faced caudoventrally. It presented a portal fissure, almost in middle, little towards medial border. The fissure was a depression bounded by the lesser omentum. The portal vein, hepatic artery, lymph vessels, hepatic nerves and the hepatic duct entered and left the gland through the portal fissure (Fig. 3). The parietal surface was convex in Marwari goat and the convexity for the most part adapted to the right half concavity of the diaphragm. The remaining surface was related to the 10th to 13th ribs and impressions of respective ribs were seen. It was not confirmed by May (1955) and Pareek (2000) who reported in the sheep that the parietal surface was related to the diaphragm and to a small extent to the last two or three ribs near their angles. But the findings of the present study were similar to Prasad and Sinha (1980) who described the costal impressions of the last four ribs on the parietal surface of the liver in buffalo.

The visceral surface of the liver was related to the reticulum, omasum, duodenum and gall bladder. The visceral surface also exhibited relation with few coils of small intestine in Marwari goat, which was not reported previously in any species of ruminants although Sission and Grossman (1958) mentioned that coil of small intestine may also lie on this surface in horses. They have also reported an impression of spleen on this surface in the horse, which was not found in sheep.

The visceral surface presented a distinct portal fissure, from where the portal vein, hepatic artery, hepatic nerve entered into the liver and lymph vessels and hepatic duct left the gland. Ventral to the portal vein three or four hepatic lymph nodes of various sizes were found at the fissure. Similar description was also mentioned by May (1955) and Pareek (2000) in sheep, Modekar et al. (2003) in the goat, Prasad and Sinha (1980) in buffalo, Raghavan (1964), Getty (1977), Nickle et al. (1979) and Dyce et al. (1996) in ruminants.

Other than portal fissure, this surface exhibited following features (Fig. 3).

- A fossa extending from the fissure upto the lateral border which was the impression of the gall bladder. There was a rough area of adhesion of gall bladder on the middle of the fossa.
- In between caudate lobe and gall bladder fossa, the duodenum and part of the caecum were related to the surface.
- Below the fossa, it presented a fissure for the round ligament called umbilical fissure.
- Below the fissure the surface exhibited abomasal impression and few coils of small intestine were also related to this surface.
- The reticular impression was present left to the above.
- The Omasal impression was situated left to the portal fissure, above the reticular impression.

Lobation

The goat liver presented distinct lobation. It was divided
into three lobes (Fig. 3) on the visceral surface the round ligament extended from the umbilical fissure to the umbilicus which divided the organ into a large dorsal and a small ventral lobe. On the parietal surface the line of attachment of falciform ligament marked the division of ventral and dorsal lobe (Fig. 2), which was also observed by Nickel et al., (1979) in ruminants, Prasad and Sinha (1980) in buffalo and May (1955) and Pareek (2000) in sheep. The visceral surface exhibited other two distinct lobes. A caudate lobe above the portal fissure, which was somewhat pyramidal, blunt pointed, overlapped the part of dorsal lobe, having a deep indentation for the right liver. A nodular papillary process was also present. The dorsal lobe was large and caudate lobe was the smallest.

References
HISTOLOGICAL AND HISTOCHEMICAL STUDY ON UROPYGIAL GLAND OF KADAKNATH BREED OF POULTRY

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ABSTRACT

The present study was carried out on twenty uropygial gland of Kadaknath breed of poultry. The samples were fixed in 10% neutral buffered formalin and were processed by routine paraffin embedding technique and paraffin sections of 5 to 7 µ were subjected to histological and histochemical study. Histologically, the uropygial gland was enclosed by a moderate thick capsule which was comprised of collagen fibres with few elastic and reticular fibres along with the blood vessels, adipose tissue, smooth muscle fibres and Herbst corpuscles. The gland parenchyma was composed of many secretory tubules, that filled with fat droplet and that opened into the central ducts. The tubular epithelial cells were classified into germinative, intermediate, secretary and degenerative layers. Lymphatic aggregations and nodules were observed towards the central cavity as well as between the ductules. Melanin pigmentation was characteristic feature regarding Kadaknath breed which were abundant between ductules and secretary tubules of uropygial gland. The capsule, intertubular septae and duct showed intense PAS and Alcian blue-PAS activity whereas intense to moderate activity of PAS were found in all four layers of tubular epithelium.

**Key words:** Kadaknath, uropygial gland, histochemistry, histology

**Materials and Methods**

The study was done on uropygial gland of twenty healthy, 6 to 8-month-old male and female birds of Kadaknath breed of poultry. These birds were sacrificed ethically and glands were fixed immediately in 10% neutral buffer formalin for 24 hours. Fixed glands were processed by routine paraffin embedding technique (Luna, 1968) and paraffin sections of 5 to 7 µ were subjected for histological and histochemical study. For general histological study the tissue sections were stained by H & E, Verhoff's staining for collagen and elastic fibres. Periodic Acid Schiff's staining for glycogen, Alcian blue PAS (pH 2.5) for mucopolysaccharides, alkaline phosphatases method for alkaline phosphate and acid phosphatases method for acid phosphate were implied (Singh and Sulochna, 1997).

**Results and Discussion**

The uropygial gland of Kadaknath birds was comprised of two lobes lies on the base of tail over pygostyle, similar observation were recorded by (Sadoon, 2011) in Starling birds and by (Salibian and Montalti, 2009) in various avian species. Each lobe had single uropygial duct and they joined together by isthmus (Karmore et al., 2011). The histologically uropygial gland of Kadaknath revealed that it was enclosed by a moderate thick capsule constituted of dense connective tissue comprised of collagen fibres with few elastic and reticular fibres with the collaboration of blood vessels, adipose tissue, smooth muscle fibres and Herbst corpuscles (Fig. 1a, 1b) which was similar findings have been reported in broiler and native chicken of uropygial gland by (Mobini and Ziali, 2011), in Goose (Shafianand Mobini, 2014) and in wild and domestic duck (Harem et al., 2005). While the smooth muscle fibres were absent in the capsule of Moorhen (Sawad, 2006) and European Starling birds (Sadoon, 2011).

The gland parenchyma was composed of many secretory

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tubules, that filled with fat droplet and that opened into central main ducts. Ducts were lined by stratified squamous keratinized epithelium. The intertubular interstitial septae were arised from capsule penetrated into the gland which contained mainly collagen and few elastic fibres along with aggregation of lymphocytes and blood vessels. The tubular epithelial cells were classified into germinative, intermediate, secretary and degenerative layers. The basal or germinative layer which was...
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layer of tubular epithelium (Fig. 4), whereas was moderate reaction in all surfaces epithelium of secretary tubuleas observed by (Shafiian and Mobini, 2014) in Goose. Intense glycogen activity due to continuous production of secretion in the gland. The positive PAS staining of the tubule secretion products supported the presence of enzyme labile sialomucins and demonstrated that lipid compounds, particularly glycolipids, occur in the secretion was observed by (Montalti et al., 2001).

Alcian blue PAS activity for acid mucopolysaccharides were moderate in capsule intertubular septae and duct. Weak activity of PAS were founded in all four layer of tubular epithelium (Fig. 5). This is an agreement with the finding of (Shafiian and Mobini, 2014) in Goose, however (Mobini and Ziaii, 2011) could not detect AP positive reaction in broiler and native chicken. The positive alcian blue PAS staining showed that carboxylated acidic mucin, probably sialidase-sensitive sialomucins, are present in the gland secretion. Since mucin are capable of forming viscous solutions, it is possible that these molecules act as lubricants on the body surfaces was observed by (Montalti et al., 2001).

Alkaline phosphatase for alkaline phosphate enzyme was intense in capsule whereas showed moderate in tubular epithelium (Fig. 6). The positive alkaline phosphatase activity in Preen gland was indicative of different compound of Preen gland release inorganic phosphatase from ester compound in alkaline pH. Ishida et al. (1973) noted that there were no activity in tubular of Preen gland but in this studies Kadaknath uropygial gland showed positive activity.

Acid phosphatase for acid phosphate enzyme activity was intense in capsule and tubular epithelium and intertubular septae (Fig. 7). The presence of acid phosphatase from ester compound of preen gland in acidic pH, is similar to the observation stated by (Ishida et al., 1973) in Fowl.

In the present study of Uropygial glands of Kadaknath breed of poultry is no significant difference among the sex, similar observation also recorded by (Salibian and Montalti, 2009).

References


 consisted of one row of flat shaped cells lied on the basement membrane. The intermediate layer was composed of 2-3 rows of polygonal cells lied on the germative layer. The secretory layer formed of 4-5 rows of pyriform or polygonal cells contained lipid droplets and secretory granules. The degenerative layer, which was adjacent to the lumen of each tubule, consisted of a few cells with pyknotic nuclei, similar observations were reported by (Shafiian and Mobini, 2014) in Goose. Each tubules was divided into two different zones, an outer sebaceous and an inner glycogen zones. Melanin pigmentation was unique characteristic feature which were observed throughout the gland parenchyma, ductules, and capsule in Kadaknath breed of poultry. (Fig. 2). It was well know fact about Kadaknath breeds its color stems from the deposition of melanin pigment.

Lymphatic aggregations and nodules were observed towards the central cavity as well as in between ductules. Wild behaviour and to many of the disease resistance could be attributed to lymphatic aggregation and nodules (Fig. 3). This is first recognition and mentioned in this present study for Kadaknath breed of poultry.

Histochemistry of gland revealed that PAS activity for glycogen were intense in capsule, intertubular septae and duct. Intense to moderate activity of PAS were founded in all four
HISTOLOGICAL STUDY OF MAMMARY DUCT SYSTEM DURING LACTATING AND NONLACTATING STAGE IN MURRAH BUFFALO

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ABSTRACT

Present experiment was conducted on mammary gland of twelve Murrah buffalo dividing into two groups (lactating and nonlactating) having six animal in each group. Mammary tissue was comprised of intralobular ducts draining the alveoli, interlobular ducts draining the lobules and interlobar ducts draining the lobes. The diameter of the olinalobular ducts and interlobular ducts were higher in lactating stage than in nonlactating stage.

Key words: mammary, duct, lactating, nonlactating, murrah, buffalo

Introduction

Mammary duct system play important role in the flow of milk from alveoli to lactiferous duct. The morphology of the duct system depends on the physiological status of the mammary gland under the hormonal influence. Revealed literature showed that there is paucity of detailed basic document on the duct system of mammary gland of Murrah buffalo during lactating and involution stage in buffalo. With considerations of above facts the experiment was undertaken to explore the histology of mammary duct system in Murrah buffalo during lactating and nonlactating stage.

Materials and Methods

The present study was conducted on mammary gland of twelve buffaloes to study the histoarchitecture and histometrical parameters of mammary duct system. The mammary gland samples of buffalo were collected from dairy farms nearby Nagpur, Durg, Rajnandgoan and Raipur after their natural death. The samples were ensured for not having any pathological lesions. The samples were categorized into two groups (six each) as lactating and nonlactating/dry by ascertaining the stage of lactation and dry period.

The samples of mammary gland were collected on ice in thermocol box brought to the laboratory. The tissue samples of 3-5 mm thickness were collected and fixed in 10% neutral buffered formalin. After fixation of tissue for 24-72 hours in fixatives, tissue were subjected to dehydration in the ascending grades of alcohol, cleared in xylene and infiltrated with paraffin wax as per the method of Drury and Wallington (1980). Tissue blocks were prepared and 4 to 7 µm thick sections were cut with the help of rotary microtome, mounted on clean albumenized glass slides, and dried on a hot plate at 45-50°C for three hours. Sections were stained and observations were recorded.

Results and Discussion

Present study revealed that the duct system of mammary gland was comprised of intralobular ducts draining the alveoli (Fig. 1), interlobular ducts draining the lobules and interlobar ducts draining the lobes (Fig. 1 and 2). Similar observations were reported by Riviere (2007) in domestic animals.

The intralobular ducts were lined by cuboidal to bistratified cuboidal epithelium in lactating (Fig. 1) and nonlactating stages in buffalo. Present findings are in agreement with Vaish et al. (2015) in the mid prepubertal goats. The diameter of intralobular duct(74.15 ± 4.12 µm) was approximately three times in lactating stage than in nonlactating stage (27.83 ± 4.23 µm). Interlobular ducts were present in interlobular tissue mostly accompanied with blood vessels. The interlobular ducts were lined by bistratified cuboidal epithelium and cuboidal to squamous epithelium throughout the study (Fig. 2). The interlobular ducts had many sacculation in their course. Interlobular ducts was twice greater in diameter in lactating stage (103.93 ± 5.45 µm) than nonlactating stage (52.83 ± 5.63 µm). This greater diameter of intralobular and interlobular ducts in lactating stage might be indicative of the greater secretory activity and carrying capacity of duct system in lactating buffalo. Present findings however, were contradictory to the observations made by Katiyar (1982) who reported larger diameter of intralobular and interlobular ducts in the nonlactating buffalo in comparison to lactating buffalo. Interlobar ducts were lined by cuboidal to squamous epithelium in nonlactating stage, however in the lactating stage it was not possible to observed the lobe under the microscope. Varying amount of secretory material was present in lumen of duct system in lactating stage (Fig.1). While, in nonlactating stage, duct system had less secretory material or empty (Fig.2). Around the ducts, lamina propria made up of collagen fibres were present. These observations are in agreement with Bacha and Bacha (2000) in mammal.

References


Fig. 1: Photomicrograph of mammary gland of Murrah buffalo during lactating stage showing alveoli (A), intralobular duct (D) and bistratified cuboidal epithelium (C) (Orcein X400)

Fig. 2: Photomicrograph of mammary gland of Murrah buffalo during nonlactating stage showing alveoli (A), intralobular duct (D), interlobular duct (ID) and interlobar duct (LD) (H & E X 100)
LOCALIZATION OF CERTAIN DEHYDROGENASES IN THE CAUDA EPIDIDYMIS OF ASSAM GOATS FROM BIRTH TO TEN MONTHS OF AGE#

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ABSTRACT

A total of 18 male Assam goats varying in age from 0-day to 10 months were used in the present study. The animals were divided into six age groups viz. group-I (0-day), group-II (2 months), group-III (4 months), group-IV (6 months), group-V (8 months) and group-VI (10 months) consisting of three animals in each group. The cryostat sections obtained from the cauda epididymides were incubated for the histoenzymic demonstration of various enzymes viz. Glucose-6-Phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), Δ⁵-3 β- hydroxysteroid dehydrogenase (Δ⁵-3 β-HSD) and 17-β-hydroxysteroid dehydrogenase (17-β-HSD). The activity of LDH remained weak to moderate in the epididymal tubular epithelium of the kids below 4 month of age (group-III). However, the enzymic activity for LDH increased to strong with the advancing age of the male goats. The tubular epithelium of the epididymis showed an enhanced activity for MDH enzyme in the male kids with advancing age. Moderate activity of G-6-PDH was observed in the tubular epithelium of the epididymis in male Assam goats at 8 to 10 months of age.

Keywords: Tubular epithelium, cauda epididymis, histoenzymology, Assam goat

Introduction

Goat rearing has tremendous potential in the North-eastern states particularly among the small and marginal farmers and landless labourers because of very low initial investment and adequate financial returns. The epididymis is the continuation of the testicular duct system connecting it with the vas deferens which provides a platform for maturation of the spermatozoa (Hafez, 1987). The enzymes located in the cauda epididymis plays a major role in this process (Deganand Lee, 1982).

Detection of various enzymes in the tissues of the epididymis had been carried out in various domestic animals like camel (Tingariand Moniem, 1979), horse (Singh et al., 1989), donkeys (Uppal et al., 2002). The aim of this present work is to study the localization of various dehydrogenase enzymes in the cauda epididymis of Assam goats at various post natal ages, which is the first report of its kind in this indigenous breed of Assam.

Materials and Methods

Eighteen male Assam goats aged from 0-day to 10 months were used in the present study. The animals were divided into six age groups viz. group-I (0-day), group-II (2 months), group-III (4 months), group-IV (6 months), group-V (8 months) and group-VI (10 months) consisting of three animals in each group. The animals were sedated by giving intramuscular injection of Siquil (triflupromazine hydrochloride) @ 1 mg/kg body weight and subsequently anaesthetized by administering intravenous injection of intravel sodium (pentobarbital sodium) @ 15 mg/kg body weight (Hall et al., 2000). After induction of proper level of anesthesia, the testicles were incised out by performing open method of castration as per standard protocol. Subsequently, the animals were given proper post operative treatment and care.

After separating from the testes, tissue samples from cauda epididymis were collected and sectioned at 10 μ thicknesses using a cryostat (Leica, U.K.). The cryostat sections were incubated for the histoenzymic demonstration of various enzymes stated as detailed hereunder.

Results and Discussion

The activity of LDH remained weak to moderate in the epididymal tubular epithelium of the kids below 4 month of age (group-III). However, the enzymic activity for LDH increased to strong with the advancing age of the male goats (Fig.1). In 6 to 10 months old bucks, comparatively more activities of LDH was observed (Fig.2) in the epididymal tubular epithelial cells of the cauda. (Allen and Sailer, 1961) reported that the LDH localized in the epididymis was suggestive to be more of a secretory type that provided lactate which could be used as a source of energy by the spermatozoa efficiently in partially anaerobic conditions. In the epididymis of Assam goats, thus the lactate secretion although appeared to occur in all the three segments of the epididymis, it was maximum in the cauda. This might be to provide adequate energy requirements for the stored spermatozoa in goats from 6 month of age (group-IV) onwards. On the contrary, Hall et al. (2000) reported maximum localization LDH enzyme in the corpus epididymis of horse, which might be a species specific characteristics. The tubular contents and the stereo cilia exhibited strong to intense reactions to LDH in 8-10 months old bucks (groups-V and VI). These findings were in corroboration with that reported in donkeys (Uppal et al., 2002).

The activity of MDH was found to be the highest in the tubular epithelium of the cauda epididymis as strong activity was noticed (Fig. 3) in the animals of all the age groups with exception of day-old kids. The activity of MDH was granular type distributed mainly in the basal border of the epithelium. The peritubular tissue showed weak activity in the animals of lower age groups (group-I and II), weak to moderate in 4 and 6 months old goats (group-III and IV)
Table 1: Histoenzymic techniques used for the demonstration of different enzymes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Enzyme</th>
<th>Substrate used</th>
<th>Incubation time</th>
<th>Method &amp; source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactate dehydrogenase (LDH)</td>
<td>Sodium-DL-lactate</td>
<td>30 min</td>
<td>(Pearse, 1980)</td>
</tr>
<tr>
<td>2</td>
<td>Malate dehydrogenase (MDH)</td>
<td>L-Malic acid</td>
<td>30 min</td>
<td>do</td>
</tr>
<tr>
<td>3</td>
<td>Glucose-6-phosphate dehydrogenase (G-6-PDH)</td>
<td>Di-Na-glucose-6-phosphate</td>
<td>30 min</td>
<td>Nitro-BT method</td>
</tr>
</tbody>
</table>

Fig.1: Photomicrograph of the cauda epididymis in an eight months old buck showing the activity of LDH enzyme (400 X).

Fig. 2: Photomicrograph of the luminal content of the cauda epididymis in a ten months old buck showing the activity of LDH enzyme (400 X).

Fig. 3: Photomicrograph of the cauda epididymis in an eight months old buck showing the activity of MDH enzyme (100 X).

Fig. 4: Photomicrograph of the cauda epididymis in a ten months old buck showing activity of G-6-PH enzyme (100 X)

and moderate activity was seen in older animals. More enzymic activity in the tubular epithelium was also reported in bull testis (Blackshaw and Samisoni, 1967). Again, among all the three segments of the epididymis, the tubular epithelium of the cauda had maximum activity of MDH in Assam goats. This high localization of MDH enzyme might be to meet an increased need for energy requirement of the stored spermatozoa in the cauda epididymis.

The G-6-PDH was associated with pentose cycle or glucose phosphate shunt and played an important role in the biosynthesis of ribose, nucleic acids and nucleotides as well as in the synthesis of amino acids (Singh et al., 1989). No activity of G-6-PDH was seen in the epididymal tubular epithelium in the male goats of all the age groups except at 8 and 10 months of age (groups V and VI), in which a diffused moderate activity was seen (Fig. 4). The peritubular tissue showed very mild activity of G-6-PDH in the goats from birth to 6 month of age (groups I to IV) and mild activity in older bucks. Moderate activity of G-6-PDH observed in the tubular epithelium of the epididymis in male Assam goats at 8 to 10 months of age (group-V and VI) might be suggestive for providing the epididymal tubular epithelial cells with various biomolecules during pot pubertal period. G-6-PDH activity was observed to be the maximum in the tubular epithelial cells of the corpus epididymis in stallion (Singh et al., 1989). However, no such trend was seen in the present study.

References
GROSS ANATOMICAL STUDIES ON PROVENTRICULUS OF POST HATCH UTTARA FOWL#

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ABSTRACT

Present study was conducted on proventriculus of Uttara Fowl at the age of 0, 7, 28 and 112 days. Proventriculus was collected from six birds of each age group for gross morphological and morphometrical studies. The proventriculus was an elongated, small, spindle shaped, thick walled organ, extended from fifth thoracic to third lumbo sacral vertebrae and its long axis was slopped from left to right. Its mucosa consists of numerous, wide, rounded, grossly visible papillae. The morphometrical observation of the proventriculus like weight, volume, diameter at the middle, wall thickness, longitudinal length and cross sectional area were showed significant difference amongst the four age groups. These parameters revealed corresponding increase with the advancing age of the birds.

Key words: Gross morphology, morphometry, proventriculus and Uttara fowl

Introduction

The indigenous fowl or poultry, forms the backbone of the backyard poultry farming in India specially in the Kumaon region of Uttarakhand, is said to be descended from the Red jungle fowl. The proventriculus (glandular stomach) is the first part of bird's stomach, where digestive enzymes like gastric pepsin, hydrochloric acid and mucous are released and mixed with food before going to the gizzard.

Materials and Methods

The study was conducted on 24 apparently healthy birds of either sex reared at the Instructional Poultry Farm, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Based on the age, birds were divided into four age groups of 0 day, 7 days, 28 days and 112 days with 6 birds in each group. The birds were sacrificed and the proventriculus were recorded by using digital Vernier calipers and digital weighing balance (Sartorius, TE 214S). Volume of the organ was measured with the help of measuring cylinder by using Archemides principle. Cross sectional area of proventriculus was calculated by dipping the cross section of proventriculus in the inkpad and followed by impression on graph paper.

Results and Discussion

Proventriculus was elongated, small, spindle shaped organ and its wall was thicker than that of the oesophagus. It was situated slightly towards the left of the median plane with its long axis sloping left to right. Ventrolaterally it related with the liver, posteriodorsally to the spleen, left abdominal airsac, ileum and caecum and anteriodorsally related to cranial thorasic airsac. These observations are in agreement with Das et al. (2013) in Kadaknath fowl while proventriculus was relatively small in the chicken and pigeon but it might be quite large in certain fish eating birds like storks and gulls (Sturkie, 1993).

The long axis of the proventriculus was sloped from left to right of the median plane. This might be due to the location of other organs like liver, heart and spleen as reported by Venkatesan et al. (2005) in Japanese quail, Das et al. (2013) in Kadaknath fowl and Nasrin et al. (2012) in broilers.

The lumen of proventriculus was narrow at both the ends, it continued anteriorly with oesophagus and posteriorly with gizzard as recorded by Das et al. (2013) and Nasrin et al. (2012) in Kadaknath fowl and broilers, respectively. At the junction of the proventriculus and gizzard there was a small constricted area referred as isthmus (Fig. 1) as reported by Das et al. (2013) in Kadaknath fowl. The mucosa was whitish in colour containing numerous low wide, rounded grossly visible papillae. This is in accordance with observations of Dahekar et al. (2014) in Japanese quail and Hodges (1974) in chickens. As observed by Lambate and Mamde (2008a) in broilers, these visible papillae were arranged in close contact with the adjacent papillae towards cranial and caudal part of the proventriculus, while the papillae in the middle part were free from each other (Fig.2).

The average weight of the proventriculus in 0, 7, 28 and 112 day old birds was 0.27±0.03 g, 0.57±0.02 g, 2.16±0.22 g, 4.56±0.19 g, respectively. In Kadaknath fowl (Das, 2010) the weight of the proventriculus in 0, 7, 28 and 112 days old birds was 0.333±0.01 g, 0.526±0.013 g, 3.939 ± 0.074 g and 9.891±0.08 g, respectively. Thus the weight of proventriculus is higher in Kadaknath fowl at 28 and 112 days old birds than that in Uttara fowl.

The average volume of the proventriculus in 0, 7, 28 and 112 days old birds was 0.40±0.04 cc, 0.63±0.08 cc, 2.25±0.25 cc and 4.00±0.05 cc, respectively. In broilers, Lambate and Mamde (2008b) reported that the average volume of proventriculus in 2, 4, 6 weeks old broiler birds were 3.02±0.12 cc, 5.56±0.24 cc and 6.70±0.12 cc, respectively which was much higher than the present findings. In Kadaknath fowl (Das, 2010) the average volume of the proventriculus in 0, 7, 28 and

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112 days old birds was 0.74±0.015 cc, 1.18±0.106 cc, 2.1±0.07 cc and 3.68±0.106 cc, respectively. This indicates that volume of proventriculus in Kadaknath fowl is higher at 0 and 7 day old birds while it is lower in 28 and 112 day old birds as compared to Uttara fowl.

The average cross sectional area of proventriculus in 0, 7, 28 and 112 day old birds was 42.83±0.94 mm², 55.83±1.86 mm², 160.83±3.00 mm², 218.33±2.78 mm², respectively. In Kadaknath fowl, the average cross sectional area of proventriculus in 0, 7, 28 and 112 day old birds was 11.5±0.65 mm², 24.3±0.90 mm², 177.1±2.53 mm² and 255.0±3.53 mm², respectively (Das, 2010). Thus the average cross sectional area of proventriculus of Uttara fowl is higher in 0 and 7 day old birds and lower in 28 and 112 day old birds as compared to Kadaknath fowl.

The average longitudinal length of the proventriculus in 0, 7, 28 and 112 day old birds was 12.52±0.87 mm, 15.65±0.46 mm, 27.59±1.22 mm, 38.22±1.25 mm, respectively. This finding is similar to that of Nasrin et al. (2012) in broilers, who have claimed that the average length of proventriculus was 1.23±0.110 cm, 2.83±0.118 cm and 3.70±0.122 cm, at D1, D14 and D28, respectively.

The average diameter of the proventriculus at the mid-length level in 0, 7, 28 and 112 day old birds was 6.42±0.20 mm, 7.77±0.40 mm, 10.98±0.53 mm, 12.80±0.20 mm. These are in accordance with Das et al. (2013) in Kadaknath fowl, who stated that the average diameter of the proventriculus at the mid-length level in 0, 7, 28 and 112 day old birds was 6.69±0.266 mm, 8.08±0.5023 mm, 10.93±0.229 mm, 14.11±0.180 mm, respectively.

The average thickness of proventriculus wall in 0, 7, 28 and 112 days old birds was 1.90±0.02 mm, 3.01±0.03 mm, 4.04±0.05 mm, 6.34±0.33 mm, respectively. In Kadaknath fowl (Das, 2010) the average thickness of the wall of proventriculus in 0, 7, 28 and 112 days old birds was 2.41±0.078 mm, 2.73±0.095 mm, 4.46±0.044 mm, 5.35±0.127 mm, respectively. These findings are on similar lines to those of present study.

Summary
The proventriculus was an elongated, small, spindle shaped, thick walled organ, extended from fifth thoracic to the third lumbo sacral vertebrae. The isthmus was a small constricted area at the junction between the proventriculus and gizzard. Its mucosa consisted of the numerous, wide, rounded, grossly visible papillae. The morphometrical observation of the proventriculus for weight, volume, diameter at the middle, wall thickness, cross sectional area and longitudinal length showed corresponding increase with the advancing of age.

References

Fig. 1: Photograph showing proventriculus and gizzard of 112 days old uttara fowl (P- Proventriculus, I- Isthmus, A -Cranial Blind sac, G- Gizzard, B-Caudal blind sac, Int- Intestinal joint)

Fig. 2: Photograph showing numerous papillae on proventricular mucosal surface in 112 day old Uttara fowl
AGE RELATED CHANGES IN ULTRASTRUCTURE AND HISTOENZYMIC DISTRIBUTION OF TESTES IN GOAT (CAPRA HIRCUS)

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ABSTRACT
Age related changes in the ultrastructural and histoenzymic status of the testes conducted on thirty goats of different age groups viz. kid, young and adult. The testes tissues were processed for ultrastructural studies by Transmission Electron Microscopy, while the histoenzymic observations were recorded by applying alkaline phosphatase, acid phosphatase, adenosinetriphosphatase, glucose-6-phosphatase, 5-nucleotidase and cholinesterase activity. The ultrastructurally sertoli cells were immature in kid while mature cells were observed in young and adult. The spermatogenic cells were mainly five types, stem cells, spermatogonial cells, primary spermatocytes, secondary spermatocytes and three types of spermatids mainly Golgi phase, head cap and acrosomal cap. On the basis of ultrastructure observation, four types of cells were observed in Leydig cells. The alkaline phosphatase activity was mild to moderate in testes of kid, whereas moderate activity was noted in young and adult goats. The acid phosphatase activity was mild to moderate in testes in all groups of animals. The glucose-6-phosphatase activity was mild in testes in kid, moderate in testes in all groups of animals. The 5-nucleotidase activity was weak in testes in kid, young and adult groups. The microscopic structure (TEM) of the testes in goat with reference to postnatal development viz. in kid, young and adult animals. In addition, the study also involved histochemical and histoenzymic changes in terms of progressive age of the animal.

Key words: Transmission electron microscopy, histoenzymology, testes, goat

Introduction
Production of spermatozoa through a process of spermatogenesis is not a simple process but involves a complicated series of changes, before they are released for fertilization from the epididymis. Testicular cell proliferation and differentiation is critical for development of normal testicular function and male reproductive maturity (Faucette et al., 2014). The chain of events in the process involves maturation and shedding of gametes. The testes produce spermatozoa and male sex hormones, the testosterone. The interstitial tissues are believed to produce hormones responsible for functional maturation and maintenance of male genital system. In the present work we have used electron microscopic studies to help elucidate the morph functional relevance of the testes.

Materials and Methods
For the present study, thirty specimen of testes of local goats ranging from 3 to 12 months onwards were collected from abattoir and differentiated into three groups as kid (3-6 months), young (6-12 months) and adult (12 months onwards). These tissue samples of 3 to 4 mm thickness were transferred to vial and fixed in 2.5% gluteraldehyde solution in 0.05M phosphate buffer at pH 7.2 for TEM study. Semi thin sections were stained with 1% Toluidene blue and examined with a light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with electron microscope for TEM (Bozzola and Russell, 1999). The alkaline phosphatase activity was observed by Gomori’s alkaline phosphatase cobalt method, acidphosphatase activity by Gomori’s modified lead nitrate method, adenosine tri phosphatase, 5- nucleotidase and glucose-6-phosphatase activities by lead method (Carleton and Drury, 1980).

Results
The present study puts on record the light and electron microscopic structure (TEM) of the testes in goat with reference to postnatal development viz. in kid, young and adult animals. In addition, the study also involved histochemical and histoenzymic changes in terms of progressive age of the animal.

Ultrastructural observations
The semi-thin sections of testes, stained with toluidene blue, showed presence of external thick fibrous capsule, the tunica albuginea. On the inner aspect of the tunica albuginea, dense connective tissue gave a way to a loose layer, provided with numerous blood vessels called as tunica vasculosa. Each seminiferous tubule in the testes was enclosed by distinct basal lamina with collagen fibres, few smooth muscles or myoid cells of contractile nature and had epitheloid organization.

Within the seminiferous tubules the spermatogenic cells were found in different phases of development and were located between the sertoli cells. In kid testes, four types of spermatogenic cells viz. stem cells, spermatogonial cells, primary spermatocytes, and secondary spermatocytes were observed. While in young and adult testes, stages of development from spermatogonial cells to the spermatids were noticed i.e. stem cells, spermatogonial cells, primary spermatocytes, secondary spermatocytes and types of spermatids.

The basal epithelium of the seminiferous tubules was longer, with irregularly shaped euchromatic nuclei. These cells had vesicular mitochondria of various shapes, Golgi complex and numerous polyribosomes and lacked the large electron dense, membrane bounded bodies seen in the first cell type of spermatogonial series (Fig. 1). The spermatogonial cells were characterized by shrunken nuclei, condensed chromatin and vacuolated cytoplasm.

The primary spermatocytes were rounded in shape with spherical nuclei. Some of the spermatocytes were...
usually notched but occasionally rounded in shape. Few primary spermatocytes were larger than the other with uniformly spheroid nuclei. The cytoplasmic organelles were similar to those of spermatogonial cells.

The secondary spermatocytes were smaller in size than that of primary spermatocytes. These cells were rounded in shape and had a centrally located nuclei and scanty cytoplasm. These cells had a very short life. The cytoplasm contained mitochondria, Golgi complex and strands of Endoplasmic Reticulum. The mitochondria were spread towards the periphery.

Three types of spermatids were identified in the spermatogenic series as round, elongating, and elongated. The newly formed spermatids showed a centrally located spherical nucleus, a well delimited Golgi complex located close to the nucleus, numerous granular mitochondria lying to the inside of the cytoplasmic membrane and a small centrioile, and such type of spermatids were categorized as round spermatids.

The elongating spermatids were seen to be characterized by presence of elongating nuclei and their cytoplasm was also elongated. The elongating spermatids contained elongated nuclei and they were usually found to be attached to the apical portion of the sertoli cells. Ultrastructurally, Golgi zone which was placed at apical portion of nuclear membrane, had formation of dense granules called as acrosomic granule.

Ultrastructurally, the acrosomic granules in the Golgi region formed the vesicle called acrosomal vesicle. The Golgi apparatus remained closely associated with the surface of the acrosomal vesicle and this phase was referred Golgi phase. As the development of acrosomal vesicle was advanced, its area of adherence to the nuclear envelope formed a thin fold that spread over the pole of the nucleus, ultimately to cover its entire anterior hemisphere as a membranous head cap, which were coextensive and constitute the acrosomal cap or acrosome (Fig. 2).

In between the spermatogenic cells, some elongated shaped sertoli cells were present rested on the basement membrane. Their nuclei were spherical, oval or pear shaped and located at the basal part of the cells. The luminal surface of these sertoli cells was deeply indented by the old spermatids. In kid, the cytoplasm of sertoli cells extended to a considerable distance towards the centre of the seminiferous tubules and this portion of the cytoplasm contained round or elongated mitochondria, polyribosomes and Golgi apparatus associated with numerous coated vesicles

Rough endoplasmic reticulum was scanty and prominent features of these cells included the presence of electron dense, membrane bound bodies and an abundance of microtubules in the cytoplasm. These cells were identified as immature sertoli cells.

In young and adult testes, the nuclei of the sertoli cells were deeply indented and within their recesses, granular endoplasmic reticulum and ribosomes were noted frequently. The nuclei were prominent with numerous vesicular elements of various sizes. These vesicles were located along the strands of nucleolonema. The outer surface of vesicular elements and tubules were surrounded by ribosome like particles. The cytoplasm of these cells exhibited an abundance of elongated mitochondria with electron dense cistae, microtubules, polyribosomes and a few lysosomes and multivesicular bodies. Golgi complexes associated with coated vesicles and sacules were observed in the cytoplasm. Perinuclear cytoplasm contained microfilaments, which appeared to keep other cellular elements a uniform distance away from the nucleus.

The transformation of indifferent sertoli cells into mature sertoli cells was associated with the appearance of vesicles in the nucleoli of these cells in young animals. Matured sertoli cells were identified by the presence of nucleolar vesicles and spermatids in the cytoplasm.

The seminiferous tubules were separated by stroma, which contained well vascularised groups of interstitial cells or Leydig cells besides stromal cell. In kid, they were comparatively more than young and adult testes. The lymphatic vessels were located near the clusters of Leydig cells.

It was observed that there were variations in the shape of the Leydig cells, size and shape of nuclei and the cytoplasmic granules.

On the basis of nuclear ultrastructure, Leydig cells were categorized into four types of cells as Type 1, Type 2, Type 3 and Type 4 (Fig. 3).

Type 1 Leydig cells were seen elongated in shape; the shape of nuclei was elongated with notch. The chromatin materials spread throughout the periphery of nucleus. The cell organelles contained mitochondria, Golgi body and smooth endoplasmic reticulum.

Type 2 Leydig cells were seen irregularly triangular shaped nuclei with scanty cytoplasmic granules. There were many small membrane bound granules.

Type 3 Leydig cells were seen with round nuclei, with distributed chromatin materials. The cytoplasm was scanty with many lipids granules.

Type 4 Leydig cells had elongated nuclei with relatively few organelles.

The difference in cellular morphology is attributable to the varied function of Leydig cells from nourishment to production of steroid hormones.

Two types of underdeveloped filament bundles were infrequently recognized in Leydig cells but not in other testicular cells. One type was the underdeveloped bundles of actin filaments, which were found in the nucleus of Leydig cells. The other type was the underdeveloped bundles of intermediate filaments, which were found in the cytoplasm of Leydig cells.

Histoenzymic observations

Alkaline phosphatase

The spermatogonial cells, primary and secondary spermatocytes showed mild activity in kid while it was mild to moderate in young and adult groups. The spermatids showed weak positive alkaline phosphatase reaction in young and adult. The sertoli cells in all groups had mild activity. All cellular components of the intertubular area demonstrated positive alkaline phosphatase activity in all groups. Mild to moderate reaction was observed in Leydig cells in kid testes but in young and adult testes, it showed moderate alkaline phosphatase activity.

Acid phosphatase

The spermatogonial cells showed mild to moderate
activity in all groups. Acid phosphatase activity was weak in primary spermatocytes of kid testes while it was mild in young and adult groups (Fig. 4). Secondary spermatocytes and spermatids showed mild activity in young and adult groups. The moderate activity was noticed in the interstitial connective tissue in kid and young testes while moderate to intense reaction was observed in adult testes. Mild to moderate acid phosphatase activity was noticed in the Leydig cells of all three groups.

Adenosine triphosphatase

The tunica albuginea of kid testes showed mild adenosine triphosphatase activity, while it was moderate in young and adult testes (Fig. 5). The basement membranes of the seminiferous tubules had mild to moderate reaction in kid and young group while in adult testes it was moderate. The spermatogonial cells showed mild to moderate activity in all animals. The primary and secondary spermatocytes showed mild activity in young and adult groups, while in kid there was no activity of adenosine triphosphatase. The sertoli cells showed mild activity in kid whereas in young and adult it showed mild to moderate activity. Moderate to intense reaction was observed in interstitial connective tissue of kid testes and it was moderate in young and adult at testes.

Glucose-6-phosphatase

The tunica albuginea of kid testes showed mild glucose-6-phosphatase activity, while it was mild to moderate in young and adult testes. The spermatogonial cells showed mild glucose-6-phosphatase activity in all groups. Primary and secondary spermatocytes showed negative reaction in all three groups while spermatids showed mild to moderate reaction in young and adult groups. The Leydig cells and interstitial connective tissue showed weak reaction in kid group while moderate reaction in young and adult at testes.

5-nucleotidase

The tunica albuginea of testes showed moderate activity in kid testes, whereas intense activity in young and adult testes (Fig. 6). In seminiferous tubules the spermatogonial cells showed mild activity in young and adult groups, while activity was not observed in kid testes. In all three groups of testes, there was negative activity in primary spermatocytes as well as in secondary spermatocytes. Spermatids and immature sperms showed mild activity in adult testes, whereas negative activity in kid and young groups. The sertoli cells showed no activity. The interstitial connective tissue as well as Leydig cells showed mild activity in young and adult testes. In kid testes, there was negative activity of 5-nucleotidase.

Discussion

Ultrastructural observations

The semi-thin sections of testes showed presence of external thick fibrous capsule, the tunica albuginea. Similar observations were seen by Gofur et al. (2008) in indigenous bull and Uppal et al. (2003) in donkeys. Each seminiferous tubule in the testes was enclosed by distinct basal lamina. The lamina propria was believed to be responsible for the rhythmic shallow contractions that could be observed in the seminiferous tubules. The lamina propria was multi layered in kid because it might be generating the germ cells. Within the seminiferous tubules the spermatogenic cells were found in different phases of development and were located between the sertoli cells.

The spermatogonial cells were characterized by shrunken nuclei, condensed chromatin and vacuolated cytoplasm. Similar characteristics of spermatogonial cells were reported by Azmi et al. (1990) in buffalo and Kamal and Devi (2012) in Assam goat. These changes were suggestive of entry of cells into progressive spermatogenic phase. It was noted that the secondary spermatocytes get transformed into spermatids in young and adult testes but this transformation of secondary spermatocytes into spermatozoa was not observed in kid animals.

Ultrastructurally the spermatids were observed mainly in three phases namely Golgi phase, head cap and acrosomal cap. These finding are similar to those reported by Goyal et al. (2000) in goat. Onyanga et al. (2000) reported the cycle began with accomplishment of spermiogenesis and ended with apical migration and close attachment of late maturation phase of spermatids at the sertoli cell apex accompanied by adluminal retention of residual bodies with dense staining inclusions in goat.

In between the spermatogenic cells, some elongated shaped sertoli cells were present rested on the basement membrane. These findings are similar to those reported by Goyal et al. (2000) in goat and Kamal and Devi (2012) in Assam goat.

In kid, immature sertoli cells had prominent features of presence of electron dense membrane bound bodies and abundant microtubules in cytoplasm, while in young and adult, the nuclei of the sertoli cells were deeply indented and within their recesses, granular endoplasmic reticulum and ribosomes were noted frequently. Azmi et al. (1990) reported the appearances of mature sertolcell coincides in time with a marked increased in the diameter of the seminiferous tubules in the goat. The increase in the diameter of seminiferous tubules also assisted the fluid transport from the interstitium through the basement membrane. Sertoli cell activity was evidenced by nucleolar transformation may be an integral part of the trigger needed for fluid transport and differentiation of the germ cells during spermatogenesis. The transformation of indifferent sertoli cells into mature sertoli cells was associated with the appearance of vesicles in the nucleoli of these cells in young animals.

The seminiferous tubules were separated by stroma, which contained well vascularised groups of interstitial cells or Leydig cells besides stromal cell. It had been established that, Leydig cell volume was correlated with the amount of smooth endoplasmic reticulum and with its capacity to secrete testosterone (Leal et al., 2004).

Four types of Leydig cells were observed viz. Type I, Type II, Type III and Type IV on the basis of ultrastructure appearance of shape of nuclei, chromatin materials and cytoplasm. Bordoloi and Dhimim (1983) reported three types of Leydig cells in the testes of goat on the basis of cellular morphology. It was noted that Leydig cells form a well-vascularized group of polyhedral cells, which occupy the space between seminiferous tubules. This is justified with the fact that, Leydig cells secrete gonadal hormone testosterone, which had an
Fig. 1: Transmission electron micrograph showing spermatogonial cells resting on basement membrane in kid testes a) Basal lamina, b) Fibroblast, c) Cytoplasmic organelle of spermatogonial cells, d) Nucleus (7720x)

Fig. 2: Transmission electron micrograph showing head cap structure of spermatid and elongating spermatid having acrosomal granules of adult testes [19300x]

Fig. 3: Transmission electron micrograph showing different types of Leydig cell in kid testes a) Type 1, b) Type 2, c) Type 3, d) Type 4 [3860x]

Fig. 4: Photomicrograph showing acid phosphatase activity (arrow) in young testes [100x]

Fig. 5: Photomicrograph showing adenosine triphosphatase activity (arrow) in adult testes [100x]

Fig. 6: Photomicrograph showing 5-nucleotidase activity (arrow) in adult testes [100x]
important role in reproductive status of animal without sexual maturity. Thus the present study puts on record the existence of 4 types of Leydig cells in the testes of goat, which appears to be a new finding.

**Histoenzymic observations**

**Alkaline phosphatase**

The positive alkaline phosphatase reaction in testes indicative of different components of testes release inorganic phosphatase from ester compounds in alkaline pH. These observations were in accordance with those reported by Pal and Bhadrawaj (1986) in buffalo. The alkaline phosphatase activity was mild in all components of testes in kid, whereas mild to moderate activity was noted in young and adult groups.

**Acid phosphatase**

As per Niemi and Karmano (1965) reported that, the activation of acid phosphatase during the maturation of spermatids could be considered as a sign of the gradual involution of cytoplasm, which would not be used for the spermatozoa. The acid phosphatase activity was mild to moderate in all components of testes in kid, whereas mild to intense activity was noticed in same components of testes in young and adult groups.

**Adenosine triphosphatase**

Gravis et al. (1976) reported ATPase was localized in the interface between spermatids and sertoli cell. The localization of ATPase in sertoli cells processes may be involved in providing an energy source for filament motility. Moderate to intense reaction was observed in interstitial connective tissue of kid testes and it was moderate in young and adult testes. This indicates that the energy requirement was more in the young and adult testes, which may due to the higher requirement of energy for the process of spermiogenesis, which mainly, occurs in the young and adult animals. Similar activities were noted by Gravis et al. (1976) in hamster, Byers and Graham (1990) in rat. The testes showed mild to moderate activity of adenosine triphosphatase in young and adult groups. In case of kid testes weak activity of adenosine triphosphatase was noticed.

**Glucose-6-phosphatase**

The glucose-6-phosphatase activity was mild to moderate in testes of all groups in goat. In bull, Blackshaw and Somisoni (1966) observed intense activity of glucose-6-phosphatase in Leydig cells and mild activity in spermatogenic cells, while the Leydig cells and interstitial connective tissue showed weak reaction in kid group while moderate reaction in young and adult groups. Glucose-6-phosphatase was demonstrated in the smooth Endoplasmic Reticulum.

**5-nucleotidase**

The tunica albuginea of testes showed moderate activity in kid testes, whereas intense activity in young and adult testes, while in other components of testes it was weak to mild in all the groups. Konrad et al. (1998) reported similar observations in human testes. A 5-nucleotidase enzyme acts as a scavenger of injured cell or membrane components or as a supplier of adenosine.

**References**


ANALGESIC EFFECT AND HAEMATOLOGICAL CHANGES AFTER EPIDURAL ADMINISTRATION OF BUPIVACAINE, BUPIVACAINE-KETAMINE AND BUPIVACAINE-TRAMADOL IN BUFFALO CALVES*

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ABSTRACT

The present study was performed on six healthy buffalo calves of either sex weighing 40-60 kg. Onset, duration and complete recovery time of analgesia were studied and haematological changes were recorded at different time intervals after epidural administration of bupivacaine and its combination with ketamine and tramadol. Long duration of analgesia and complete recovery was prolonged in combination with tramadol as compared to bupivacaine alone and its combination with ketamine, onset of analgesia was minimum in bupivacaine and its combination with tramadol as compared to bupivacaine alone and its combination with ketamine. Non significant (P>0.05) changes were observed in packed cell volume (PCV%), haemoglobin (g%), total erythrocyte count (million/ cumm) and total leucocytes count (thousand/cumm) in all three groups.

Key words: Bupivacaine, ketamine, tramadol, analgesic effect and haematological changes

Introduction

Epidural anaesthesia is accomplished by introduction of a local anaesthetic or analgesic combination into the epidural space. An epidural local anaesthesia with or without opioids, decrease the risk of post operative pulmonary complications such as atelectasis and pneumonia by 50-70% compared with systemic opioids (Igg et al., 2002). Bupivacaine hydrochloride is most widely used local anaesthetic for epidural use because of its tendency to block sensory fibres preferentially to relative sparing of motor fibres (Alvarez et al., 1983). Ketamine as epidural analgesia suppress myelinated nerve conduction, interacts with monoaminergic and opioid receptors. Epidural administration of tramadol seems to produce satisfactory antinociception as an analgesics technique for surgery in the hind limb of the dog (Alonso et al., 2005).

Materials and Methods

The present study was conducted on 6 healthy buffalo calves of either sex weighing 40-60 kg. All the calves were dewormed by bolus panacure (fenbendazole) at the dose rate of 7 mg/kg b.wt. one week prior to experiment. All animals were maintained under similar standard management condition and standard feed ration. Each animal was subjected to the following three treatments at an interval of 8 days. The epidural catheter was placed at the sacrococcygeal space. In treatment I-bupivacaine hydrochloride @ 0.15 mg/kg body weight in the sacrococcygeal space. In treatment II- bupivacaine hydrochloride @ 0.15 mg/kg body weight and ketamine @ 2 mg/kg body weight in the sacrococcygeal space. In treatment III- bupivacaine hydrochloride @ 0.15 mg/kg body weight and tramadol hydrochloride @ 2.5 mg/kg body weight in the sacrococcygeal space. The epidural injection was well tolerated by all experimental animals and there was no sedation observed between mixing of bupivacaine with the ketamine and tramadol. Packed cell volume (PCV%), haemoglobin (g%), total leucocytes count (thousand/cumm) and erythrocyte count (million/ cumm) were recorded at 0 min, 10 min, 20 min, 40 min, 60 min, 80 min, 2 hrs, 3 hrs, 4 hrs, 12 hrs, 24 hrs, and 48 hrs. The '0' hr values from each animal were recorded immediately before the start of the treatment, as the control value. The onset and duration of analgesia was determined by the pin prick method. The onset of analgesia was ascertainated by noting the time of loss of tail reflex, pedal reflex and pin prick reflex. Complete recovery from the 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. All these haematological parameters were studied as per the procedure described by Jain (1986).

Results and Discussion

Time of onset of analgesia was prolonged in bupivacaine 7.20±0.10 minutes in comparison with bupivacaine-ketamine 5.87±0.53 minutes and bupivacaine-tramadol 4.98±0.68 minutes. The duration of analgesia was 93.34±2.71 minutes, 113.48 minutes and 184±4.84 minutes in treatments I, II and III, respectively. There was a significant difference (P<0.05) between three treatments. The total duration of complete recovery was 160.23 ± 3.5, 185.05 ± 2.4 and 210 ± 3.28 minutes in treatment I, II and III, respectively. There was a significant difference (P<0.05) between three treatments.

Dhage and Pawshe (2010) compared bupivacaine and ketamine groups in goats after epidural administration and found the prolonged period of onset of analgesia in bupivacaine (10.51±0.72 min) compared to ketamine group (6.80±0.25 min).

The packed cell volume (PCV%) did not vary significantly in all three treatment groups and there was no significant

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change in values of PCV between groups. The minimum and maximum values of PCV ranged from 28.79±0.69 at 3 hrs to 32.68±0.89 at 48 hrs, 28.16±0.32 at 3 hrs to 30.37±0.77 at 0 minute and 29.75±0.62 at 2 hrs to 33.27±0.83 at 0 minute in treatment I, II and III, respectively.

The haemoglobin concentration (g%) also did not vary significantly in all three treatment groups and there was no significant change in the values of Hb between groups. The minimum and maximum values of Hb ranged from 10.37±0.23 g% at 80 minutes to 12.43±0.29 g% at 48 hrs, 9.84±0.53 at 60 minutes to 11.67±0.19 g% at 48 hrs and 9.39±0.17 at 80 minutes to 10.78±0.45 g% at 0 minute in treatment I, II and III, respectively.

There was no significant change in erythrocyte count (million/cumm) in all three treatment groups and value obtained ranged from 6.08±0.28 at 40 minutes to 6.70±0.36 at 0 minute in treatment I. The maximum and minimum values of erythrocyte count ranged from 6.23±0.21 at 24 hrs to 6.02±0.24 at 80 minutes and 5.78±0.29 at 0 minute 5.36±0.15 at 10 minutes millions/cumm of blood in treatment II and III, respectively.

The leucocytes count (thousand/cumm) also did not vary significantly in all three treatment groups. The minimum and maximum values of leucocytes count ranged from 6.83±0.36 to 6.88±1.12, 6.69±0.10 to 6.77±0.14 and 6.24±0.07 to 6.40±0.08 thousands/cumm per ml of blood in treatment I,II and III, respectively.

These findings for the haematological parameters (Hb, TEC, TLC, PCV and DLC) are in accordance with Ahmad and Shukla (2011).

Onset of analgesia was reduced in the bupivacaine-ketamine group when compared with the bupivacaine group because of competitive antagonism of ketamine to NMDA receptors and cause hyperalgesia (Yanli and Eren, 1996). Prolonged duration of analgesia (4hrs) following the administration of tramadol has been reported in dogs and horses (Guides et al., 2005). These results support the prolonged duration of analgesia observed in our study after epidural injection of bupivacaine-tramadol group in comparison with bupivacaine and bupivacaine-ketamine group.

Epidural administration of bupivacaine/ketamine combination resulted in fast onset and moderate duration of analgesia of caudal areas and this combination could be used to perform operations without any marked side effects (Dadafarid and Najafpour, 2008).

Ketamine-lidocaine combination provides very effective and long duration of analgesia (Gunduz et al., 2005). Long duration anaesthesia could commence soon after epidural administration of lidocaine-tramadol combination and might be used without re-administration of anaesthetic agent in long duration obstetrical and surgical procedures in cattle. Lidocaine-tramadol combination produced a significantly (P<0.05) longer duration of analgesia than lidocaine alone (Bigham et al., 2010). It also has been reported that ketamine releases the endogenous opioids to cause analgesic effects (Pekoe, 1982). Caudal epidural ketamine administration induces analgesia without sedation in cows and duration of analgesia was dose dependent with ataxia (Lee et al., 2003).

References
Introduction

Skin of canines is most common seat for tumours. Skin tumours in the dog are more likely to be benign than malignant (Wilcock, 1993). Jain (1996) stated that normochromic anaemia was seen in malignancies in the dogs. Of all species, dog develops neoplasms twice as frequently as humans, with incidence of skin and mammary tumours being the highest. Fayolle et al. (1987) studied the routine haematology in canine mammary tumours and found non-significant changes in haematological parameters. Stockhaus et al. (1999) also found non-significant haematological observations in cases of mammary tumour in female dogs.

Materials and Methods

A total of 750 cases were presented during the period from October 2010 to September 2011 and out of these 40 dogs were affected with skin and subcutaneous neoplastic growths at various body parts. Twenty five clinical cases of skin and subcutaneous tumours in dogs of different age groups and breeds were selected for the present study. The animals were divided into three groups. All the dogs of Group I, II and III were premedicated with atropine sulphate @ 0.65 mg (total dose) i/m followed by xylazine sedation @ 1 mg/kg body weight or medetomidine @ 20 µg/kg, i/m and general anaesthesia was achieved by using ketamine @ 5 mg/kg or propofol @ 5 mg/kg body weight i/m. After proper premedication and anaesthesia, in group I, fifteen dogs which were affected with benign tumours were treated surgically. In group II, five dogs which were affected with malignant tumours were treated surgically while remaining five dogs of group III which were affected with malignant tumours were treated by lumpectomy plus antineoplastic therapy (i.e. Vincristine sulphate @ 0.025 mg/kg body weight iv followed one week later by Methotrexate @ 0.3 mg/kg body weight) intravenously at weekly interval under fluid therapy. Blood samples were collected from the dogs before surgery and at 60, 120, and 180 minutes during and post surgical interventions.

The venous blood (approximately 3 ml) was collected by vein puncture either from cephalic or saphenous vein in the vials containing ethylene diamine tetra acetic acid (EDTA). Before mixing the EDTA, slides were immediately prepared for DLC. The estimation of haematological parameters were done by automated haematology blood cell counter, Sussi. The blood samples (3 ml) from the dog were collected before surgery, post surgery and after application of antineoplastic drugs to estimate the biochemical parameters. These parameters were estimated by standard methods using semi-automated analyzer (Logotech-Techo-168). The haematological parameters evaluated were haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC) and differential leucocyte count (DLC) (Table 1).

Results and Discussion

Haemoglobin showed a non-significant decrease after lumpectomy alone in group I and II at various intervals. These values ranged between 10.12±0.83 g/dl to 13.65±0.2 g/dl. In animals of group III, there was a non-significant increase in the haemoglobin level between 60 to 120 minutes followed by decrease at 180 minutes. These values ranged between 10.16±0.34 g/dl to 11.96±0.23 g/dl. The packed cell volume showed a non-significant decrease between 60 to 120 minutes which returned to normacy by 180 minutes in all the treatment groups at various intervals. The PCV values ranged from 33.50±1.65 % to 36.92±1.78 % in different groups of animals at various intervals. There was increased TLC count at 0 hour interval before surgery. The TLC decreased significantly (P<0.01) from 60 minutes up to 180 minutes post surgery. The values ranged between 9.20±0.16 to 17.81±0.12 before and after surgery. The haematological parameters revealed significant reduction in total erythrocyte count (TEC), total leucocyte count (TLC) and total platelet count (TPC) in both the groups.

Differential Leucocyte Count (DLC)

A non-significant increase in neutrophils (%) observed between 60 to 120 minutes in all the three groups during post-surgical treatment. The increased values ranged from 64.67±0.71 per cent to 71.0±0.71 per cent. The values returned towards normalcy by 180 minutes post surgery. Combination chemotherapy might cause decrease in the neutrophils (Walter and Biller, 2006). A non-significant decrease in lymphocytes (%) was observed between 60 to 120 minutes followed by an increase in lymphocytes at 180 minutes after surgical treatment.
in all the groups of animals. The decreased values ranged between 23.80±0.49 per cent to 30.73±0.53 per cent. In
neutropenia as a compensatory mechanism lymphocyte count increases in the blood or due to change in neutrophil
lymphocyte ratio (Jacob, 2008). The monocyte count fluctuated non-significantly at different time intervals after various
treatments. These values ranged from 3.13±0.17 per cent to 4.20±0.51 per cent in all the groups of animals at various
intervals. The eosinophil count fluctuated non-significantly at various intervals after different treatment and the values ranged
from 1.00±0.32 per cent to 1.60±0.51 per cent in all the groups of animals at various intervals. There were non-significant
changes in basophils in all the groups of animals at various intervals post treatment and the values ranged between
0.00±0.00 per cent to 0.60±0.02 per cent.

Based on these observations it can be inferred that alterations in haematological values due to skin and
subcutaneous neoplasms, probably depend upon immune status, hormonal changes, diet and the relative number in
different geographical areas.

References

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### Table 1: Haematological observations before and after treatment in dogs affected with skin and subcutaneous neoplasm

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (n=15 in I and n=5 in II,III)</th>
<th>0 minute</th>
<th>Post operative time interval (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>60 minutes</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>I</td>
<td>13.65±0.23</td>
<td>12.92±0.22</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>12.28±0.24</td>
<td>11.12±0.40</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>11.16±0.34</td>
<td>11.48±0.23</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>I</td>
<td>35.98±1.66</td>
<td>35.62±1.59</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>36.92±1.78</td>
<td>36.88±1.48</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>33.88±1.10</td>
<td>33.84±1.80</td>
</tr>
<tr>
<td>TLC (x 10^6 cu mm ^{-1})</td>
<td>I</td>
<td>17.20±0.13</td>
<td>12.51±0.09</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>17.81±0.12</td>
<td>13.81±0.08</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>16.41±0.12</td>
<td>12.81±0.08</td>
</tr>
<tr>
<td>DLC Neutrophils (%)</td>
<td>I</td>
<td>64.67±0.71</td>
<td>64.93±0.75</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>70.00±0.63</td>
<td>70.80±0.97</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>68.20±0.37</td>
<td>68.60±1.44</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>I</td>
<td>30.73±0.53</td>
<td>30.47±0.62</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>25.40±0.24</td>
<td>24.60±0.98</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>26.20±0.49</td>
<td>25.80±1.24</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>I</td>
<td>3.13±0.17</td>
<td>3.20±0.17</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3.20±0.20</td>
<td>3.80±0.37</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>4.00±0.45</td>
<td>3.20±0.20</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>I</td>
<td>1.20±0.24</td>
<td>1.20±0.24</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.20±0.37</td>
<td>1.40±0.51</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1.40±0.40</td>
<td>1.60±0.51</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>I</td>
<td>0.20±0.11</td>
<td>0.13±0.09</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.20±0.20</td>
<td>0.20±0.20</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.20±0.10</td>
<td>0.60±0.14</td>
</tr>
</tbody>
</table>

**P<0.01 = Significant at 1% level
SUCCESSFUL MANAGEMENT OF EYE CANCERS IN DEONI CATTLE: A REVIEW OF 10 CASES

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ABSTRACT
A study was conducted in 10 clinical cases of eye cancer in Deoni animals. Out of 10 cases 8 were Deoni bullocks and 2 were Deoni cows. The age of bullocks ranged between 8-10 years where as the age of cows ranged between 5-7 years. The diagnosis was done by history, clinical signs and histopathological examination. Superficial keratectomies followed by cautery with 1% silver nitrate solution were done in all cases. After follow up of one year, recurrence was seen in two cases in same operated eye where above surgical treatment was followed. Along with this, administration of autogenous vaccine and BCG vaccine was carried out. No recurrence was seen even after one year.

Key words: Deoni cattle, eye cancer, silver nitrate, autogenous and BCG vaccine

Introduction
Eye cancer is the most common neoplasm in cattle (Radostits et al. 1995). Eye cancer is seen in 0.8 to 1.6% of cattle population in United States (Cleaver et al., 1972). In some areas, it is nearly 5% (Moulton, 1961); among the eye tumours, 91% are malignant and most common is the carcinoma (Moulton, 1961; Priester and Mantel, 1971 and Kircher et al., 1974). In some parts of Maharashtra, incidence of eye cancer was found to be 2-8% (Panchbhai et al., 1987).

Cancerous growth of eye may be classified as plaque, papilloma and invasive squamous cell carcinoma (Bhaskar, 2011). Treatment method for eye cancer in food animals in United States include radiation therapy, immunotherapy and electro-thermal therapy (Gelatt and Williams, 1985). These are costly and not possible in Indian field conditions. Surgical excision of the growth followed with cautery with 1% silver nitrate (Tyagi and Singh, 1993). However, extensive follow up of the cases was not done. Hence, the present work was taken with the objectives to surgical excision of tumour cautery with 1% silver nitrate solution and immunotherapy and follow up of these cases up to 1 year.

Materials and Methods
A study was taken in 10 clinical cases of eye cancer in 8 Deoni bullocks were aged between 8-10 years of age and 2 Deoni cows were aged between 5-7 years. Out of 8 Deoni bullocks, 4 bullocks had plaque growth on limbus and remaining 4 bullocks had papilloma growth either on cornea or sclera. Out of 2 Deoni cows, 1 cow had plaque adjacent to sclera and another cow had papilloma on eye lid region. Design of study is given in Table 1.

Animals were off fed for 48 hours prior to operation and were operated in standing position under xylazine sedation @ 0.03 mg/kg b.wt., i/m and auriculopalpebral and Petersons orbital nerve block with 2% lignocaine hydrochloride. Similar anaesthetic techniques were followed by other authors. Soma (1971) used auriculopalpebral and Petersons nerve blocks for eyelids and eyeball in bovines. Gelatt (1991) mentioned that eyelid and orbital surgery can be performed by using Peterson nerve block in standing cattle. Superficial keratectomy with cautery with 1% silver nitrate solution with the ear bud was followed. The eye was fixed by placing stay sutures on the sclera using silk. Prophylactic dose of ciprofloxacin injection @ 2 mg/kg, i/m was 3 hours before surgery. Membranoplasty was done to protect operated eye up to 15 days. Post operatively, the eye was cleansed with alum solution and ciprofloxacin eye drops instilled through the surgical window of membranoplasty. Along with 1 ml ciprofloxacin injection was administered subconjunctivally and 15 ml meloxicam was administered intramuscularly.

Excised tumour mass was collected in 10% formal saline. The tissues were processed as per standard technique. The paraffin sections were cut to a size of 3-5 micron thickness. The slides were stained with haematoxylin and eosin for histopathological studies (Culling, 1974).

Follow up of these animals was done up to 1 year to know recurrence of tumor.

Results and Discussion
The clinical cases of different size of eye cancer were subjected to superficial keratectomy followed by cautery with 1% silver nitrate solution with ear bud. Similar operative procedure were performed by Pandey et al. (1989) where eye cancer in cattle were treated by surgical excision followed by cautery of growth bed with silver nitrate. He observed healing of wound in 20 to 25 days in all the 36 animals. Membranoplasty was done to protect operated eye up to 15 days. Bullocks and cows showed normal healing of the excised wound at the site of surgery without lachrymation and inflammatory changes were minimum and no recurrence of tumour up to 3 months. However, mere inflammatory changes and cloudiness were noticed around surgical site. Vision was retained from rest of the cornea. After 3 months, no inflammatory changes and cloudiness were noticed around surgical site. Normal vision was retained.

As follow up of these animals up to 1 year, the recurrence
of cancerous growth on the same eye was noticed in one papillomatous bullock on sclera and one papillomatous cow on eye lid. Similar recurrence reports were reported by other authors. Bhume et al. (1992) reported different treatments in 22 clinical cases of bovine ocular squamous cell carcinoma. The animals were observed for 200 days postoperatively. They reported recurrence in all the cases treated with surgical excision and subsequent cauterization with silver nitrate. Complete cure was observed in all the cases after exenteration of eye ball.

Panchbhai et al. (1989) treated the ocular squamous cell carcinoma of cornea and conjunctiva of both the lids and third eye lid in cattle with surgical intervention followed by autogenous vaccine along with BCG. Complete cure was observed in 37.5% cases. In another group where the surgery along with cauterization of growth bed with marking nut oil was done, 12.5% cure was observed.

Radhakrishnan et al. (1999) reported successful treatment of bovine ocular squamous cell carcinoma through immunotherapy and surgery. They used BCG as immunotherapeutic agent. Grossly, the plaques were round, raised, smooth and size in millimetre. Papillomatous growths were irregular or ulcerated, cauliflower like appearance and size in centimetre (Fig. 1 and 2). The growths were whitish to pinkish coloured. In some cases, show fragile bleeding surface and foul odour. Similar findings were observed by Gharagozlou et al. (2007) and Kachhawa et al. (2015) where they reported that papillomatous growth vary from few centimetre to several centimetres, had cauliflower like appearance. The surfaces

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**Table 1: Design of research work**

<table>
<thead>
<tr>
<th>Bullock/Cow No.</th>
<th>Histopathological Diagnosis</th>
<th>Location</th>
<th>Technique used</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Deoni bullocks (8-10 years)</td>
<td>Plaque</td>
<td>Limbus</td>
<td><strong>Anaesthesia</strong>: Xylazine(^1) @ 0.03 mg/kg b.wt. i/m. and auriculopalpebral and Petersons orbital nerve block with 2% lignocaine hydrochloride(^2). <strong>Surgery</strong>: Superficial keratectomy with cauterization by 1% silver nitrate solution. Membranoplasty to protect operated eye. Ciprofloxacin eye drops and injection(^3) 1 ml subconjuctivally and Meloxicam(^4) inj. 15 ml intramuscularly. Follow up was done for 1 year.</td>
</tr>
<tr>
<td>4 Deoni bullocks (8-10 years)</td>
<td>Papilloma</td>
<td>Cornea/Sclera</td>
<td></td>
</tr>
<tr>
<td>1 Deoni Cow (5-6 year)</td>
<td>Plaque</td>
<td>Adjacent to sclera</td>
<td></td>
</tr>
<tr>
<td>1 Deoni Cow (5-6 year)</td>
<td>Papilloma</td>
<td>Eye lid</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Xylazine- injections, Indian immunologicals Ltd, Hyderabad; \(^2\)Lignocaine hydrochloride-injections, Astra Zeneca Pharma Ltd, Bangalore; \(^3\)C-Flox injection, 50 ml, Intas Pharmaceuticals, Matoda, Ahmadabad; \(^4\)Melonex injection, 100 ml, Intas Pharmaceuticals, Matoda, Ahmadabad

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Tyagi and Singh (1993) stated that Bacillus Calmetta Guerin (BCG) and methanol extract of mycobacterial cell membrane were used non-specific immunotherapeutic agents for bovine squamous cell carcinoma where recurrences of the tumors were not seen provided those were caused by viruses.
were ulcerated and bleed easily.

On histopathological examination, these were characterised by keratin “pearl” formation with highly anaplastic, small and hyperchromatic cells with no keratinisation. Inflammatory cells infiltration was also evident indicative of Bovine Ocular Squamous Cell Carcinoma (BOSCC). Similar findings were observed by other authors. Kircher et al. (1974) described carcinoma histopathologically and observed tumour that arises in the nests of the cell in the basal layer of plaques or papillomas. Spradbrow et al. (1977) detected the characteristic features of invasive carcinomas as branching down growth of the columns of the cells from the surface epithelium.

No recurrence of cancerous growths was noticed in these animals on 1 year follow up. In conclusion, surgical excision of tumour followed by cauterization with 1% silver nitrate solution and immunotherapy is best suggestive technique to prevent the recurrence of growths on eye. This technique is beneficial in initial stages of cancerous growth and also saves the vision of animal.

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DIAGNOSIS AND MANAGEMENT OF GASTROINTESTINAL FOREIGN BODY OBSTRUCTION IN THREE DOGS

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Introduction

Foreign body obstruction is a common cause of mechanical gastrointestinal obstruction in dogs. Indiscriminate eating habits or tendency of dogs to play with foreign objects may result in blockage of GIT. GIT obstruction is an emergency, any delay in treatment can be fatal (Kumar et al., 2000). Diagnosis of foreign body obstruction can be made from clinical presentation, radiographic, ultrasonographic and endoscopic examination. Abdominal pain, vomiting, anorexia, melena and absence of defaecation are the common clinical signs (Dunn, 1999). Radiopaque foreign bodies can be detected on plain radiographs (Penninck and Mitchell, 2003). Foreign bodies lodged in intestines and stomach are removed surgically by antimesenteric enterotomy and gastrotyom procedures (Fossum, 2002). Time delay in diagnosis, correction of primary disorder and associated haematobiochemical complications, have a significant impact on the postoperative recovery and survivability of the animal. Present report includes haematobiochemical changes, diagnosis and surgical treatment in canine patients suffering from gastrointestinal foreign body obstruction.

Case history and observations

Study includes 3 dogs (two male and one female) presented to the university teaching hospital for gastrointestinal obstruction with symptoms of abdominal pain, anorexia and absence of defaecation. The animals were having age varying between 1-2 years. Diagnosis was made from history, clinical presentation and radiography. Animals were having moderate to severe dehydration, graded as per Levitt and Bauer (1992). GIT obstruction was identified on lateral radiographs. Lateral radiograph of case 1 showed radiopaque foreign body in the intestinal loops with grossly distended intestinal loops proximal to the obstruction (Fig. 1). In second animal radiograph showed a circular density in mid abdominal region, which was later, found to be a crazy ball. Involvement of urinary bladder calculus was ruled out with the help of pneumocystography (Fig. 2). In third animal radiograph showing heterogeneous density in the cranial and mid abdomen suggested the foreign body to be lodged in gastric area (Fig. 3). Haematological examination of the animals showed lowered levels of Hb (6.33±0.60 g/dL) and PCV (17.67±1.20%), marked leukocytosis (17.27±1.41 thousands/µL), neutrophilia (87.00±1.00) and lymphocytopenia (12.50±0.50). Hypoproteinaemia (2.30±0.15 g/dL) and hypoglycaemia (4.83±0.17 g/dL), hypoalbuminaemia (2.53±0.13 g/dL), and lymphocytopoenia (12.50±0.50). Hypoproteinaemia leukocytosis (17.27±1.41 thousands/µL), neutrophilia (87.00±1.00)

Treatment and Discussion

After establishing the condition, animals were stabilized by administration of intravenous fluids, broad spectrum antibiotics and multivitamins. Surgical management was done using anaesthetic protocol comprising of a preanaesthetic combination of Glycopyrrolate (Pyrolate®, Neon laboratory limited) @ 0.01 mg/kg bwt s/c followed by Diazepam @ 0.5 mg/kg bwt (Lorit®, Neon Vet) intravenously. Induction was achieved by thiopental sodium (Thiosof®, Neon Vet) @10 mg/kg bwt., followed by maintenance with Isofluorane (isorane®; Piramal Healthcare) mixed with oxygen. Surgical procedure included midline laparotomy in dorsal recumbency. In two animals intestinal foreign bodies were removed by performing antimesenteric enterotomy (Fig. 4 and 5). In third animal gastrotyom and antimesenteric enterotomy (Fig. 6) were performed to clear the impaction of intestine and stomach. Standard methods of enterotomy and gastrotyom were followed taking care to prevent abdominal contamination by spillage of GIT contents in the abdomen. Polygactin No. 3/0 (Vicryl®, Johnson and Johnson Ltd) was used for closure of gastrotyom and enterotomy incisions by double layer of Lambert and Cushing inversion suture patterns. Intestinal defect was covered by a layer of omentum. Linea alba incision was closed in routine manner by simple interrupted suture pattern followed by subcuticular suture pattern using no. 2/0 Vicryl. Skin was closed by simple interrupted pattern with nylon No. 1/0. Postoperatively animals were managed by intravenous fluids for 1-5 days, parenteral broad spectrum antibiotics for 5 days and analgesics for 3 days. Oral feeding with liquid diet like rice water and milk was started after 3 days. Normal feeding was started after 5-7 days.

Anorexia, depression, dehydration, lack of defaecation and abdominal pain, the most commonly recorded complaints at the time of presentation, have earlier also been reported by, Levitt and Bauer (1992), Tyrell and Beck (2006) and Papazoglou et al. (2010) in dogs with gastrointestinal disorders. Moderate to severe dehydration recorded made the role of intravenous fluid administration during the peri-operative period very important as it might have helped in correcting dehydration and fluid imbalances consequent to mechanical obstruction. GIT obstruction in present study was caused due to ingestion of foreign objects like stones, ball and coir of sofa. Radiography was found useful for the diagnosis. Habit of eating unusual objects like stones, bones, coins, balls, clothes, rags, metallic spoons, rubber nipples and magnets has been reported in young dogs (Mohindroo et al., 2006; Hayes, 2009; Rossmass et al., 2011). More activity and playful habit of young dogs are responsible for ingestion and subsequent lodgment of foreign body in GIT (Koike et al., 1981; Applewhite et al., 2002; Han et al., 2008).
Haematological alterations like anaemia; depicted by lower levels of Hb and PCV, neutrophillic leukocytosis and lymphocytopenia may be due to blood loss owing to haemorrhage at the site of obstruction, long duration of anorexia and response of the body towards inflammation. Similar haematological findings in animals suffering from gastrointestinal problems have been reported by Wilson and Burt (1974) and Gal et al. (2007). Biochemical changes like hypoproteinaemia, hypoalbuminemia and hyperglobulinaemia indicate the response of body to inflammatory reaction and increased secretion of albumin into the intestine. Hypoglycaemia indicate anorexia. Strombeck and Guilford (1990), Willis and Farrow (1991) and Kahn (2005) attributed hypoproteinaemia and hypoalbuminemia to GIT blood loss in chronic cases of intussusception, extravasation of plasma proteins from mucosal vasculature and interstitial spaces due to mucosal damage by the foreign body. Degradation of protein and reduced nitrogen absorption due to bacterial overgrowth in stagnant intestinal loops may further aggravate the problem.

Animals suffering from complete obstruction of GIT are medicinal and surgical emergencies. Early diagnosis and surgical intervention are important as the chance of intestinal perforation and peritonitis increase with delay in surgery. Antimesenteric enterotomy and gastrotomy are the standard techniques for removal of foreign bodies lodged in intestine and stomach. Different suture patterns have been used by various workers. Inversion sutures have been reported to cause reduction in the lumen diameter while eversion sutures increase adhesion formation (Weisman et al. 1999; Brown 2003; Holt and Brockman 2003). In present study intestine and stomach were closed by double layer of Lambert and Cushing suture patterns using number 3/0 Vicryl. Omentization of the intestinal defect helped in providing additional blood supply to the area and protection from leakage. Postoperative mortality was recorded in one animal which was having multiple sites of obstruction. Other animals recovered uneventfully. The animal who could not survive was having compromised haematobiochemical profile and was presented late for surgery. Other two animals regained their normal appetite within one week of surgery. Haematobiochemical parameters returned within normal range during a period of 1-2 weeks postoperatively. Postoperative complications like recurrence, suture dehiscence and peritonitis which have been reported by earlier workers (Wilson and Burt, 1974; Weaver, 1977) were not noticed in the present study during a follow up period of 1 year. Peri-operative antibiotic therapy helped in reducing bacterial growth and thereby circumventing the chances of peritonitis secondary to peritoneal contamination which may occur during intestinal surgery (Walshaw, 1985).

In the present study, radiography was found useful for diagnosis of the GIT foreign bodies. Early presentation, diagnosis and surgical intervention improved the outcome of gastrointestinal affections. Good surgical technique followed by adequate postoperative care and fluid therapy are useful in saving lives of many canine patients.

References
Fig. 4: Intra-operative photograph showing mechanical obstruction of intestinal loops with foreign body. Observe the difference in size of affected jejunal loops in comparison to healthy loop (a). Retrieval of stone from jejunal loops (b).

Fig. 5: Intra-operative photograph showing mechanical obstruction of intestinal loops with a foreign body (a). Crazy ball retrieved from jejunal loops (b).

Fig. 6: Intra-operative photograph showing impacted foreign material lodged in the stomach (a). Obstructing material/coir of sofa retrieved from the jejunal loops (b).

ABOMASAL IMPACTION DUE TO PHYTOBEZOARIASIS IN BUFFALO

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ABSTRACT

A phytobezoar is known as a fibre ball. They are accumulations of indigestible plant material in the digestive system of ruminants. Based on their composition, bezoars are further categorized into five subtypes that include phytobezoars, trichobezoars, lactobezoars, medicinal or pharmacobezoars, and concretions. A three and half years old crossbred buffalo was presented with the history of recurrent bloat, respiratory distress, inappetance to anorexia since 20 days and absence of defaecation. On post-mortem examination a nearly 3.0 kg of Phytobezoar recovered from the abomasum of animal. Surgery is the only way to treat a phytobezoar obstruction.

Key words: Abomasal impaction, phytobezoar, buffalo

Introduction

Bezoars are recognized as a form of concretion, which develop within the gastrointestinal tract of animals and humans. Based on their composition, bezoars are further categorized into five subtypes that include phytobezoars, trichobezoars, lactobezoars, medicinal or pharmacobezoars, and concretions (Anderson and St-Jean, 2002; Kishan et al., 2003; Ku, 1996). However, trichobezoars, phytobezoars, and a mixture of both, which are named phytotrichobezoars, are the most common subtypes reported in veterinary medicine (Oehme and Prier, 1986). Gastrointestinal phytobezoars are also reported in humans (Holloway et al., 1980), horses (Mealey et al., 1995; Cummings et al., 1997), cattle (Radostits et al., 2000), sheep, and goats (Bath et al., 1992; Oehme and Prier, 1986). Overall, the formation of bezoars appears to be most prevalent in ruminants, particularly in small ones. Trichobezoars have also been reported to cause obstruction in the lower gastrointestinal tract (Radostits et al., 1994). Phytobezoars (fibre balls) consists largely of cereal awns and hairs impregnated with some phosphate salt and rolled into balls and are usually rounded and smooth (Gahlot et al., 2006). The results of a field study (Azizi and Mirza Aghazadeh, 2008) and clinical observations have shown that abomasal phytobezoariasis is one of the most common causes of death (Azizi et al., 2010). But reports on phytobezoars in buffaloes are few. This report presents an unusual case of abomasal impaction due to phytobezoariasis in buffalo.

Case history and clinical examination

A three and half years old crossbred buffalo was presented to TVCC with the history of recurrent bloat, respiratory distress, inappetance to anorexia since 20 days and absence of defaecation. The case was treated by local veterinarian/quacks for simple bloat. Clinical examination revealed that the rectal temperature was within normal range, respiratory rate was 48 breaths/min and heart beat was 78 beats/min and conjunctival mucous membrane was pale pink and CRT <3 sec. Hyper motility of rumen (7/5 min.) was observed. Ruminal fluid analysis revealed 7.5 pH and absence of protozoal motility. A high pitched sound was heard on auscultation and percussion of the left paralumbar fossa due to bloat. Per rectal examination did not reveal any obstructions.

TREATMENT AND DISCUSSION

The treatment was given symptomatically i.e. inj. RL 4 lt. iv, inj. 5% DNS 3 lt. iv, inj. Rumeric 10 ml i/m, inj. Synkomet 10 ml i/m, powder Opener 125 gm with castor oil 200 ml and powder magnesium sulphate 500 gm orally for 3 days. The watery faeces came next day onwards but the animal collapsed on third day. On post-mortem examination a nearly 3.0 kg of phytobezoar recovered from the abomasum of animal.

A phytobezoar is known as a fibre ball. They are accumulations of indigestible plant material in the digestive system of ruminants. These occur in areas where fibrous feeds make up a substantial part of the cows diet, initially these fibrous materials accumulate in the rumen, contractions of the rumen then cause these fibres to roll around and form a ball. The balls can be variable in size, from marble size to grapefruit, even football size (Al Hindi, 2004). In the present case also unusually large phytobezoar of the size of a football 62 cm circumference at the centre that was porous and fragile. Veeriah et al. (2008) also recorded a fatal phytobezoar in a she buffalo and reported obstruction of reticulo-omasal orifice leading to excessive ruminal contraction, regurgitation, aspiration pneumonia and death. Bath and Bergh (1979) and Schneider and Hugo (1980) have also indicated that bezoars cause abomasal impaction manifested by anorexia and heavy mortality in young ruminants.

The occurrence of abomasal phytobezoariasis in ruminants is rare and, usually, it occurs in the sporadic form (Ravikumar et al., 1989; Sargison et al., 1995). However, large numbers of sheep and goats could be affected simultaneously by the disease due to some particular management circumstances (Bath et al., 1992). It has been identified that bezoars are the most common foreign bodies (35.3%) in ruminants (Asma Amer

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Hammad Hamid, 2015). An enzootic like occurrence of abomasal phytobezoarasis has been observed among Herrick sheep flocks in Urmia (Azizi and Mirza Aghazadeh, 2008).

Gastrointestinal bezoars are prevalent phenomenon in ruminant and most occur in inefficient management conditions, abomasal bezoars are relatively common in young beef calves (Doustar et al., 2012). Formation of bezoars is common for plant hairs that are apparently poorly digested by ruminal microorganisms and accumulate, especially in the abomasum. Here under the influence of abomasal movement, innumerable hairs conglomerate (possibly around a small milk clot as nucleus) and adhere together firmly in packed, dense layers to form balls of various shapes and 10 to 150 mm in diameter. Up to 30 balls might be present in the abomasum (Chisholm et al., 1992).

In the affected animals, phytobezoars are formed within the abomasum over a period of a few months. There are limited clinical manifestations in the latent phase. Then, due to gradual loss of appetite and the abomasal impaction, the affected animal becomes progressively weak, which finally causes emaciation and death if the animal is not treated surgically (Azizi et al., 2010).

The phytobezoar was fatal as it has obstructed the reticulo-omasal orifice leading to excessive ruminal contractions, regurgitation, aspiration pneumonia and death. These are often revealed at post-mortem examination as reported by Sastry (1983). There are no special paraclinical or ancillary tests found in the literature to diagnose abomasal phytobezoars in sheep. However, plain lateral radiography could help diagnose substantial abomasal phytobezoars or the use of contrast media with barium sulphate, as performed in the residual bodies in Herrick sheep (Torkamani, 2003). However, the latter technique requires an extensive period of time. Surgery is the only way to treat a phytobezoar obstruction, most animals that are still on their feet at the time of surgery will recover and many are back on their milk within 48 hours. The outlook is very poor for animals that are down and unable to rise (Asma Amer Hammad Hamid, 2015).

Acknowledgements
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MANAGEMENT OF OESOPHAGEAL OBSTRUCTION DUE TO PHYTOBEZOARS IN A CATTLE

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The oesophagus is a musculo-membranous tube which connects the pharyngeal cavity to the stomach. It is divided into cervical, thoracic and a very small abdominal part (Sisson and Grossman, 1967). Oesophageal obstruction occur in cattle due to foreign body or feed particles (O’ Connor, 1965; Singh et al., 1993), trichobezoar and phytobezoars (Radostits et al., 2000). Intraluminal blockage of the oesophagus, popularly called choke, may occur due to vegetables (e.g. turnips), fruits (e.g. large size lemons and apples), phytobezoars and various foreign bodies. Extraluminal causes; large peri-oesophageal abscess, enlarged mediastinal lymph nodes and tumours, may also lead to partial obstruction of the lumen of the oesophagus due to pressure. In ruminants, obstruction occurs mostly in the cervical region and however, obstruction of the thoracic oesophagus is rare.

Case history and observation

A cattle was presented in TVCC, Bikaner with a history of stretched neck and swelling in the ventral neck region with inability to swallow feed and water. The patient remained thirsty and made attempts to drink water which often returned back through the nostrils carrying food particles with it. Based on the clinical observation and history it was diagnosed as obstruction (Phytobezoars) in oesophagus with the confirmation after plain (Computed) radiograph. Computed radiograph reveals an oval shape mass (Fig. 2) in oesophagus.

Treatment and Discussion

Restrainting the cattle in lateral recumbency and applying mouth gag (Fig. 1). A conservative treatment was aimed at reducing the oesophageal spasm and to dislodge the obstruction by external or internal manipulations. Once the spasm of the oesophagus was controlled and some degree of relaxation was achieved, it was possible to clear the cervical obstruction by placing the thumb or fingers distal to the foreign body and gradually forcing it upwarded until it reached the pharynx. The phytobezoars was removed with the help of fingers. Xylazine sedation (0.1mg/kg b.wt. i/m) was used for relaxation of oesophagus. Animal was administered inj. streptomycin 2.5 gm and inj. phenyl butazone 3 mg/kg body weight i/m for 5 and 3 days, respectively. Animal was cured. Obstruction of oesophagus occurs mostly in the cervical region and obstruction of the thoracic oesophagus is rare in ruminants (Singh et al., 1993). The foreign body, which might have been passed initially to the rumen, could have come back during the act of rumination towards the mouth and caught half way (Ramadan and Abdi-Bey, 1990). The high incidence of cervical obstruction might support the idea that oesophagus in the caudal cervical region was narrower than earlier region or the pressure exerted by the first rib and the trachea could act as a predisposing factor (O’ Connors, 1965). The undigested plant fibres of fodder and hairs may be rolled during ruminal contraction into ball called trichophytobezoars and such animals consumed little concentrate feed in comparison to others (Reddy et al., 2003). These trichophytobezoars are usually rounded and smooth, but some are convoluted like the surface of the cerebrum (Jubb and Kennedy, 1963; Runnels et al, 1976). However, in the present paper an oval and smooth phytobezoar was recovered. In agreement with Singh and Nigam (1980), radiographic observation was advantageous to ascertain the site and the nature of the foreign bodies which either migrated or lodged in the various regions.

Fig. 1: Restraining the cattle in lateral recumbency, applying mouth gag after xylazine sedation and removing phytobezoars

Fig. 2: Plain (Computed) radiograph before and after removal of phytobezoars

References


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SURGICAL MANAGEMENT OF DERMOID CYST IN A CALF

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Introduction

Ocular dermoid is a skin or skin like appendage usually arising on the limbus, conjunctivae and cornea. It can be unilateral or bilateral and may be associated with other ocular manifestation or with other malformation. Hairs from the lesion is predominantly responsible for the associated irritation resulting in chronic inflammation of conjunctivae and cornea and may cause visual impairment (Barkyoumb and Leipold, 1984 and Moore et al., 1999). Dermoids may affect the eyelids, conjunctiva, nictitans, sclera and cornea which are most commonly present unilaterally. Bilateral ocular dermoids have been reported in cattle (Croshaw, 1959; Yeruham et al., 2002). The present paper deals the surgical management of bilateral dermoid cyst in a female calf.

Case history and Observation

A six-day-old calf weighing approx. fifty kg was found in good bodily condition and no further abnormalities were detected on physical examination was presented in Ranchi Veterinary Clinical Complex with an abnormal appearance of both eyes since birth. Clinical examination revealed the slight vision in right eye whereas, no vision in the left eye. There was a large fleshy mass containing hairs was attached to sclera and cornea of both eyes with excessive lachrymation (Fig. 1). The case was diagnosed to be a congenital dermoid cyst.

Treatment and Discussion

The calf’s eyelashes were trimmed, then the eye was washed with normal saline solution to remove the contaminants and then dried with sterilized gauge. Lignocaine hydrochloride was infiltrated in upper and lower eyelids after controlling the animal in lateral recumbency. Eye speculum was used for proper exposure of operative field. The dermoid was grasped with allis tissue forceps and the chromic catgut no.1/0 was used for ligation and suturing of stamp of dermoid mass. The mass was excised and bleeding was controlled by instillation of adrenaline solution. The same procedure was carried out with another eye. Eye was flushed with NSS solution 2-3 times until blood clot was removed from the eye (Fig. 2). Post-operatively, gentamicin and cortisone eye drop was intilled @ 4 drops b.i.d in both the eyes followed by systemic administration of inj. gentamicin @ 3 ml intramuscularly for 5 days. The calf made uneventful recovery with appearance more vision and absence of lachrymation in the right eye whereas, vision was absent in the left eye even after 15th day post-operatively.

Ocular dermoid in cattle are not common with an estimated prevalence of 0.002-0.4% (Brunedall, et.al., 2008). Ocular dermoids have been reported in cattle of many breeds worldwide (Yeruham et.al., 2002). The apparent predisposition in Hereford is largely based on report by Barkyoubm and Leipold (1984). Excessive lacrimation in the present case was due to irritation caused by hair and cyst in the eyes.

Ocular dermoids may be associated with other congenital ocular or multiorgan abnormalities, which was not found the present case. Barkyoubm and Leipold (1984) described cardiac defects (teratology of fallot and patent ductus arteriosus), polycystic kidney disease and small masses protruding into the external nares in some of the 75 calves reported with ocular dermoid, although they did not specify the no. of calves affected and whether calves showed one or combination of the three abnormalities.

References

CLINICAL MANAGEMENT OF HAEMANGIOSARCOMA OF SPLEEN IN A DOG

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Key words: Haemangiosarcoma, dog, fine needle aspiration cytology, blood transfusion

Introduction

Haemangiosarcoma, a malignant neoplasm of endothelial cells, occurs more frequently in dogs than in other domestic animals. Hemangiosarcoma (HSA), also known as malignant haemangioendothelioma or angiosarcoma, is a malignant neoplasm of vascular endothelial origin. Haemangiosarcoma occurs more frequently in dogs than in any other species (Douglas, 2007) and is a rapidly progressive, fatal neoplastic disease. Although, the etiology is unknown, reports in humans have been related to exposure to thorium dioxide, arsenicals, vinyl chloride, and androgens (Falk et al., 1981). The most common primary site in the dog is the spleen (Schultheiss, 2004; Srebernik and Appleby, 1991). Other frequent sites include thtaitrium, skin and subcutis, and liver. Cases have also been reported in the lung, kidneys, oral cavity, muscle, bone, urinary bladder, left ventricle, uterus, and retroperitoneum (Brown, 1985 and Liptak et al., 2004).

Haemangiosarcoma is the most common splenic neoplasm encountered, but it is by no means the only differential for splenomegaly or splenic masses in dogs. The "double two thirds rule" has been applied to caninesplenic masses: approximately two thirds of those dogs with splenic masses will have a malignant tumour, and approximately two thirds of those malignancies will be HSA (Spangler and Cubertson, 1992 and Spangler and Kass, 1987). However, one study found that approximately 70% of dogs with splenic masses presenting With nontraumatic haemobabdomen were HSA (Pintar et al., 2003). Several differentials for solitary splenic masses (e.g., HSA, haematoma, haemangiomata, other sarcomas) can have a similar gross and ultrasonographic appearance. Histopathology is necessary to establish a definitive diagnosis in all cases. Results of clinical studies show that, to date, there is no effective therapy. Conventional methods of diagnosis and treatment are limited (Hirsch et al., 1981).

Materials and Methods

A six-year male Labrador dog was presented on sternal recumbency with the history of chronic inappetance, haematemesis, bloody diarrhoea and ascites. The dog was treated at local veterinary hospital for one week but there was no recovery of clinical signs. Clinical examination revealed normal vitals except for dyspnoea and tachycardia, dehydration (STT<5 sec), pale mucus membranes with marked anaemia and distension of abdomen (Fig. 1). On palpation fluid thrill (ascites) was noticed. As the dog is dyspnoeic, it was decided to aspirate/remove some amount of fluid. On aspiration blood tinged fluid was noticed. Blood and serum samples were collected for haematology and biochemical analysis. Radiograph, electrocardiogram and abdomen ultrasound scan were performed. Treatment was initiated symptomatically with diuretics (Inj. Lasix @ 2 mg/kg b.wt. iv), parenteral coagulants (Inj. Ethamsylate @ 125 mg total dose, iv), fluid therapy (Inj. DNS @ 10 ml/kg slow iv), antemetics (Inj. Emeset @ 0.2 mg/kg b.wt. iv) and antibiotic (Inj. Augmentin @ 20 mg/kg b.wt.). The vitals were monitored continuously all through the treatment and also during the diagnostic procedures. Later, based on results, the cause in the present case was found to be haemangiosarcoma of spleen and hepatoomegaly was also present. As the dog was anaemic, not fit for surgery and chemotherapy. Hence, the above therapy was continued to subside the clinical signs. Further, the next day blood transfusion was done (Fig. 3). Haematonics (Inj. Imferon @ 1 ml, i.m, daily) for one week. Syrup. Dextrone 5 ml bid per os and hepatoprotective agents (Silymarin @ 10 ml per os bid) were advised.

Results and Discussion

A complete blood profile was conducted, including a chemical blood profile. Anaemia ([Hb - 5 g/dl], PCV (17%), TEC (2.08 x 10⁶ mg/dl), slight increase in TLC (9.01 x 10⁶ mg/dl), thrombocytopenia (platelets - 98,000/μl), neutrophilia (73%), lymphocytosis (22%), normal range of monocytes (3%) and eosinophils (2%) were the haematological findings. Howell-Jowel bodies were the significant red blood cell morphology recorded. Johnson et al. (1989) reported Howell-Jowel bodies as one of the abnormal red cell morphology in dogs with splenic neoplasia. Anaemia is a common finding in animals with metastatic or widespread neoplasia. Anaemia of chronic disease, hypersplenism, microangiopathic haemolytic anaemia and blood loss from the tumour are likely to be responsible for anaemia associated with splenic neoplasia (Johnson et al., 1989). Hammer et al. (1991) reported that thrombocytopenia was the most common abnormality, being present in 75% of the patients with haemangiosarcoma. Serum biochemistry revealed increase in ALT (137 U/L), AST (102 U/L), BUN (47 mg/dl), total bilirubin (1.5 mg/dl), direct bilirubin (0.4 mg/ dl), creatinine (1.8 mg/dl) and decrease in total protein (4.8 mg/dl). Pintar et al., 2003 stated that serum biochemistry changes are typically nonspecific and can include hypoalbuminaemia, hypoglobulinaemia, and mild elevations in liver enzymes. On ECG electric alternans was noticed. Mark and Meg (2010) stated that electrical alternans is observed when large volumes of effusion (fluid) is present. Diagnostic imaging is one of the best methods for viewing the abdominal cavity and making an initial diagnosis.

But in the present case, X-ray of abdomen was not helpful as the dog had ascites which masked the appearance of abdominal organs (Fig. 2). Ultrasonography of abdomen revealed anechoic fluid and heteroechoic appearance ranging from anechoic to hyperechoic appearance masses in the spleen extending till
bladder. Hepatomegaly was also recorded. The findings are in accordance with Cuccovillo et al. (2002) and Wrigley et al. (1988). Ultrasonography assisted fine needle aspiration cytology of suspicious mass (simple and cost-effective) was performed. Douglas (2007) reported that fine needle aspiration cytology is of low diagnostic utility due to the haemodilution that usually accompanies sampling. But in the present study, fine needle aspiration revealed the mass as neoplasia. However, the case was referred to other veterinary hospital for conducting biopsy and the mass was confirmed as haemangiosarcoma. Later, thoracic radiography of the chest cavity was also performed to detect metastasis into the lungs and no abnormality was detected. Surgery remains the primary method of treatment for almost all dogs with HSA. But in the present case as the dog is severely anaemic blood transfusion was done to stabilise (Fig. 3). Blood was transfused from the apparently healthy dog @ 10 ml/kg body weight. According to Alex et al. (2015) dogs that subsequently received transfusions had higher mean illness severity score, heart rate, respiratory rate, blood lactate concentration, and prothrombin time, with lower mean PCV, platelet count, serum total solids and albumin concentrations, and base deficit than dogs that did not receive transfusions. Dogs that received transfusions had higher odds of death or euthanasia while hospitalized and lower odds of surviving to 30 or 180 days after hospital discharge than dogs that did not.

Symptomatic treatment was initiated with diuretics (Inj. Lasix @ 2 mg/kg b.wt. iv) for ascites, parenteral coagulants (Inj. Ethamsylate @ 125 mg total dose, iv) for haemachezia, fluid therapy with caution as the dog was anemic (Inj. DNS @ 5 ml/kg slow iv), antemetics (Inj. Emeset @ 0.2 mg/kg b.wt. iv) to control emesis and antibiotic (Inj. Augmentin @ 20 mg/kg b.wt.) to control secondary bacterial infection. The owner was informed about the prognosis and the mean survival time following surgery. However, as the owner was not interested in surgery the case was symptomatically managed till the clinical signs resolved. The dog resolved from diarrhoea and vomiting on day 5, ascites on day 7 and hemoglobin level was 8 g/dl on day 10.

References
A COMPARATIVE OESTRUS AND FERTILITY STUDY ON EVALUATION OF THREE SYNCHRONIZATION PROTOCOLS*

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ABSTRACT

Post-partum cyclic Rathi and its crossbred cows were subjected to oestrus synchronization using either Ovsynch (OVS) (n=12), double prostaglandin injections (PG) 11 days apart (n=12) or CIDR-PG-E2 (CPE) protocols (n=12). Oestrus was detected by twice visual observations and cows were inseminated at standing oestrus using frozen semen. The proportions of cows responding were 100%, respectively for three protocols. OVS, PG and CPE groups oestrus duration was 27.75±1.19 hours, 90±4.31 hours and 37±2.74 hours, respectively. The proportion of cows conceiving was 75, 66.67 and 75% for the OVS, PG and CPE groups, respectively. It was concluded that the OVS protocol is better for oestrus synchronization of Rathi and its crossbred cows.

Key words: Cycling cows, ovsynch, CIDR, prostaglandin, synchronization, conception rate

Introduction

Synchronization of oestrus implies the manipulation of oestrus cycle or induction of oestrus to bring a large percentage of a group of females into oestrus at a short, predetermined time (Oddie, 1990). However, the labour saving aspect is compared to the economic returns available when oestrus synchronization is used as a “reproductive management tool” (DeJarnette, 2001). Numbers of oestrus synchronization programmers are available in cattle based on the use of various hormones like progesterone, prostaglandin Fα and their various combinations with other hormones like oestrogen and gonadotrophin releasing hormones (Islam, 2011). Synchronization protocols that regulate follicular development with a GnRH injection 7 days prior to a luteolytic dose of PGF2α not only improve oestrus detection rates and synchrony of oestrus, but also induce fertile oestrus cycles in both cyclic and anoestrus bovine females (Thompson et al., 1999; Stevenson et al., 2000). In this study, Rathi cows of dairy farm were taken to study the oestrus and fertility response in a least time period.

Materials and Methods

Bos indicus or their crosses cows were taken from the private dairy herds of Bikaner region. General health point of view only normal and cycling cows were taken. A total of 12 cyclic cows (n=12) were taken in each group of protocols of oestrus synchronization. Hormones/hormonal device were used EAZI breed CIDR insert contains 1.38 g of prostaglandiner, inj. Receptial contains 0.0042 mg buserelin acetate per ml inj. Pragama contains 250 µg cloprostinol per ml, inj Progynon depot contains 10 mg estradiol valerate per ml.

Protocols of Oestrus synchronization

A. GnRH - PGF2α-GnRH (Ovsynch/OVS)

All the 12 selected cyclic cows were taken as a group and were given an inj. Buserelin acetate 20 µg i/m. The day of first inj. was considered d 1. On day 5th inj. cloprostinol 0.5 mg i/m was given and again on day 7th second inj. Buserelin acetate 20 µg i/m was given again. After this all the treated cows were observed daily morning-evening for 7 days for the occurrence of signs of oestrus. The time of start of signs of oestrus and duration of oestrus were recorded. A day after the end of protocol is on 8th day both the ovaries of all cows were scanned to note the number of follicles developing and to record the size of the dominant follicle. All the cows which showed signs of oestrus were inseminated artificially 12 to 18 hours.

B. Two shots prostaglandin (PGF2α) based (PG protocol)

One shot of inj. Cloprostinol 0.5 mg i/m was given and was considered as d 1 and then again were given second shot of same dose on d 11. The time of start of signs of oestrus and the total duration of oestrus were recorded for all the cows showed signs of oestrus and were inseminated 12 to 18 hours after the onset of oestrus.

C. Progesterone ‘CIDR’-prostaglandin-oestrogen based (CPE)

CIDR was inserted in all the 12 cows. The day of insertion was considered as d 1 of the start of protocol. Simultaneously, all the cows were given an inj. of estradiol valerate 2 mg i/m. CIDR was withdrawn on day 8 from insertion and inj. Cloprostinol 0.5 mg i/m was given. On day 9, all the cows were given an inj. of estadiolvalerate 1 mg i/m. After the end of this protocol all the treated cows were observed daily morning-evening for 7 days for the occurrence of signs of oestrus. All the cows showed signs of oestrus were inseminated artificially 12 to 18 hours after the onset of oestrus.

Pregnancy diagnosis

All the covered but non returned cows were scanned ultrasonographically or examined manually through rectum for

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the confirmation of pregnancy at 35 to 60 days of pregnancy.

**Results and Discussion**

Oestrus synchronization is good management tool for programmed breeding, hence leading to the programmed feeding and easier management of the cows being at the same stage of gestation and finally calving spread over a shortest possible period (Satter, 2002).

In OVS protocol the onset of oestrus was also significantly (P<0.01) earliest due to the effect of 2nd shot of GnRH, this protocol is considered most effective as in the shortest duration of within 16 hours (34-18=16 hours) all the cows showed onset of oestrus. Also the maximum CR (conception rate) 75% was recorded in this protocol. Geary and Whittier (1998) reported over all 57% CR in cows when used Ovsynch protocol of oestrus synchronization and Geary et al. (1998) reported that Ovsynch protocol is better in induction of oestrus and conception rate than syncromate B protocol. However, in both studies they worked on beef cattle where CR is considered quite lower than syncromate B protocol. Higher, in both studies they worked on beef cattle where CR is considered quite lower than that of in dairy type of breeds.

The mean diameter of the dominant follicle in PG protocol recorded was 10.33±0.55 mm, which was significantly (P<0.01) lower than the other two protocols. Another peculiarity of this protocol was that there was longest mean duration of onset of oestrus as 90±4.30 hours. This long duration is in accordance with the reportings of many other workers. (Dailey et al., 1983, Stephens and Rajamahendran, 1998; Kafi and Mousavi, 2000 and Rajamahendran et al., 2001). In this protocol, like in others, all cows of the group came in oestrus (100% induction of oestrus) but the onset was variable than other protocols as have also been reported by other workers as mentioned. CR in this protocol recorded was 66.66 % (8/12). However, Satter (2002) recorded 46% CR in his work with such type of protocol and reported that this low CR was due to the hot and humid season in which the work was done. Likewise Kafi and Mousavi (2000) reported 69% oestrus induction rate and 41% CR in the same type of protocol.

In CPE protocol 100% cows showed synchronized induction of oestrus. Ayalon and Marcus (1975) have also recorded 100% onset of oestrus in cows when used prostogestin impregnated vaginal sponges for 14 days and also reported 60% CR. Expression of signs of oestrus in the cows of this protocol was quite usual. Like other two protocols, all the cows showed occurrence of oestrus (100%) but 2 cows were not given the artificial insemination service because of mucopurulent discharge at the time of oestrus. This may be due to carrying mild degree of infection either carried previously or also suggestive of contamination in the application of CIDR leading to the condition. Though, the number of cows in the protocols was small but 70% CR was achieved which was higher than the PG protocol. Kafi and Mousavi (2000) reported 82% onset of oestrus and 63% CR in cows when applied CPE type of protocol for synchronization of oestrus.

**References**


EFFECT OF CYSTEINE HYDROCHLORIDE ON POST THAW VIABILITY AND ACROSOMAL INTEGRITY OF JERSEY BULL SEMEN

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ABSTRACT
A study was undertaken to assess the efficacy of cysteine hydrochloride concentrations on post-thaw progressive motility, live sperm, acrosome integrity and sperm abnormalities following freezing and thawing in Jersey bulls semen. Semen was collected from 3 purebred Jersey bulls twice a week and 18 collections (6 collections per bull) were utilized in this study. After collection and primary evaluation, samples were divided into four fractions and were diluted in egg yolk tris glycerol extender with addition of cysteine @ 10, 12 or 15 mM (CYS-10, CYS-12 and CYS-15, respectively) and without cysteine (EYTG-C) which acted as control. Filling and sealing of diluted semen samples in PVA straws, equilibration at 4°C for 4 h, freezing in programmable bio-freezer and plunging of straws into liquid nitrogen were followed as per standard procedures. After thawing at 37°C for 30 sec, semen characteristics such as progressive motility, live sperm percentage and acrosome integrity were significantly higher whereas sperm abnormalities were significantly lower in samples containing cysteine @ 10 mM over the control and other dilutors. The results revealed that addition of cysteine @ 10 mM as an additive to semen in tris egg yolk extender, significantly improves the post-thaw semen characteristics, whereas adverse effect of cysteine was evident in its higher concentrations i.e. 12 mM and 15 mM.

Key words: Cysteine, post-thaw semen quality, tris, bull semen, cryopreservation

Introduction
Crossbreeding of non-descript cows with high yielding exotic bulls is a well adopted technique for improvement of genetic merit and production potential of future progeny. Long term storage of semen facilitates easy transport over distances and enables maximized use of a bull, even after the sire’s death. Freezing and thawing exerts physiological as well as chemical stress on the sperm membrane (Chatterjee et al., 2001), associated with an oxidative stress induced by free radical (Salvador et al., 2006). Dilution of semen reduces the anti-oxidant reserves of seminal plasma, making the spermatozoa more susceptible to cryodamage (Muzaffer et al., 2012). Therefore, it becomes all the more necessary to incorporate anti-oxidants to semen extenders as protective agents.

Garner et al. (1994) reported that post thaw sperm quality and fertility from a routine sperm function assay would be beneficial in predicting bulls fertility. The combined assessment of acrosome integrity and semen motility had a significant effect on fertility (Kjaestad et al., 1993), thus is a reliable basis for post thaw estimation of semen quality.

Cysteine is a thiol compounds used in intracellular glutathione biosynthesis (Meister and Tate, 1976). Addition of cysteine to the semen dilutors have improved post thaw semen quality in different species (Uysal and Bucak, 2007; Perumal, 2008; Beheshti et al., 2011). Thus, the present work was undertaken to assess the beneficial effects of Cysteine, on post-thaw semen quality following freezing and thawing in Jersey bulls.

Materials and Methods
Three adult, sexually mature Jersey bulls maintained at frozen semen bull station, Hakkal, Jammu were selected for this study. The bulls were maintained under scientific and uniform conditions of feeding and management throughout the experimental period. Semen was collected twice a week between 7.00 AM and 8.00 AM using artificial vagina following standard protocol using a male partner as dummy. On each collection day, 2 ejaculates were collected at an interval of 15-20 min and the semen was kept in a water bath at 37°C till further processing. Total 18 collections from 3 bulls (6 collections/bull) were utilized in this study.

After initial evaluation, each semen sample based on at least 75% initial motility was divided into four equal fractions; one fraction was diluted in egg yolk tris glycerol extender without any additive which acted as control (EYTG-C), second fraction was diluted in egg yolk tris glycerol with addition of cysteine @ 10 mM (CYS-10), third fraction was diluted in egg yolk tris glycerol with addition of cysteine @ 12 mM (CYS-12) and fourth fraction was diluted in egg yolk tris glycerol with addition of cysteine @ 15 mM (CYS-15). The semen was diluted in such a way that it contained 80 million spermatozoa/ml post dilution. The diluted semen was filled in 0.25 ml poly vinyl chloride straws using an automatic filling and sealing machine. After equilibration for 4 hrs, sealed straws were frozen using programmable bio-freezer where the temperature was brought down from 4 to -140°C in 7 min. The straws were transferred to pre-cooled plastic goblets and plunged into liquid nitrogen. After 10-15 days of preservation in liquid nitrogen, at least 2
straws from each bull in each collection were thawed at 37°C for 30 sec and evaluated for various parameters such as progressive motility, livability, sperm abnormalities and acrosomal integrity. The data recorded was analyzed as per the procedure described by Snedecor and Cochran (1994).

**Results and Discussion**

The effect of addition of cysteine hydrochloride to tris egg yolk extender on post-thaw semen progressive motility percentage, live percentage, intact acrosome percentage and total sperm abnormalities are presented in Table 1. In the present study, the post-thaw progressive motility, livability and acrosomal integrity were significantly higher (P<0.05) and sperm abnormalities were significantly lower (P<0.05) in extenders containing cysteine hydrochloride @ 10 mM compared to control and other cysteine diluents. However, adverse effect of cysteine was evident at higher concentration diluents. The present findings authenticate earlier reports on supplementation of antioxidants for improving the viability and motility of liquid storage or cryopreserved sperm cells (Baumber et al., 2000; Maxwell and Stojanov, 1996; Perumal et al., 2011). Beneficial effect of cysteine might be due to its free radical scavenging property and reduction in lipid peroxidation of sperm plasma membranes.

Freezing-thawing process produces physical and chemical stress on the sperm cell membrane which in turn reduces sperm viability and fertilizing ability (Uysal and Bucak, 2007). The cold shock exerted on sperm cells during freezing to thawing process is associated with oxidative stress induced by free radicals (Sanocka and Kurpisz, 2004; Salvador et al., 2006).

Protection of sperm motility, viability and stabilization of sperm cells membrane during cryopreservation are of prime importance. Perusal of results indicates beneficial effect of cysteine @10 mM on sperm survival; where by increasing concentration of cysteine (12 mM and 15 mM) had adverse effect during the freezing thawing process. Similar results were reported by Uysal and Bucak (2007) by addition of different concentration (5 mM, 10 mM and 20 mM) of cysteine in tris based extender and better post thaw motility of ram semen by addition of cysteine @ 10 mM. Funahashi and Sano (2005) and Szczesniak-Fabianczyk et al. (2003) reported that a semen extender with cysteine improved the viability, chromatin structure and membrane integrity of boar sperm cells during liquid preservation. Moreover, in horses (Baumber et al., 2000) and in rams (Sarlos et al., 2002) there was no effect of Cysteine hydrochloride on the acrosomal membrane integrity. In man, semen showing high concentration of thiols is considered subfertile (Ebisch et al., 2006).

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**References**


<table>
<thead>
<tr>
<th>TYPE OF DILUENT</th>
<th>Progressive motility (%)</th>
<th>Live sperm (%)</th>
<th>Acrosome integrity (%)</th>
<th>Sperm abnormalities(%)</th>
</tr>
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<tr>
<td>EYTG-C (Control)</td>
<td>53.88±0.43 a</td>
<td>54.38±0.49 a</td>
<td>74.83±0.44 a</td>
<td>10.38±0.28b</td>
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<td>64.67±0.49 a</td>
<td>78.17±0.45 a</td>
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<td>CYS-12</td>
<td>50.05±0.43 a</td>
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<td>69.83±0.44 a</td>
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<tr>
<td>CYS-15</td>
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<td>40.83±0.49 a</td>
<td>59.83±0.44 a</td>
<td>22.11±0.28 b</td>
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Means bearing different superscripts within a row differ significantly (P<0.05)
OVULATORY RESPONSE AND EMBRYO RECOVERY IN RAMBOUILLET SHEEP USING FSH


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ABSTRACT

Rambouillet sheep (n=15) were subjected to superovulation treatment using FSH (Folltropin-V, Bioniche Animal Health, Canada) during the period between August and November, 2013. The sheep were randomly divided into two groups. Group 1 (n=7) animals were administered 80 mg of NIH-FSH-P1 in six divided doses for 3 days at 12 hour interval in a tapering doses schedule (20 mg, 20 mg, 15 mg, 15 mg, 5 mg and 5 mg) where as ewes of Group 2 (n=8) were given 100 mg NIH-FSH-P1 in the tapering dose schedule (25 mg, 25 mg, 15 mg, 15 mg, 10 mg and 10 mg) as that of group 1. All the ewes received progesterone (P) impregnated vaginal sponges for 12 days with FSH treatment initiated either on day 9 or 11 and 200 IU eCG along with first/ fifth FSH injection. P, sponges were withdrawn at the time of penultimate FSH injection. Oestrus detection was done using apronized rams from 24-48 hr after sponge withdrawal and those in oestrus were naturally served for 2-3 times at 12 hr interval. Embryos were collected by retrograde surgical technique between day 3 and 5 after oestrus. Animal with two or more corpora lutea (CL) were considered to have responded to superovulation (SOV) treatment. A 57.14 % (4/7) and 75% (6/8) ewes of group 1 and 2, respectively responded to SOV treatment. The ovarian response was highly variable and ranged between two and eight CL irrespective of groups. The mean ovarian response was 2.0±1.06 (4/7) and 2.37 ± 0.8 (19/8) in groups 1 and 2, respectively. The mean number of transferable embryos in groups 1 and 2, respectively was 1.0± 0.57 (4/4) and 1.83 ±0.75 (11/6). The mean ovarian response as well as embryo recovery did not differ significantly (P >0.05) between groups. From the results it may concluded that the reasons for poor ovulation rate (over all 2.2 ± 0.63; 33/15), high anovulation rate (over all 3.4 ± 0.93; 51/15), poor embryo recovery rate (45.45%; 15 embryos/33 CL) as well as less no. embryos recovered per animal (over all 1.50 ± 0.5) need to be investigated and warrant further studies.

Key words: Sheep, superovulation, FSH, embryo

Introduction

Long generation interval coupled with limited prolificacy is the major limiting factor for faster multiplication in sheep. The technique of multiple ovulation and embryo transfer (MOET) has been developed in this species to accelerate genetic improvement by increasing the number of offspring produced. However, successful integration of Embryo Transfer Technology (ETT) into breeding programme largely depends on the number of viable embryos recovered as well as the conception rate following ET. The uncertainty as well as variability in ovulatory response is a frustrating task for extensive exploitation of superior ewes to realize more number of offspring. The response to superovulation as well as the yield of viable embryos depend on various factors such as photo period (Mutiga and Baker, 1984), season (Mufli et al., 1997, Chagas et al., 2003), ovarian follicular status (Gonzalez-Bulnes et al., 2000), hormone used (Driancourt and Fry, 1992), dose schedule (D’Alassandro et al., 2005), repeated superovulation (Magarey et al., 2003) etc. Information on the superovulation of Rambouillet sheep in tropical climate is very scanty. The present experiment was an attempt to assess the ovulatory response and embryo recovery in Rambouillet sheep following administration of purified FSH in two different dose schedules.

Materials and Methods

The experiment was conducted during the peak breeding period between August and November at Govt. sheep breeding farm, Panthal, located near Katra, District Reasi of Jammu (J & K) at latitude of 32°58’29”N and 74°58’35”E and at an altitude of 1671 meters. The annual rainfall in the area is about 130 cm and the maximum and minimum temperature during this period was 29.6°C and 11.2°C, respectively. Parous, cycling, non-pregnant Rambouillet sheep (n=15) of 6-8 years age and weighing 40-50 kg were randomly divided into two groups. They were grazed daily from 07.00 AM to 06.00 PM on green pastures interspersed with seasonal shrubs. Additionally each ewe was given daily 6 kg green/dry fodder (Oat/maiz/barseem) and 500gm concentrate mixture. Animals of group 1 (n=7) animals were administered i/m 80 mg of NIH-FSH-P1 (Folltropin V, Bioniche Animal Health, Canada) in six divided doses for 3 days at 12 hr interval (6 AM and 6 PM) in a tapering dose schedule (20 mg, 20 mg, 15 mg, 15 mg, 5 mg and 5 mg) where as ewes of group 2 (n=8) were given 100 mg NIH-FSH- P1 in the same tapering dose schedule (25 mg, 25 mg, 15 mg, 15 mg, 10 mg and 10 mg) as that of group 1. All the ewes received progesterone (0.35 gm) impregnated vaginal sponges for 12 days with FSH treatment initiated on day 10 and 200 IU eCG (Folligon, Intervet, Holland) along with first/ fifth FSH injection. The vaginal sponges were withdrawn at the time of penultimate FSH injection. Oestrus in gonadotropin treated ewes was detected by using apronised ram of high sexual vigor every 8 hrs for 30 minutes, starting at 24-48 hrs after sponge withdrawal and those in oestrus were naturally mated for 2-3 times at 12 hr interval using Rambouillet ram of proven fertility.

Between day 3 and 5 after super oestrus, the ewes were...
withheld feed and water from 24 and 12 hours, respectively prior to surgery. The abdominal area anterior to the udder was shaved and disinfected with 70% alcohol and the ewes were sedated by administering xylazine @ 0.05 mg/kg body weight followed by ketamine @ 2-5 mg/kg body weight 10 minutes later. The surgical area was locally infiltrated with 4-5 ml of 2% lignocaine hydrochloride (Lox, Neon Laboratories Limited, Mumbai, India). Each ewe was subjected to laparoscopic ovarian examination to assess the ovarian response and those having ≥2 functional corpora lutea (CL) with good morphological appearance consistent with their age, were subjected to mid ventral laparotomy for embryo collection. Following laparotomy, the ovarian response on each ovary was quantified by counting the number of CL and anovulatory follicles of ≥ 5 mm (Naqui and Gulyani, 1999). A PVC tube of 2 mm inner diameter was inserted into the fimbria and each fallopian tube was flushed retrogradely (Hunter et al., 1955) by injecting 20 ml Dulbecco’s phosphate buffered saline containing 0.1% BSA from utero-tubal junction towards fimbriated end. The collected tubal washings were examined under stereozoom microscope (Olympus, Japan) at 20-63x magnification. The embryos were evaluated morphologically for their stage and grade as per IETS recommendations. The data was analysed as per Snedecor and Cochran (1994).

Results and Discussion

A total of 33 ovulations (2.2 ± 0.63) were observed and 15 viable embryos (mean 1.5 ± 0.5) were recovered from 10/15 (66.67%) ewes that responded (≥2CL) to superovulatory treatment (Table 1). The ovulatory response varied considerably among ewes (range 2-8 CL) which may be attributed to ovarian status of the donor at the time of gonadotropin treatment (Gonzalez-Bulnes et al., 2000). Wide variation in ovarian response was also reported elsewhere in Rambouillet (Naqui and Gulyani, 1999) and Corridale (Mufli et al., 1997) breeds of sheep super ovulated with FSH-P. The incidence of super ovulatory response was 57.14 and 75.0%, respectively in groups 1 and 2 and the difference was not significant (P>0.05). In the present study, the mean ovarian response was 2.0±1.06 (14/7) and 2.37±0.8 (19/8) in groups 1 and 2 respectively with no significant difference (P>0.05) between groups. It is quiet unusual that the mean ovarian response was exceedingly low irrespective of the groups, in contrast to previous reports (Ishwar and Memon, 1996; Naqui et al., 2001; Forcada et al., 2011). This could be attributed to variation in breed, hormone used and dosage schedules (Torres and Cognie, 1984). It has been observed that the reproductive performance of Rambouillet sheep was low (0.34 - 0.67 lambs/ewe/year) in sub temperate and semi arid environments (Tomer and Mahajan, 1980; Nivasasrkar et al., 1983). In general the animals with high fecundity are known to be more responsive to gonadotropin simulation than those with low fecundity (Cahill and Dufour, 1979). The mean number of transferable embryos also did not differ significantly (P>0.05) between groups (1.0±0.57 vs. 1.83±0.75). In the present experiment, the low mean viable embryo recovery might be the direct reflection of poor ovarian response. However, all the recovered embryos were found to be viable indicating the fertility of ram as well as lack of deleterious effect of hormonal treatment on sperm transport. In general, fertilization rates are compromised in high responding donors due to either impaired sperm transport (Evans and Armstrong, 1984) or sub optimal ovum quality (Moor et al., 1985). The embryo recovery rate was observed to be low in this study (45.45%; 15 embryos/33 CL). Zhongle Chang et al. (2006) reported a recovery rate of 45-92% in different age groups of goats. From the results, it may concluded that the reasons for poor ovulation rate (over all 2.2 ± 0.63; 33/15), high anovulation rate (over all 3.4 ± 0.93; 51/15), poor embryo recovery rate (45.45%; 15 embryos/33 CL) as well as less no. embryos recovered per animal (over all 1.50 ± 0.5) need to be investigated and warrant further studies.

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THE EFFECT OF CLOPROSTINOL ON PERSISTENT CORPUS LUTEUM IN CATTLE

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ABSTRACT

The present investigation was conducted on cows maintained at Radha Vallabh Goushala, Dherewala Pillibanga (Rajasthan). The 50 cows were examined per rectum to check the condition of genitalia for PCL in which 9 showed a condition of PCL. These PCL cases were treated with 2 ml of Estrumate intramuscularly on the same day. It was found that 85% of animals as determined by the examination of reproductive tracts became normal and consequently 60% were pregnant at first service whereas 25% were repeat breeder.

Key words: Cow, persistent corpus luteum, PGF2α

Introduction

The ability of a cow of successfully mating, conceiving, parturiting and raising a healthy calf each year is essential for economical milk production. Therefore, causes for failures in reproduction must be identified and overcome. In a normal phenomenon of ovulation, the cells that develop within the follicle undergo a differentiation process by the action of pituitary hormones. This process is called luteinization and gives rise to the second ovarian structure, the corpora lutea (Rowlands and Weir, 1984; Christensen and Gillum, 1969). This structure is often referred to simply as the CL and plays a major role in secreting the hormone progesterone. The CL goes through a maturation and regression cycle much the same as the follicle (Weir and Rowlands, 1977). The CL remains fully functional from Day 5 to Day 15 of the cycle and if the female does not become pregnant then begins to regress (Ahmad et al., 1995). The CL regresses and no longer secretes progesterone and the follicle of the next oestrus cycle begins to develop (Stevens et al., 1995). As the CL regresses further, it converts in to the corpus albicans and remains visible on the ovary for several subsequent cycles (Hafez, 2006). Any condition that prolongs the period of time that blood levels of progesterone remain high will have the same effect as pregnancy in stopping the regular 21-day-cycle (Gordon, 1996). Occasionally, the CL does not regress normally (persistent CL) even though the animal does not become pregnant and reduce the fertility and productivity. PCL continue to produce enough progesterone to prevent further follicular development, ovulation and oestrus. Severe endometritis may be associated with PCL due to toxic damage to the endometrium, which prevents proper secretion of luteolytic prostaglandins. This can also occur with pyometra, foetal mummification and maceration, i.e. conditions that simulate pregnancy (Boyd, 1977). Peters and Lamming (1986) reported PCL to be rare (under 2%) in cows with normal uterus. The present study was designed to determine the efficacy of PGF2α for correcting this condition in the cows.

Materials and Methods

The present investigation was carried out on cows (n=50) maintained at Radha Vallabh Goushala, Dherewala Pillibanga (Rajasthan). The cows in anoestrus for a long time were examined per rectum to check the condition of genitalia for PCL. The cows having PCL were treated with PGF2α, 2 ml of Estrumate (Cloprostinol; MSD Pharmaceutical) intramuscularly. The effects of this treatment were determined by putting these animals to teasers. The animals, which showed oestrus, were inseminated. The cows were examined for returning to oestrus or subsequent pregnancy status.

Results and Discussion

The results regarding the PCL in cows are shown in Fig. 1. It was found that 18% (9 cows) were suffering from this condition, whereas remaining (82%) had anoestrus due to the malnutrition or some other genital disorders. It was found that the treatment regressed PCL in approximately 78% of the animals (7 cows) whereas 22% were still having PCL intact. This was also confirmed by the oestrus behaviour of the animals. Moreover, of the animals, which showed response to this treatment, 85.71% (6 cows) were pregnant.

It has been shown that luteolysis in cow is by the production of PGF2α (Flint et al., 1992; Silvia et al., 1991). Several studies have shown that the levels of PGF2α are reduced in the presence of a viable conceptus (or its signal) in vivo and in vitro (Bazer, 1992; Danet-Desnoyers, 1994; Lashari and Tasawar, 2010). The longer survival of PCL could be attributed either to weaker luteolytic signal or strong luteotrophic support. It has been shown that treatment with PGE2 protects the CL from spontaneous regression (Pratt et al., 1977) as well as luteolysis induced by exogenous PGF2α (Henderson et al., 1977). The results of the present study indicate that after exogenous PGF2α treatment, 78% animals showed response by accepting the male. It was assumed that in these cows, the PGE2 concentrations were not as high as that could protect the PCL in the presence of exogenous PGF2α treatment and thereby PCL regressed. Moreover, in remaining cows (22%) the concentrations of PGE2 were higher and in this condition 2 ml of PGF2α was not enough to overcome the antiluteolytic effect of PGE2. To verify this assumption some more work.
need to be done. However, this possibility gets support from earlier report of Boyd (1977) who found that severe endometritis may be associated with a PCL due to toxic damage to the endometrium, which prevents proper secretion of luteolytic prostaglandins. The results of this study also indicate that majority of animals who showed response to this treatment got pregnant with the first insemination (85.71%) and remaining repeated (14.29%). It indicates that this treatment had the potential to improve the fertility in lactating cows and thereby enhance the life time productivity of cows.

References

EARLY EMBRYONIC RESORPTION IN A POITOU JENNY: A CASE REPORT

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Keywords: Poitou jenny, early embryonic death, pregnancy, ultrasonography, embryonic vesicle

In equines, early embryonic loss was reported mainly from studies in mares (Woods et al., 1987; Ball, 1993; Newcombe, 2000; Vanderwall, 2008). The donkey species is useful to provide draught power for various services such as transport of goods, construction work, tracking and tourism (Varshney and Gupta 1994), production of mule (Legha et al., 2012) but ignored when breeding practices is concerned. Use of ultrasonography has greatly improved the diagnosis (Singh et al., 2013) and monitoring of early conceptus development and the embryonic resorption. Early pregnancy diagnosis (EED) is essential for equine management, especially to reduce the need for continued oestrus detection, reintroducing the non pregnant animals to breeding programme within least time lost; and thus to minimize economic losses to the farm. EED can be defined as pregnancy failure that occurs between fertilization and day 42 of gestation (Vanderwall, 2008). Repeated diagnoses are needed to identify early embryonic failure. EED is multi-factorial in nature, in which external factors such as environmental, managerial and patho-physiological factors are involved. External environmental factors include stress, nutrition, season of the year, climate, sire effects, and rectal palpation (Pycock, 2009). Patho-physiological factors which include a number of abnormal maternal factors, like hormone deficiencies and imbalances, uterine environment and age has been implicated.

Case history and investigations
An exotic Poitou jenny (Martina franca) aged 13 years, maintained under standard feeding and farm management conditions at National Research Centre on Equines, Bikaner sub campus started showing signs of oestrus from day 33 post ovulation. The jenny had oestrus period of 8 days when bred twice with frozen semen artificial insemination (AI) of a Poitou jack on 6th and 8th day of oestrus. Ovulation was confirmed by ultrasonography on 8th day of oestrus. Ultrasonography examination was performed on day 15 post ovulation to confirm pregnancy using per rectal ultrasonography with 5 MHz linear transducer (Kontron Medical, France). On 25th day post ovulation, pregnancy was re-confirmed. The viability of embryo was assessed and reconfirmed on days 33, 34, 35 and 36 post-ovulation with decreasing diameter of embryonic vesicle since the jenny showed signs of oestrus. The embryonic vesicle disappeared completely on 37 day post ovulation.

Observations
On day 15th and 25th post ovulation embryonic vesicle was measured as presence of small round anechoic vesicle centrally located in uterine body measured 20.1x17.3 (average 18.7 mm) and 30.2 x 41.8 mm (average 36 mm) in size (Fig. 1 and Fig. 2), respectively. The embryo was visible as echoic mass present within the vesicle on day 25th post ovulation (Fig. 2).

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Fig. 1: Embryonic vesicle detected at day 15 post ovulation

Pregnancy was reconfirmed on 30th day post ovulation with embryonic vesicle measuring 49.5 x 40.8 x 48.2 mm in size (Fig. 2).

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On day 33<sup>rd</sup> post ovulation, the jenny was examined for the size of embryonic vesicle since she showed signs of oestrus. The embryonic vesicle was measured smaller (11 x 12.8 mm; average 11.9 mm) in size (Fig. 4).

On day 36<sup>th</sup> post ovulation the embryonic vesicle disappeared completely. Both the uterine horns (Fig. 5 & 6) and the uterine body (Fig. 7) did not show presence of any embryonic vesicle.

The left ovary had a graffian follicle sized 24.4 x 23.9 mm (average 24.15 mm; Fig. 8), while right ovary had graffian follicle of 22 x 21.1 mm in size (average 21.55 mm; Fig. 9).
On day 7th of oestrus (day 40th of previous ovulation) the Graaffian follicle’s size was 40.2 x 38.1 mm in size (average 39.15; Fig. 10). Ovulation occurred on day 8th of oestrus.

Centre on Equines, India for providing necessary facilities to carry out the work successfully.

**References**


NEONATAL HYPOXAEIA IN A CALF BORN TO THE DAM WITH PLACENTA PRAEVIA AND DYSTOCIA

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Introduction
Placenta praevia in human obstetrics immediately warrants laparohysterotomy for deliverance of live foetus otherwise causes foetal death due to hypoxaemia and cardio-respiratory collapse. Detachment of foetal membranes from maternal attachments and their expulsion prior to foetal birth usually leads to deliverance of dead foetus in veterinary obstetrical cases (Schuijt and Ball, 1980). Present paper reports about successful cardiopulmonary resuscitation of hypoxaemic unconscious and apnoeic foetus born to a cow having dystocia and placenta praevia.

Case history and clinical observations
A rare case of bovine dystocia was presented at hospital with signs of parturition viz., restlessness, suspended ruminations, anorexia, severe abdominal contraction and straining with elevated tail, followed by rupture of water bag but neither the foetus was delivered nor any body part of foetus appeared at vulvular commissure of dam. After about 14 hours, the animal was eating and ruminating normally with part of placenta hanging out. The foetal membranes with few cotyledons were found hanging out at vulvular commissure. Foetal body parts or extremities were not visible at vulva or inside the vagina. Per vaginal examination revealed that cervix was completely dilated with foetal head lodged in the cervical canal. Retropulsion and further examination revealed that the foetus was in anterior presentation, dorso-sacral position with flexed carpal and fetlock joints of both the fore limbs. The foetus did not show any sign of vitality viz. foot withdrawal reflex, sucking or tongue withdrawal reflex and eye blink reflex. There was complete uterine inertia and no efforts were made by the animal to parturiate.

Delivery and resuscitation of neonate
The dystocia was relieved by mutation and judicious traction applied on the calf. The calf did not show any observable sign of vitality, it was unconscious with apnoea, very weak feeble pulse and bradycardia. The nostrils of neonate were cleared of mucus; insufficient oxygen uptake and carbon dioxide release lead to hypoxaemia and respiratory acidosis, simultaneously metabolic acidosis also develops due to accumulation of acid products of anaerobic respiration. These events ultimately lead to central depression and constriction of arteriole with resultant decrease in pulmonary circulation and cardio-respiratory collapse. Schuijt and Ball (1980) described that detachment of foetal membranes from maternal attachments and their expulsion prior to foetal birth usually followed by deliverance of a dead foetus. In present case the calf born to a cow having placenta praevia though appeared dead but it had very weak signs of vitality (feeble pulse, apnoea and bradycardia) due to compromised cardiopulmonary functions. Hall and Clarke (1983) suggested that intermittent compression over the lung area on the chest triggers a reflex spontaneous respiration, a remarkably effective circulation can also be maintained by applying intermittent compression over cardiac area on intact chest wall.

Discussion
In present case the first and second stages of labour were passed away and the dam was in third stage of labour. Detachment of foetal membranes was initiated prior to expulsion of foetus that hampered supply of oxygenated maternal blood to foetus. Lodgment of foetal head in hyperventilated cervical canal deprived it from oxygen uptake by natural breathing thereby foetus was subjected to hypoxia. Muir and Hubbell (1991b) also described that hyperventilation causes hypoxia in neonates. Thomson et al. (1996) opined that hypoxia in neonate causes bradycardia as observed in present case. Drost (1980) and McDonell (1996) suggested that delayed passage of foetus through the birth canal in the face of a faltering placenta compromises the oxygenation of the calf; insufficient oxygen uptake and carbon dioxide release lead to hypoxaemia and respiratory acidosis, simultaneously metabolic acidosis also develops due to accumulation of acid products of anaerobic respiration. These events ultimately lead to central depression and constriction of arteriole with resultant decrease in pulmonary circulation and cardio-respiratory collapse.

References

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SPONTANEOUS EXPULSION OF MUMMIFIED FOETUS IN A COW: CASE REPORT

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Key words: Cow, regression of CL, mummified foetus, vaginal cavity

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Introduction
Foetal mummification occurs with an incidence of 0.13-1.8% in cattle, (Arthur et al., 1996). In foetal mummification cases, resorption of the foetal body fluids and most of the cases the mummified foetus become dry and paper like. It is called papyraceous mummification (Arthur et al., 1996). Foetal mummification occurs after the first trimester of gestation (Roberts, 1986) but it cannot be diagnosed, because the placenta and corpora luteum (CL) are capable of producing sufficient progesterone. Foetus can remain in the uterus for between 150 and 200 days or a normal gestation period in undiagnosed cases (Johnson et al., 1981). The causes of mummification are poorly described, and infectious agents like Campylobacter foetus, molds, leptospirosis and BVD-MD virus may cause foetal death without abortion may result into mummification in cattle (Drost, 2007). In the present paper, a unique case of papyraceous mummified foetus that was present in the vaginal cavity due to the regression of corpus luteum.

Case history and observations
A 7-year-old Holstein Friesian cow in her fourth parity had a history of hanging the dry paper like foetal membranes from the vaginal cavity. And that animal was inseminated at nine and half month back. Feed intake was normal, temperature was 38.2ºC, and heart rate was 62/min. Rectal examination reveals the non-gravid uterus. There was no presence of corpus luteum and follicular cyst on both the ovaries. On the vaginal examination, dry and rigid foetal mass were palpated in the vaginal cavity and dry paper like foetal membrane was surrounded the foetus and part of it was hanging outside the vaginal cavity. Cervix was fully closed. Finally, the case was diagnosed as foetal mummification and expulsion of mummified foetus from the uterus due to the regression of corpus luteum.

Treatment and Discussion
The lubrication of the vaginal cavity was done by liberal application of the obstetrical gel (Cetrimide). Mild traction was applied on the foetal mass; the mummified foetus was delivered along with the foetal membranes. After the delivery of the foetus dam was administered with antibiotics, anti inflammatory and antihistaminic drugs on the day. Uneventful recovery of the dam was noticed. In the present case, gross examination of the foetus revealed dry and hard foetus with attachment paper like foetal membrane at the head region (Fig. 1). A rapid functional regression of the CL due to uterine PGF2α surge which characterised by inhibition of progesterone release and followed by a structural regression (Pate, 1994). Most mummified foetuses will remain until treatment is given to expell them or until they are removed by ceaserean section (Rajoriya et al., 2014). But most of the mummified foetus cases regression of CL only achieved by administration of PGF2α analogues (Yilmaz et al., 2011). Some of the mummified foetus may pass into the vaginal cavity because of regression of the corpus luteum and the cow returns to heat (Jackson, 2004). Cows usually conceive on the first or second oestrus cycle after expulsion when prognosis for fertility after foetal expulsion is good (Roberts, 1986).

References

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EPIDEMIOLOGICAL STATUS OF PESTES DES PETITS RUMINANTS (PPR) VIRUS INFECTION IN SMALL RUMINANTS OF PUNJAB, INDIA

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Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, India

ABSTRACT

The present study reports the seroprevalence of PPR in sheep and goat populations of the north-western Indian state of Punjab, bordering Pakistan. Serum samples (n=446) from sheep and goats were collected using random sampling software 'survey toolbox' and monoclonal antibody (MAb)-based competitive ELISA was used for the detection of antibodies to PPR virus. Risk factors associated with the prevalence of anti-PPR antibodies were analyzed. The competitive ELISA revealed an overall apparent prevalence of 55.38%. Species seroprevalence was found to be significantly higher (chi square 13.549 p<0.01) in goats (63.16%) as compared to sheep (45.73%). Further, prevalence of specific antibodies was found to increase significantly with age (chi square 60.566, p<0.01) in both the species and significantly higher (chi square 19.727, p<0.01) in females in comparison to males. Animals under grazing had significantly higher antibody prevalence (chi square 74.380, p<0.01) in comparison to stall fed ones as indicated by risk ratio (18.676). Observed higher seroprevalence of the disease in the state of Punjab shows the need for implementation of regular vaccination and restriction of interstate movements of small ruminants for effective control of the disease.

Key words: Epidemiology, PPR, goats, sheep

Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of domestic and wild small ruminants caused by a virus, peste des petits ruminants virus (PPRV) of genus Morbillivirus, family Paramyxoviridae (Gibbs et al., 1979). PPR has been reported from various parts of Africa, Middle East, Indian sub-continent and Central Asia (Diallo et al., 2007). The disease is one of the major transboundary animal diseases posing a severe threat to international trade (Domenach et al., 2006). PPR has been identified as one of the priority animal diseases whose control is considered important for poverty alleviation in Western Africa and Southern Asia (Perry et al., 2002). Though the disease was reported for the first time in India by Shaila et al. (1989), analysis of previous data suggested the presence of disease in southern India since 1980s (Taylor et al., 2002). The disease has been reported in neighboring Pakistan more recently (Athar et al., 1995; Hussain et al., 1998). Since then, outbreaks of disease in small ruminants have been reported from both the countries (Joshi et al., 1996; Nayak et al., 1997; Rao et al., 1998; Shankar et al., 1998; Aruni et al., 1999; Katoch et al., 1999; Dhand et al., 2002; Kumar et al., 2002; Sharma et al., 2007, Zahur et al., 2008, Abubaker et al., 2009). Abubaker et al. (2009) have reported the sero-prevalence and risk factors associated with the incidence of PPR in small ruminants in Pakistan including the state of Punjab bordering India. However, studies on sero-prevalence of the disease in India are limited (Hinsu et al., 2001; Singh et al., 2004a; Raghavendra et al., 2008) and no reports are available from the state of Punjab. The state of Punjab is situated in the north-western part of India bordering Pakistan in the west. Though the state has no major population of small ruminants, prevalence studies in the state will help in understanding the epidemiology of PPR in surrounding regions like, Rajasthan and Jammu and Kashmir (in India) and Pakistan where the disease is responsible for severe economic loss. Hence, the present study was designed to investigate the sero-prevalence of the disease in Punjab (India) using random sampling software 'survey toolbox' (Cameron, 1999). This is the first report on seroprevalence of the disease from the Indian state of Punjab. Further, the study appears to be first of its kind in India using such software in small ruminants and hence the results from the study could reflect those at the state and national level.

Materials and Methods

Study area

Punjab is the north-western state of India bordering Pakistan on the west and situated between the 29.30°N to 32.32°N latitude and 73.55°E to 76.50°E longitude. Climatically, the state has 5 seasons- spring (February-March), summer (April to June, maximum temperature 43.6°C), rainy season (July-August), autumn (September to November) and winter season (December-January, minimum temperature 4.4°C). Punjab state is broadly divided into 3 agro-climatic zones viz. Sub Mountain Region (annual rainfall 800-900 mm), Central Plain Region (annual rainfall 500-800 mm) and Southern-Western Region (<400 mm). Approximately 70% of human population lives in villages and agriculture is the main occupation. India has 200 million cattle, 76 million buffaloes, 110 million goats and 46 million sheep, of which Punjab has 2.64 million cattle, 6.17 million buffaloes, 0.41 million goats and 0.44 million sheep (www.husbandrypunjab.org).

Selection of villages and animals for sero-surveillance

To draw a simple random sample from the population of animals, two-stage sampling procedure was adopted. In the first step, villages were selected followed by selection of animals. A computerized list of the villages of the state was used as the sampling frame and villages were selected (n=48) from the state using simple random sampling, without replacement using 'Random Village' program of Survey toolbox (Cameron, 1999). The animals were selected using systematic random sampling and approximately 10% animals (n= 446) were selected from the selected flocks.

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Sample collection and serology

Blood samples (5 ml) were collected from the jugular vein of healthy animals; the sera were separated and stored at -20°C until they were tested for antibodies to PPR virus using competitive ELISA (cELISA). A standardized questionnaire for animals sampled at the farms was filled at the time of sampling. A monoclonal antibody (MAb)-based cELISA was used for the detection of antibodies to PPRV having sensitivity of 90.5% and specificity of 99.8% (Singh et al., 2004b).

Statistical analysis

True prevalence was calculated at 95% confidence interval (CI) using the 'True prevalence' program of survey toolbox, in which sensitivity, specificity and sample size were taken into consideration. The data was analyzed using SPSS (Statistical Package for Social Sciences) for Window version 11.0.1®SPSS Inc. USA computer software program. The associations were evaluated between binary outcome variable and a variety of risk factors such as species, age, sex, and feeding patterns.

Results

For seroprevalence study a total of 446 animals were selected from 48 villages of Punjab. The serum samples from these animals were analyzed with competitive ELISA, which revealed prevalence of 63.16% and 45.73% in goats and sheep, respectively; the overall apparent prevalence of disease being 55.38%. Given the sensitivity and specificity of test at 90.5 and 99.8%, respectively, true prevalence was calculated to be 61.11% (95% CI 58.68-63.53).

The cELISA used in the present study to detect the presence of antibodies to PPRV was able to clearly differentiate the exposed (infected) population from the unexposed (not infected) population. However, considerable differences were observed between the exposed sheep and goat populations when the results of the cELISAs were plotted as a frequency of the per cent colour inhibition. Among the samples considered negative for PPRV (% colour inhibition of less than 50%) the greatest number of samples had a per cent colour inhibition of between 6 to 16 and 11 to 21 for goats and sheep, respectively. Alternatively, among the samples considered positive for PPRV (% colour inhibition of greater than 50%) a peak frequency distribution of between 81% and 91% colour inhibition was observed for the goat population (Fig. 1).

Various host factors associated with the prevalence of anti-PPR antibodies were studied. Species (Table 1) prevalence was found to be significantly higher (chi square 13.549 p<0.01) in goats (63.16%) as compared to sheep (45.73%). Prevalence was significantly higher (chi square 19.727, p< 0.01) in females in comparison to males and risk of infection was twice more in females (Table 2). In different age groups (<1 year, 1-2 years, 2-3 years, 3-4 years, 4-5 years and >5 years) prevalence of specific antibodies to PPR virus was found to increase significantly with age (chi square 60.566, p<0.01) in both the species (Fig. 2) indicating exposure of animals to infection during the lifetime. Grazing animals had significantly higher antibody prevalence (chi square 74.380, p<0.01) in comparison to stall fed animals (Table 3) as indicated by risk ratio.

Discussion

Though Punjab is not a major small ruminant rearing state and this report is a small sample study, the information obtained is of epidemiological significance since the state has some major small ruminant migration routes across its territory. The state has geographical continuity and similar ecological features as bordering Pakistan, where the disease has greater significance to the livestock industry. Sero-prevalence of PPR has been reported recently from southern peninsular India (Raghavendra et al., 2008) using samples collected from most parts of India (Singh et al., 2004a). However, the state of Punjab was not included in their study as the state has a small ruminant population. Though no animal trade occurs across the border between Indian Punjab and Pakistan, trading of farm goods is practiced leading to movement of men and materials related to agriculture at Wagha (International border between India and Pakistan). The endemic nature of PPR in Pakistan and the high prevalence the disease in the plains of Pakistan Punjab has been reported recently (Zahur et al., 2008; Abubaker et al., 2009). Unusual increase in the occurrence of the outbreaks in the months April to June coincides with the movement of sheep flock of nomads from the desert state of Rajasthan. Since the April-June period is hot and dry summer in Rajasthan, it is usual for nomads in this region to migrate towards the Himalayan plane of Jammu through the state of Punjab. By the time the Punjab region has finished harvesting the wheat crop and it is possible that wheat stubs in the harvested fields might cause abrasion of oral mucosa during grazing making them more prone for the entry of virus (Dhand et al., 2002; Sharma et al., 2007). Interestingly, in a similar study a higher incidence of PPR was reported in the months of March and April in Pakistan during 2006-2008 (Abubaker et al., 2009). Molecular characterization of virus isolates from either side of the border is needed for investigating the possible cross border transmission of the virus.

The prevalence (55.38%) in the present study is quite higher compared to earlier studies. In similar studies from southern peninsular India Raghavendra et al. (2008) had reported the sero-prevalence of 41.35% in sheep and 34.91% in goats. However, Singh et al. (2004a) reported the lower prevalence in northern India (10-30%) compared to southern and south-western India (30%-60%). Interestingly, similar to the present observation (Abubaker et al., 2009) has reported the sero-prevalence of 54.09% in sheep and 44.15% in goats from Pakistan. The high prevalence of antibodies to PPR virus in the absence of vaccination indicates the widespread infection in the small ruminants of the state.

The cELISA used in the present study to detect the presence of antibodies to PPRV was able to clearly differentiate the exposed (infected) population from the unexposed (not infected) population. However, considerable differences were observed between the exposed sheep and goat populations when the results of the cELISAs were plotted as a frequency of the per cent colour inhibition. The findings suggest that only those goats capable of mounting a strong humoral antibody response to PPRV (high percent colour inhibition) were capable of surviving infection.

Our results show that the risk of disease was twice as high in goats in comparison to sheep. Similar studies conducted earlier have also reported higher prevalence of disease in goats as compared to sheep (Rajeshwari et al., 2000; Singh et al., 2004a), although, Abraham et al. (2005) reported higher antibody prevalence in sheep (13%) in comparison to goats (9%). Thus, for any control programme to be successful, both the species will have to be included in the programme. The observed prevalence of specific antibodies which was found to be increasing with age indicates the exposure of animals to infection during the lifetime.
Table 1: Species wise antibody prevalence of PPR

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>Positive</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>247</td>
<td>156</td>
<td>63.16</td>
</tr>
<tr>
<td>Sheep</td>
<td>199</td>
<td>91</td>
<td>45.73</td>
</tr>
<tr>
<td>Total</td>
<td>446</td>
<td>247</td>
<td>55.38</td>
</tr>
</tbody>
</table>

Chi square - uncorrected 13.549 (P < 0.01)
Chi square-Mantel-Haenszel 13.518 (P < 0.01)
Chi square-corrected (Yates) 12.852 (P < 0.01)
Odds ratio 2.035 (95% CI=1.392-2.974)
Risk ratio 1.381 (95% CI=1.161-1.648)

Table 2: Sex wise antibody prevalence of PPR

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>Positive</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>389</td>
<td>231</td>
<td>59.38</td>
</tr>
<tr>
<td>Male</td>
<td>57</td>
<td>16</td>
<td>28.07</td>
</tr>
<tr>
<td>Total</td>
<td>446</td>
<td>247</td>
<td>55.38</td>
</tr>
</tbody>
</table>

Chi square - uncorrected 19.727 (P < 0.01)
Chi square-Mantel-Haenszel 19.682 (P < 0.01)
Chi square - corrected (Yates) 18.480 (P < 0.01)
Odds ratio 3.746 (95% CI=2.045-6.859)
Risk ratio 2.116 (95% CI=1.443-3.297)

Table 3: Association of feeding pattern with antibody prevalence of PPR

<table>
<thead>
<tr>
<th>Feeding pattern</th>
<th>Total</th>
<th>Positive</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grazing</td>
<td>387</td>
<td>245</td>
<td>63.31</td>
</tr>
<tr>
<td>Stall-fed</td>
<td>59</td>
<td>2</td>
<td>3.39</td>
</tr>
<tr>
<td>Total</td>
<td>446</td>
<td>247</td>
<td>55.38</td>
</tr>
</tbody>
</table>

Chi square - uncorrected 74.380 (P < 0.01)
Chi square - Mantel-Haenszel 74.213 (P < 0.01)
Chi square - corrected (Yates) 71.975 (P < 0.01)
Odds ratio 49.173 (95% CI=13.021-185.206)
Risk ratio 18.676 (95% CI=5.554-67.901)

Fig. 1: Identification of uninfected versus infected in sheep and goat populations

Similar results had been reported by various workers (Singh et al., 2004a; Agrawal et al., 2006). Evidence of infection was twice more frequent in females compared to males which were similar to earlier reports (Hinsu et al., 2001; Agrawal et al., 2006). The reason for higher antibody prevalence in females could be that most of the males are sold at much earlier age for meat purpose retaining only a few for breeding, whereas females are mostly kept for reproduction and milk production. Feeding pattern has significant impact on PPR antibody prevalence which was reflected in the observation that grazing animals had significantly higher antibody prevalence in comparison to stall fed animals as indicated by risk ratio. As the grazing animals have higher chance of picking up the infection from direct and indirect contact with source of the virus.

Observations of the study suggest that PPR is endemic in the state of Punjab and outbreaks reported were coinciding with the annual migration of animals from across the states. Since, similar pattern of outbreaks and sero-prevalence of the disease were reported from neighboring Pakistan it seems that similar epidemiological factors operate on either sides of the border. Further molecular studies of the virus isolates from the outbreaks are required to investigate the possible transmission of the virus across the borders. Strict movement restrictions and regular vaccination of both goat and sheep populations is required for effective control of the disease in the region. Further, regional and international approaches are needed for prevention and control of the disease and to reduce its economic impact.

Acknowledgements

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References


Fig. 2: Age-wise prevalence of antibodies to PPRV
EVALUATION OF SERO-PREVALENCE OF BRUCELLOSIS IN GOATS

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ABSTRACT

A total of 100 serum samples of goats were evaluated to determine the seroprevalence of brucellosis. The Brucella reactors were determined by using Brucheck Dot ELISA test. Overall seroprevalence of brucellosis was 22%. The adult goats showed higher seroprevalence (33.33%) as compared to goats below one year (12.72%) of age group. Susceptibility to brucellosis in adult animals might be attributed to their breeding suitability. The female goats showed higher seroprevalence (22.85%) as compared to male goats (20.00%).

Key words: Goats, brucellosis, seroprevalence, Dot-ELISA

Introduction

The goat has tremendous potential to be projected as the future animal for rural prosperity under the changing agro-climatic conditions and depleting resources. Brucellosis is a world-wide contagious disease of zoontic importance and of public health significance (Anonymous, 1986; Gerhardt et al., 2002). Brucellosis is endemic in India. Brucella melitensis biotype-1 in sheep, goats and man are pre-dominant infective biotypes. Clinical signs vary according to the animal hosts and the infecting Brucella spp. (Megid et al., 2010). The disease is characterized by abortion, retention of placenta, decreased milk yield and subsequent high rate of infertility. During last 3-4 decades, brucellosis in small ruminants has gained much attention because of its role in spread of infection to cattle and human beings. All abortions in late gestation in domestic animals should be treated as suspected brucellosis and should be investigated. Sero-prevalence of caprine brucellosis has been reported from different parts of India and abroad. Dubey and Mathur (1980) reported 1.78 % prevalence of brucellosis in sheep and goats in Rajasthan. Shaukat (1985) and Godara (1998) reported incidence of brucellosis to be 3.94 and 18.51 per cent in sheep and goats, respectively by using serum tube agglutination test (STAT).

Brucellosis in human beings cannot be managed without its control and prevention in animals. Brucella melitensis requires major consideration as an agent of bio-terrorism and agro terrorism. It is believed that fewer than 10 cfu are capable of infecting humans and infection can occur from aerosol infection. This would require mass therapy of human populations and destruction of animal populations, with associated problems (Radostits et al., 2007). The present investigation was carried out to determine the seroprevalence of disease for further formulation of strategic approach for its prevention and control.

Materials and Methods

A total of 100 blood samples of goats were collected and serum was separated. The Brucella reactors were determined by using Brucheck Dot ELISA test kit. This dot-ELISA kit detects antibodies to Brucella melitensis. Sample antibodies bind to Brucella melitensis antigen molecules attached to the nitrocellulose membrane of the spikes of the 12 spiked plastic comb. Binding of these antibodies is detected by reaction with horse radish peroxidase (HRP) labeled affinity-purified antibodies to goat and sheep immunoglobulin. Attached HRP-labelled antibodies are detected by addition of enzyme substrate and visualized by subsequent bluish-brown color product development. Strong colour development indicates the presence of antibody to Brucella melitensis in the sample. No colour development indicates the absence of antibody to Brucella melitensis in the sample. This test can be used to detect antibodies in serum.

Results and Discussion

Out of 100 serum samples, 22 (22%) were found positive for brucellosis by Dot ELISA test (Table 1 and Fig. 1). Singh et al. (1998) used rapid, easy and field-based qualitative dot-enzyme linked immunosorbent assay in the screening of field goats. They found that overall incidence of brucellosis in goats was 4% in seven districts of U.P. and Punjab. In Bikaner region of Rajasthan, Maheswari (2012) reported seroprevalence of brucellosis in goats by using RBPT and STAT as 10% and 6%, respectively. Godara (1998) reported 18.51% and 8% positive samples on STAT in sheep and goats, respectively in Western part of Rajasthan. Teshale et al. (2006) reported seroprevalence of brucellosis in sheep and goats in pastoral region of Ethiopia by using RBPT and indirect ELISA as 1.9% and 9.7%, respectively. Maher and Venkataraman (2007) reported overall prevalence of B. melitensis infection in goats as tested by RBPT, STAT and indirect ELISA to be 17.68, 16.02 and 24.86%, respectively in different areas of Chennai.

Out of 100 serum samples, 55 samples were from below one year of age group animals and 45 were from adult age group goats. The adult goats showed higher seroprevalence of 33.33% by Dot-ELISA as compared to below one year (12.72%) of age group (Table 2).

Congenital infection may occur in calves born from infected dams but its frequency is low. The infection occurs in uterus. Infection of foetus during pregnancy does not necessarily result in abortion. Infected kids and lambs may born alive. Latent
infection can also be acquired from the ingestion of infected colostrum and milk and this may be the reason of presence of positive reactors in young ones (Radostits et al., 2000).

Chandra et al. (2005) reported higher prevalence in adult (1.65%) than in young goats (0.58%). Susceptibility to brucellosis in adult animals might be attributed to their breeding suitability. Sexual maturity is attained in adult age. Different stress factors may also contribute to increased susceptibility of animal towards infection due to release of naturally occurring corticosteroids which are strongly immunosuppressive in nature (Wadhwa, 2007).

Out of 100 serum samples, 35 samples were from male animals and 65 were from female animals. The female goats showed higher seroprevalence of 24.61% by Dot-ELISA as compared to male goats (11.42%).

Out of 100 serum samples, 30 samples were from male animals and 70 were from female animals. The female goats showed higher seroprevalence (22.85%) as compared to male goats (20.00%) by Dot-ELISA (Table 3).

Avinash et al. (2014) reported seroprevalence in goats as 7.14% by Dot-ELISA and the prevalence was found to be highest among goats in Karnataka. A total of 252 serum samples were collected from the goats of Karnataka and subjected to 5 different serological tests, i.e. Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), 2-mercaptoethanol test (2-MET), Indirect ELISA (I-ELISA) and Dot-ELISA to detect the Brucella antibodies. Test-wise, the seroprevalence in goats was 5.15% by RBPT, 6.34% by STAT, 1.98% by 2-MET, 9.52% by indirect ELISA and 7.14% by Dot-ELISA. The prevalence of brucellosis was found to be highest among goats of northeast Karnataka followed by northwest Karnataka, central Karnataka and south Karnataka. Indirect ELISA detected maximum number of positive samples. The study used five serological tests to determine the apparent seroprevalence of caprine brucellosis in Karnataka. Taking indirect ELISA as reference, the tests revealed the relative sensitivity values in the following order: Dot-ELISA > STAT > RBPT > 2-MET.

Kartik (2015) reported similar findings that overall sero-prevalence of brucellosis by Dot ELISA test was 20%. Out of 100 serum samples 20 (20%) were found positive for brucellosis by Dot-ELISA test. The adult goats showed higher sero-prevalence of 30.00% by Dot-ELISA as compared to below one year (13.33%) age group. The female goats showed higher sero-prevalence of 24.61% by Dot-ELISA as compared to male goats (11.42%).

Amit et al. (2016) reported that overall sero-prevalence of bovine brucellosis by RBPT and STAT was 5.55% and 6.79%, respectively.

The Dot ELISA test is better test for sero-diagnosis of brucellosis in goats in field conditions because it is easy, rapid, highly specific and sensitive test.

Table 1: Overall seroprevalence of brucellosis in goats

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Samples tested</th>
<th>Samples positive</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dot-ELISA</td>
<td>100</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 2: Age wise seroprevalence of brucellosis by Dot-ELISA

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Age</th>
<th>Samples tested</th>
<th>Samples positive</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Below 1 yr</td>
<td>55</td>
<td>07</td>
<td>12.72</td>
</tr>
<tr>
<td>2</td>
<td>Adult</td>
<td>45</td>
<td>15</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Table 3: Sex wise seroprevalence of brucellosis by Dot-ELISA

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sex</th>
<th>Samples tested</th>
<th>Samples positive</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>30</td>
<td>06</td>
<td>20.00</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>70</td>
<td>16</td>
<td>22.85</td>
</tr>
</tbody>
</table>

References

SOMATIC CELL COUNT IN DIFFERENT STAGES OF LACTATION IN ORGANIZED VS UNORGANIZED LACTATING DAIRY COWS-A REPORT

Ramya, K¹, D. Anandha Prakash Singh², S. Anbazhagan, V. Anusuya³ and L. Gunaseelan⁴
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ABSTRACT

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Subclinical mastitis in dairy animals remains a hidden threat to the dairy farmers as it does not present apparent signal. One of the techniques used to monitor the level or occurrence of subclinical mastitis in herds or individual cows or quarters is to determine the somatic cell count (SCC) of milk samples. The somatic cells of milk are mainly leukocytes, which include macrophages, lymphocytes and neutrophils which are involved in the defence against invading microorganisms. Thus, the number of SCC in milk sample is an indicator of subclinical mastitis. The present study was undertaken to compare the health status of lactating dairy cows of organized (ILFC, VCRI, Namakkal) and unorganized dairy farms, respectively at different stages of lactation. The midstream milk samples from the dairy cows were analyzed as per the protocol mentioned in the ABT-SCC Quick count kit (TANUVAS). The results were read and compared with the score card provided with the kit. The SCC of animals in organized farm were in the range of 1,00,000 to 2,00,000 per ml of milk and among the animals in unorganized farm, the animals in late stage of lactation had SCC of 3,00,000 per ml and other animals had 1,00,000 per ml. The somatic cell counts of the animals under study were within the agreed limit for subclinical mastitis i.e., 5,00,000 per ml of milk. The result of the present study reveals that regardless of the management practices followed in organized and unorganized farms the animals are acquainted to their environment and generate resistance and are free from subclinical mastitis.

Key words: Somatic cell count, subclinical mastitis, stage of lactation, leucocytes

Introduction

Mastitis in both clinical and subclinical forms is a frustrating, costly and extremely complex disease that results in a marked reduction in the quality and quantity of milk. Annual losses in the dairy industry due to mastitis was approximately 526 million dollars in India, in which subclinical mastitis are responsible for approximately 70% of these capital losses (Varshney and Naresh, 2004). The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, subclinically infectedudder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk et al., 2003). Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing (Holdway, 1992). Although there are no visible or palpable external changes, the infection is present and inflammation is occurred in the udder (Blowey and Edmondson, 1995). The invisible changes include increase in the somatic cell count, and other changes in the composition and quality of milk.

The somatic cells of milk are mainly leukocytes, which include macrophages, lymphocytes and neutrophils (Harmon and Reneau, 1993). The goal of the leukocytes is to embody and digest the invading microorganism of the mammary gland. Thus, the counting of somatic cells (SCC) is a tool to detect the increase of leukocytes in the blood. Although sub clinical mastitis does not present apparent signal, it limits the economical exploration of the cow. It can develop into an acute mastitis if there is a decrease in resistance, and its detection depends of laboratory tests (Torres, 1985).

The occurrence of environmental mastitis may be related to the type of housing, bedding and season of the year. Confined animals are under higher risk to have environmental mastitis than animals free in the field. Nevertheless, the agglomeration of animals in areas with shade in the field during summer results in a concentration of ambient pathogenic greater than 10,000,000/grams of dry material of the soil. Organic material used as bed in those systems of free stall, such as straw, saw dust or wood shavings result in a higher concentration of ambient pathogenic than inorganic beds such as sand and lime.

Materials and Methods

The present study was conducted to compare the somatic cell count in animals maintained at organized and unorganized farm, to ascertain out the presence of subclinical mastitis. The study was made on cows milked twice daily with regular intervals. The milk samples from the organized farm was collected from the animals maintained at Instructional Livestock Farm Complex (ILFC), Veterinary College and Research Institute, Namakkal, and the milk samples from unorganized farms were collected from the dairy animals reared by small animal holders around the college. All dairy cows were apparently healthy with clinically sound udder secreting apparently normal milk. The milk samples were collected from the cattle at different stages of lactation and age. The milk samples were collected in a clean environment, after washing the teats with potassium permanganate solution and thoroughly wiping with dry clean cloth. The midstream milk

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### Table 1: Somatic cell count and the record of ILFC dairy cattle

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Breed</th>
<th>Stage of lactation</th>
<th>Parity</th>
<th>Somatic cell count No. of cells x10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Jersey Cross</td>
<td>Late (7 months and above)</td>
<td>4-7</td>
<td>2 x 10^5</td>
</tr>
<tr>
<td>6</td>
<td>Jersey Cross</td>
<td>Early (up to 3 months)</td>
<td>1-4</td>
<td>1 x 10^5</td>
</tr>
</tbody>
</table>

### Table 2: Somatic cell count and the record of dairy cattle reared in unorganized farms

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Breed</th>
<th>Stage of lactation</th>
<th>Parity</th>
<th>Somatic cell count No. of cells x10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Jersey Cross</td>
<td>1-5 days post calving</td>
<td>3</td>
<td>4 x 10^5</td>
</tr>
<tr>
<td>2</td>
<td>Jersey Cross</td>
<td>Early (up to 3 months)</td>
<td>1-4</td>
<td>3 x 10^5</td>
</tr>
<tr>
<td>5</td>
<td>Jersey Cross</td>
<td>Mid (4-6 months)</td>
<td>1-4</td>
<td>1 x 10^5</td>
</tr>
<tr>
<td>4</td>
<td>Jersey Cross</td>
<td>Late (7 months and above)</td>
<td>1-4</td>
<td>3 x 10^5</td>
</tr>
</tbody>
</table>

Samples were collected in a sterile container from each quarter and pooled and used as composite milk sample. The milk samples from the dairy cows were analyzed as per the protocol mentioned in the ABT-SCC Quick count kit (TANUVAS).

**ABT-SCC test**

A drop of milk samples was taken in the ABT-SCC tube and a drop of SCC substrate solution was added to it and mixed well. Then 3 drops of enhancer solution was added to the ABT-SCC tube and mixed well and incubated at room temperature for 40 minutes. The colour development was read and compared with colour card provided with the kit. The scores obtained were multiplied by 1000 to obtain the somatic cell count of milk sample.

**Results and Discussion**

The objective of the study was to explore the health status of uninfected quarters of mammary gland by enumerating the
somatic cells in the midstream milk sample. The results obtained were in accordance with observations made by several authors. The present study reveals that the somatic cell count of milk from the uninfected quarter of animals from organized and unorganized farms did not show significant difference regardless of other factors such as stage of lactation, parity, breed, age etc. The results obtained can be attributed to the management practices such as milker hygiene, sanitization of milking machine dry off treatment and controlling other predisposing factors followed at the organized farm where the animals are maintained in a semi confined environment and unorganized farms where the animals are maintained in an open environment.

**Age**

The somatic cell counts of milk from uninfected quarters have averaged 2,60,000 cells/ml in quarters with no previous history of mastitis and 6,00,000 cells/ml in quarters with a previous history of infection, with a resultant overall average for uninfected quarters of 3,14,000 cells/ml. Cell counts in composite samples taken from cows with all four quarters free of infection have been reported to average from 1,13,000 to 2,51,000 cells/ml depending on the cow’s age (Eberhart et al., 1979). Cows harbouring commensals have been reported to have somatic cell counts in composite samples that average from 1,90,000 to 5,19,000 cells/ml depending on the cow’s age (Eberhart et al., 1979), and an average of 2,27,000 cells/ml has been reported when all age groups were considered (Schultz, 1977). This increase is primarily due to an increased prevalence of infection in older cows and is not due to any large increase due to age perse (Marshall and Edmondson, 1962; Reichmuth, 1975). Examining the somatic cell count of mastitis free cows some have found no increase with age (Duitschaever and Ashton, 1972).

**Stage of lactation**

The somatic cell count of two of the cattle which has the history of recent parturition had 4x10^8 cells/ml of milk which is in accordance with Brolund (1985), Schepers et al. (1997). In long term uninfected cows the number of cells in milk follows an approximate inverse lactation curve (Brolund, 1985; Schepers et al., 1997), somatic cell counts have been found to be elevated immediately after calving, regardless of whether the cow is infected or not. The elevation in count has been variously reported to last for five days (Reichmuth, 1975) to two weeks (Natzke et al., 1972; Cullen, 1968).

The somatic cell count of the milk sample is influenced by additional factors like stress, management factors (Kelly et al., 2009), nutrition, breed, genetic makeup, diurnal variations, season etc., in addition to the above mentioned factors (Chassagne et al., 2005; Marta Woloszyn, 2007).

The sources of variation that influence cell counts tell us that SCC is a fairly unrefined measure of infection status. It is wise to make a decision based on herd management practices relating to somatic cell count on multiple readings and trends, as single-test SCCs are not very useful to undertake mastitis control actions on. However, despite these short comings the somatic cell count remains an important and practical tool for measuring the general health of a herd or of individual cows.

**References**


EFFICACY OF IVERMECTIN AGAINST GASTROINTESTINAL PARASITES IN HORSES (EQUUS CABALLUS)

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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT
A trial was conducted to confirm the efficacy of ivermectin against gastrointestinal parasites of horses. A total of 20 positive cases for gastrointestinal parasitism were selected for treatment irrespective of sex, breed and age. The samples were collected from various places of Bikaner. In these trial 20 horses were treated with single dose of ivermectin tablet (Virbac, Animal Health India Pvt. Ltd., Mumbai, each tablet contains 80 mg ivermectin) at 0.20 mg/kg body weight once on 0 day after 15-day administration of drug in each positive animal, faecal sample were collected for egg per gram (EPG) count to note the efficacy of ivermectin. The mean egg per gram (EPG) of gastrointestinal parasites from average count of 825 ± 58.77 to 20 ± 9.17 with the average reduction of 97.57% on 15th day.

Key words: Ivermectin, horse, gastrointestinal parasites, efficacy, faecal

Introduction
Parasitic infestations are the major veterinary problems in most of the developed and underdeveloped countries of the world. Some parasitic infestations even cause death when the control measures are neglected (Hassan et al., 2005). Large and small strongyles are the significant pathogens of horses and in addition to that ascarids, thread worms, hair worms, pin worms and tapeworms are also found naturally in horses (Saeed et al., 2010). Equine parasite control remains a complex and constant challenge for both owners and their veterinary advisers. Horses are infested by a wide range of helminth species and differences in life cycles, epidemiology, pathogenicity and drug susceptibility (Nielsen et al., 2010).

Ivermectin was the first macrocyclic lactone anthelmintic, introduced as a veterinary antiparasitic agent in France in 1981. The pharmacokinetic behaviour of ivermectin has been investigated more extensively than that of the other members of the endectocides and ivermectin is so far the most widely used endectocide across animal species (Gokbulut et al., 2010). Ivermectin is a broad-spectrum parasiticide which, in the horses, is highly effective against bots, intestinal nematodes, the migratory stages of Strongylus vulgaris, Habronema, Draschia, and Onchocerca spp. Ivermectin was introduced for horses first as a micellar formulation for parenteral use, but some adverse reactions were reported and the product was withdrawn from the market place. Adverse reactions were not seen when the same formulation was given per os with a paste formulation or with a liquid formulation for horses (Owen et al.,1988).

Ivermectin is highly effective against adult ova-producing nematode parasites. Within 7-14 days after treatment faecal ova count are usually near zero (Kachhawa et al., 2013). The aim of the study was to investigate efficacy of ivermectin against gastrointestinal parasites in horses.

Materials and Methods
A total of 100 horses were randomly selected with irrespective to age, sex, breed and out of these 20 positive cases were selected for efficacy trial. The faecal sample was examined for the presence of infestation and intensity of gastrointestinal parasitic load in term of eggs per gram of faeces (EPG). The efficacy of ivermectin was evaluated in natural gastrointestinal parasitic infestation of horses. The samples found positive for gastrointestinal parasites were analyzed by McMaster egg counting technique for their EPG. Animals were administrated orally tablet ivermectin @ 200 µg/kg body weight as a single dose (Virbac, Animal Health India Pvt. Ltd., Mumbai, each tablet contains 80 mg ivermectin).

After administration of drug in each positive animal, faecal sample were again collected on 15th day for EPG count to note the efficacy of drug.

\[
\text{Efficacy of treatment} = \frac{\text{Mean EPG value before treatment} - \text{Mean EPG value after treatment}}{\text{Mean EPG value before treatment}} \times 100
\]

The evaluation of efficacy of anthelmintic drug was done strictly on the basis of WAAVP guidelines as described by Wood et al. (1995) by comparing the mean EPG values before and after treatment.

Results and Discussion
Therapeutic efficacy of ivermectin against gastrointestinal parasites of horses presented in Table 1. Ivermectin tablet given orally as a single dose at the rate of 0.20 mg/kg body weight reduced the mean egg per gram of gastrointestinal parasites from average counts of 825 ± 58.77 to 20 ± 9.17 with the average reduction of 97.57 per cent on 15th day.

The efficacy of ivermectin in present study was 97.57 per

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Table 1: Therapeutic efficacy of ivermectin against gastrointestinal parasites infested horses on 15th day

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose and route of administration</th>
<th>Egg per gram (MEAN±SE) On 0 day</th>
<th>Egg per gram (MEAN±SE) On 15 day</th>
<th>Per cent efficacy on 15th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivermectin</td>
<td>0.20 mg/kg body weight, orally</td>
<td>825±58.77</td>
<td>20 ± 9.17</td>
<td>97.57</td>
</tr>
</tbody>
</table>

Different superscripts differ significantly (P<0.01)

cent analyzed by comparing reduction of mean epg counts between 0 day as pre-treatment and on 15th day as post treatment. A significant (P<0.01) decrease in the mean epg counts was recorded ingastrointestinal parasite infested horses.

The efficacy of ivermectin against gastrointestinal parasites in horses has been recorded earlier by many workers. It has been reported that 95% (Gokbulut et al., 2010); 96.21% (Aftab et al., 2005); 96.9 to 100% (Larsen et al., 2011), 98.42% (Kachhawa et al., 2013).

Ivermectin is highly effective for the treatment and control of a broad range of small and large Strongyle species as well as other species of gastrointestinal parasites (Hassan et al., 2005). In present study, the prevalence of nematode was high in comparison to other gastrointestinal parasites.

Gamma-amino-butyric acid (GABA) is the neurotransmitter substance mediating transmission of inhibitory signals from the inter neurons to the motor neurons in the ventral nerve cord of nematode parasites. The overall GABA-mediated chloride ion conductance effect may be due to (a) ivermectin acting as a GABA agonist either at the GABA binding site or elsewhere on the protein, (b) stimulation of presynaptic GABA release, or (c) potentiation of GABA binding to its receptors. The paralysis is the most evident effect of ivermectin in parasites (Barragy, 1987).

References

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Due to increasing cost of publication, it has been decided to levy following charges from author(s) w.e.f. January, 2017 issue:

1. Processing Charges : Rs. 700/- for each article.
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CHIEF EDITOR
GASTROINTESTINAL HELMINTHIC INFESTATIONS IN LOCAL CATTLE OF DIBRUGARH DISTRICT OF ASSAM

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ABSTRACT
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A total of 200 faecal samples were randomly collected from either sex of local cattle of different age groups. Egg per gram of faeces (EPG) was determined by the Stoll’s technique. The most prevalent gastrointestinal helminth parasite eggs detected were Fasciola, Paramphistomum, Cooperia, Toxocara, Strongyloides, Haemonchus and Ostertagia. Before occurrence of flood, EPG count of different helminths was less than 400, which markedly increased beyond 400 after flood. Our study clearly indicated that flood had a significant role in multiplication of different helminthic infestations.

Key words: Gastrointestinal helminths, flood, clinical manifestation, cattle, Dibrugarh

Introduction
Gastrointestinal (GI) nematode infestations of cattle are constraints on the efficient raising of cattle on pasture throughout the world (Gasbarre et al., 2001). These parasites have two main features of epidemiological importance: there is no multiplication of their pre-parasitic stages and their infective stages, whether acquired directly by the host or indirectly via intermediate hosts, always give rise to a single adult male or female parasite in the definitive host (Chiejina, 1994). Therefore, the number of infective stages present in the host environment at any given period is related to the number of worm eggs passed by the host and this largely determines the number of parasites potentially capable of being established in a susceptible host. However, characteristics such as breed, age and nutritional status of the host as well as characteristics of the environment also have a considerable influence on the parasites and their capacity to infect and inflict damage to the host (Rivera et al., 1983). Most studies on GI nematode ecology in cattle have concluded that climatic conditions play an important role in the survival and transmission of parasite eggs and larvae (Rivera et al., 1983). Helminth infestation, especially sub clinical gastrointestinal nematode infestations are among the major health problems limiting the productivity in dairy animals (Dimaner et al., 2000; Johannes et al., 2009). Therefore, to be effective, control measures depend on a sound understanding of the epidemiology of the disease in both the host and the environment. Keeping in view the immense economic losses of GI helminthic infestation, the present study was planned to know the prevalence of these infestations during pre and post flood situation under agroclimatic condition of rural Assam.

Materials and Methods
The investigation was carried out from February, 2013 to September 2013 in Phutahola village of Dibrugarh district of Assam. The village, located at a distance of 5 km way from the Krishi Vigyan Kendra, Dibrugarh, was selected on the basis of previous agro meteorological records of recurrent flash flood condition that lead to water stagnation for prolong period in the open grazing pasture. For the study, a total of 200 local cattle of 1-6 years of age of either sex were selected. The cattle were reared in open grazing method comprising of loosing the animals in the morning in the pasture and bringing the animals evening to the shelter. Two samples were collected from the same cow i.e. one during February to April and other during July to September. Monthly temperature, rainfall and humidity of the study area during the study period is shown in Table 1. Faecal samples were collected from the cattle on random basis. Faecal samples were collected per rectum and put into faecal pots, labelled and kept cool prior to transportation to the laboratory where they were examined immediately or stored in refrigerator for a maximum of 6 hours before processing. The samples were processed by Standard Floatation and Sedimentation techniques to investigate the eggs of helminth parasites. The ova of parasites were identified from their morphological features (Soulsby, 1982). Quantitative examination of faeces was conducted to record the intensity of parasitic infestation (EPG; egg per gram of faeces) by Stoll’s technique. The quantum of infection among the animals was derived in terms of percentage positive of the total samples examined.

Results and Discussion
Out of 200 local cattle examined during the study period, 114 animals (57.0%) were shedding helminth eggs in the pre flood situation and 156 (78.0%) in the post flood situation. The most commonly occurring parasite egg recovered from faeces were Fasciola, Paramphistome, Coccidian oocyst, Toxocara, Strongyloides, Haemonchus, Ostertagia. During pre-flood condition, out of 57% positive cases, 16% and 41% cattle excreted eggs in their faeces ranged from < 200 and 201- 400, respectively. Number of eggs beyond 400 was not encountered during pre-flood situation. In the post flood condition, out of 78% positive cases, 1.5, 11, 21 and 44.5% cattle shed eggs in their faeces that ranged from < 200, 201-400, 401-600, 601 and above, respectively (Table 1). Several earlier workers reported the presence of numerous helminths in cattle of Assam (Borkakoty, 1984; Borthakur and Das, 1998; Hafiz and Bhattacharyya, 2009).

The onset and advancement of monsoon rains have a profound effect on the incidence and seasonality of fascioliasis in India. Most of the available information on the prevalence of...
from December onwards, the mean total EPG count decreases with the temperature during the monsoon also favours bacterial infections. During post flood situation (from July to September, 2013), prevalence of Fasciola spp. ranged between 200-400 and 401-600 EPG while EPG of Fasciola, Coccidian oocyst and Strongyloides spp. ranged from 401-600 EPG.

In our study, prevalence of helminth eggs was slightly higher during post flood situation as compared to pre flood condition. The EPG count for Fasciola, Coccidian oocyst and Strongyloides spp ranged between 200-400 and Paramphistome, Toxocara, Haemonchus and Ostertagia spp. were less than 200 during pre flood situation. During the post flood situation (from July to September, 2013), prevalence of Fasciola spp. was highest ranging above 600 EPG. Prevalence of Paramphistome, Strongyloides, Haemonchus spp. ranged from 401-600 EPG while EPG of Coccidian oocyst, Toxocara, Ostertagia spp. were in between 200-400.

Fasciola gigantica comes from abattoir surveys and coprological studies on animals visiting dispensaries and is thus biased. It is however, apparent that the prevalence of fascioliasis in tropical country like India is largely determined by rainfall and production systems (Copeman and Copland, 2008). The rate of prevalence and intensity of various gastrointestinal parasites are severely affected during drought conditions (Chauhan et al., 1981).

In our study, prevalence of helminth eggs was slightly higher during post flood situation as compared to pre flood condition. The EPG count for Fasciola, Coccidian oocyst and Strongyloides spp ranged between 200-400 and Paramphistome, Toxocara, Haemonchus and Ostertagia spp. were less than 200 during pre flood situation. During the post flood situation (from July to September, 2013), prevalence of Fasciola spp. was highest ranging above 600 EPG. Prevalence of Paramphistome, Strongyloides, Haemonchus spp. ranged from 401-600 EPG while EPG of Coccidian oocyst, Toxocara, Ostertagia spp. were in between 200-400. Higher rate of infection could be possibly due to continuous rearing of the animals in the pasture where flood water retained for prolong period and thereby exposing to infestation and availability of infective larvae on the water stagnated ground as reported earlier in sheep (Singh et al., 2005; Sharma et al., 2007). Earlier report from India revealed that incidence of helminths especially Strongyloides and Haemonchus in sheep was higher during August to October (Sharma et al., 2007). High rainfall and humidity lowers the resistance of animal that in turn establishes heavy infection in the host. Moreover, high humidity and moderate temperature during the monsoon also favours bacterial multiplication aggravating the parasitic load. With the onset of winter from December onwards, the mean total EPG count decreases and again accelerates with the increase in temperature (Rahman et al., 2012).

Few numbers of animals showed clinical signs of parasitic infestation like diarrhoea, gradual emaciation, body weight loss, foul smelling mucus discharged stool. Increased parasite burden both inside host as well as on pasture during rainy season has been reported in dairy animals (Sanyal and Singh, 1995; Jitendra and Bhatt, 1999; Rahman et al., 2012). The study indicated that under normal condition, the animals harboured worm burden without any clinical signs but would reach threshold pathogenic level in the monsoon and post monsoon seasons. The EPG count revealed that July to September were the months with the highest risk of GI helminths and pasture contaminations.

Based on the findings of study it is concluded that monsoon has a drastic effect on harbouring gastrointestinal helminthic infestation in flood prone areas of Dibrugarh district of Assam. It is hoped that this study would pave the way for timely eradication and control of parasitic diseases with prophylactic use of anthelmintic and ultimately improving the living status of livestock holders of Assam.

Table 1: Monthly temperature, rainfall and humidity pattern during the study period (Source AWS data, KVK-Dibrugarh)

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (mm)</th>
<th>Temperature (°C)</th>
<th>Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>Jan</td>
<td>6</td>
<td>24.27</td>
<td>8.62</td>
</tr>
<tr>
<td>Feb</td>
<td>4.75</td>
<td>29.03</td>
<td>11.67</td>
</tr>
<tr>
<td>Mar</td>
<td>132</td>
<td>29.34</td>
<td>16.35</td>
</tr>
<tr>
<td>April</td>
<td>186</td>
<td>27.77</td>
<td>19.21</td>
</tr>
<tr>
<td>May</td>
<td>335.5</td>
<td>29.85</td>
<td>22.04</td>
</tr>
<tr>
<td>June</td>
<td>345.25</td>
<td>33.33</td>
<td>24.99</td>
</tr>
<tr>
<td>July</td>
<td>346.5</td>
<td>32.22</td>
<td>25.39</td>
</tr>
<tr>
<td>August</td>
<td>200.5</td>
<td>35.21</td>
<td>27.25</td>
</tr>
<tr>
<td>September</td>
<td>57.7</td>
<td>34.</td>
<td>30.12</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of GI helminthes under pre and post flood condition (Nos and %age in parenthesis).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of helminths</th>
<th>Pre flood situation (n=200)</th>
<th>Post flood situation (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;200</td>
<td>201-400</td>
</tr>
<tr>
<td>1</td>
<td>Fasciola spp.</td>
<td>-</td>
<td>41(20.5)</td>
</tr>
<tr>
<td>2</td>
<td>Paramphistome spp.</td>
<td>8(4.0)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Coccidian oocyst</td>
<td>-</td>
<td>28(14.0)</td>
</tr>
<tr>
<td>4</td>
<td>Toxocara spp.</td>
<td>12(6.0)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Strongyloides spp.</td>
<td>-</td>
<td>13(6.5)</td>
</tr>
<tr>
<td>6</td>
<td>Haemomchus spp.</td>
<td>8(4.0)</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Ostertagia spp.</td>
<td>4(2.0)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>82</td>
</tr>
</tbody>
</table>
FILARIAL PARASITE FROM A FREE-LIVING GREAT PIED HORNBILL
(BUCEROS BICORNIS) FROM PAKKE WILD LIFE SANCTUARY AND TIGER RESERVE, ARUNACHAL PRADHESA, INDIA

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ABSTRACT

Parasitic infection in free living great pied hornbill (Buceros bicornis) is scanty. Present communication deals with the occurrence of a filarial parasite from a male B. bicornis from the Pakke Wildlife Sanctuary and Tiger Reserve, Arunachal Pradesh, India. A male bird was rescued from the wilderness of the Pakke Wildlife Sanctuary which was found to have severe traumatic injury. Even after providing intensive veterinary-medical care the bird succumbed on 9th day after rescue. Post-mortem examination of the carcass revealed presence of filarial parasites located in the subcutaneous tissues and pleural sac of the bird. Parasitological evaluation of the samples confirmed filarial parasites that belonged to the family Onchocercidae and sub-family Lemdaniniae.

Key words: Great pied hornbill, Buceros bicornis, Ameeria spp., Pakke Wildlife Sanctuary and Tiger Reserve, Arunachal Pradesh

Introduction

Hornbills are medium to large sized free ranged birds of the family Bucerotidae. They possess massive bills with a variable sized casque. They are arboreal in habit and mainly feed on wild Ficus figs and various berries. Occasionally, the feed consist insects and small animals. In wilderness, they are found in pairs, often in small groups. They nest in holes of tall, wood trees (Fig. 1). Eggs are incubated by the female. As the female sits for incubating the eggs, the male paints the nest entrance with mud leaving a narrow vertical slit to feed the female from outside (Fig. 2). The male makes repeated visits to the nest to feed the female. There are various species of hornbills found in the country i.e. Mulbar gray hornbill (Ocyeros giseus), Indian grey hornbill (O. bistris), Malabar pied hornbill (Anthracoceros coronatus), Oriental pied hornbill (A. albirostris), brown hornbill (Anorrhinus tickellii), great pied hornbill (Buceros bicornis) and rufous-necked hornbill (Aceros nipalensis). Amongst these, the great pied hornbill is the most spectacular one (Fig.1) with size varying from 95 to 105 cm (Grimmett et al., 2015). Where the later species is endemic, the males are captured or poached for collection of feathers, casques and beaks as trophies. They also bear local traditional ethnic values. If the male fall victim of poaching or dies accidentally, the life of the female in incubation becomes at stake. Clandestine logging has become a contributory threat factor for the nesting habitats. The existing world population is roughly estimated as 13,000 - 27,000 mature birds; IUCN has enlisted B. bicornis as Near Threatened (NT) species (Bird Life International, 2016). In India, the species is fairly common, however, the number is declining.

The Pakke Wildlife Sanctuary (PWLS) and Tiger Reserve is the abode of a good number of B. bicornis in addition to 268 species of resident birds (Varma et al., 2008). PWLS is an IUCN category II habitat of wildlife. It is located in East Kameng district of Arunachal Pradesh, established in 1986. The area coverage of the Sanctuary is 861.95 km² (332.80 sq mi) and located between the latitudes 26°54’2" -27°16’2” N and longitudes 92°36’2" - 92°09’2” E at an elevation of 2040 m (6,690 ft). The sanctuary has a good precipitation (2,506 millimeters or 98.7 inch) with an annual average summer temperature of 36°C (97°F) and 12°C (54°F) winter temperature. The forest type of the sanctuary is Assam valley tropical semi-evergreen forest. It has six types of vegetation, i.e., Sub-Himalayan light alluvial semi evergreen forests (2B/C/151), Eastern Hollock forests (3/152(b)), Upper Assam valley tropical evergreen forest (1B/C.2B), Tropical riverine forests (4E/RSI) and secondary moist bamboo tract (E1/2/SI) (Champion and Seth, 1959). B. bicornis is declared as the state bird of Arunachal Pradesh and legally it is protected under Schedule I, Part III of the Wildlife (Protection) Act (1972) of India. There is trade ban on B. bicornis according to CITES Appendix I. The species can be bred in captivity in zoos (Jensen, 2008). There is least information on the veterinary parasitic diseases of this NT species (Reddy and Rao, 1983; Ali, 1961, Galama, 1996; Galama et al., 2002 and Hossain, 2012) probably due to rarity of availing materials from the wilderness. We record here a chance case of natural filarial infection in a free ranged B. bicornis from PWLS.

Materials and Methods

Source of materials

An adult male great pied hornbill (Buceros bicornis) weighing 3.28 kg was rescued at Lanka in the Pakke Wildlife Sanctuary and Tiger Reserve, East Kameng District of Arunachal Pradesh.
Arunachal Pradesh by the staffs of the sanctuary. The bird was brought for veterinary-medical care to the Centre for Bear Rehabilitation and Conservation (CBRC) jointly operated by the Department of Environment and Forest, Government of Arunachal Pradesh and Wildlife Trust of India (WTI) located at Seijosa during the month of August, 2015. On clinical examination by the resident veterinary surgeon the bird was found to have severe injury caused due to hit by catapult. The traumatic injury was detected in the rump associated with consistent loss of feather around the injured area. The bird also became unable to fly. Though the patient was provided with adequate veterinary-medical care, yet, it succumbed on
9th day after rescue at the CBRC facilities. A post-mortem examination was conducted by the second author to ascertain the cause of death. It revealed that, the bird died due to major organ failure. In addition to the lesions in the organs and body, presence of a few helminth parasites could also be noticed in the subcutaneous tissues and the pleural sac (Fig. 3 and Fig. 4). The parasites were collected, fixed and preserved in 2% formalin. The samples were sent for parasitological evaluation to the Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22. The unknown parasitic species is the material for the present communication.

Parasitological evaluation
The parasitic samples were cleaned in distilled water and put in lactophenol for 48 hours for clearing. Thereafter, temporary mount was prepared and examined under a compound microscope for evaluation of morphological characteristics of the parasites following standard published keys (Sood, 2006).

Results and Discussion
In the present investigation, four nematode parasites could be isolated, of which two were males and two females. The latter two were significantly damaged during collection. Based on the morphological features of the exhibits under study, they could be assigned to the family Onchocercidae (Leiper, 1911) and sub-family Lemdaninae (Lopez-Neyra, 1956). Further morphological features were similar to the genera *Ameeria* (Ali, 1961; Sood, 2006) (Fig. 5, Fig. 6 and Fig.7), pending species affiliation. Prevalence of helminth parasites in hornbills are scantily reported by a few authors. Previously, Ali (1961) described the morphology of filarial parasites recovered from the orbital cavity and under neath skin along trachea of a captive common grey hornbill (*Tockus birostris*) from Hyderabad. Based on measurements of 2 males and 6 females the author assigned the parasites to belong to a new genera *Ameeria* Ali, 1961 and a new species as *A. sultanae* (Ali, 1961). Reddy and Rao (1983) reported the presence of *Ascaridia galli* in the intestine of a captive great hornbill (*B. birostris*) from the Nehru Zoological Park at Hyderabad. Galama (1996) reported the occurrence of *Ascaris, Trichostomylus* and *Strongylodes in B. bicornis*. Galama et al. (2002) in a compilation on hornbill management and husbandry guidelines opined that a nematode parasite *Syngamus trachea*, commonly known as gape worm infection may be one of the causes of mortality of young birds in nest. The parasite is transmitted from adult birds to the young. Hossain (2012) could recover *Heterakis* sp. and stomach worm during post-mortem examination of a great pied hornbill (*Buceros bicornis*) from Bangabandhu Sheikh Mujib Safari Park, Dulahazara, Cox’s Bazar of Bangladesh. It could be observed that, the life cycle of the parasites mentioned above or their pathology in *B. bicornis* has not been worked out. Hence inference has to be drawn from the known species that occurs in domesticated birds (Soulsby, 2012). Veterinary health care and management of captive wildlife especially endangered species in exhibits, breeding, rescue or rehabilitation center is a challenge for the wildlife veterinarians due to lack of baseline information on pathogens. Parasites are common pathogens of wild life (Islam, 2012) which might endanger the life of precious wildlife. The north-eastern region of India being biodiversity hotspot region needs attention in respect of generating baseline information on the parasitic fauna and their pathology in wildlife. Present investigation could gather data on the occurrence of a filarial nematode *Ameeria* Ali, 1961 for the first time from a free ranged *B. bicornis* from its in situ habitat.

Acknowledgements
Authors acknowledge the Department of Forests and Environment, Govt. of Arunachal Pradesh and Wildlife Trust of India (WTI), Noida, for the materials and the Dean, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati - 781 022 for the facilities to carry out this study.

References
PREVALENCE OF SUBCLINICAL KETOSIS IN WESTERN RAJASTHAN*

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ABSTRACT

Dairy farming is a business that people engage for financial gain. Diseases in farm animals have a significant economic impact on livestock production. This paper presents to find out the prevalence of subclinical ketosis in dairy cows of western Rajasthan. The subclinical ketosis was diagnosed on the basis of plasma BHBA and NEFA levels during post-partum period. The overall prevalence of subclinical ketosis was recorded as 23.57 per cent. The prevalence was highest in Pali-Marwar districts (28.08 per cent). The prevalence of subclinical ketosis is high comparatively literature cited due to non-scientific feeding.

Key words: Prevalence; subclinical ketosis, dairy cow

Introduction

The prevalence of animal diseases in the world has been reduced in the last four decades due to its economic importance; there are still some of the livestock diseases that cause reduction in production efficiency leads severe economic losses (Johnchriste and Thirunavukkarasu, 2006; Nagategize and Kaneene, 1985). Prevalence is a snapshot of the amount of a disease existing in a herd at a point in time. Prevalence is calculated by dividing the number of disease positive cows by the number of cows sampled at that point or period in time. Ketosis, a disease of heavy lactating animals, occurs during peak milk yield causing a great economic loss to the milk industry. Diagnosis of bovine ketosis at an early (subclinical) stage is a must to prevent the economic loss to the farmers in terms of reduced milk yield. Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. Dairy cows suffer from negative energy balance (NEB) during the first week of lactation due to energy expenditure associated with milk production and limited feed intake, resulting in NEB, a high mobilization of lipids from body fat reserves and hypoglycaemia (Drackley, 1999; Bobe et al., 2004; Dokovic et al., 2011). Nutrition, age, heredity, body condition score (BCS), management and energy imbalance as various risk factors are possible causes of NEB, periparturient fatty liver and ketosis (Drackley, 1999; Bobe et al., 2004). The economic impacts of maladaptation are important and include increased risk of metabolic disease, reduced milk production, early removal from the herd, and poor reproductive performance. The present study was found out the prevalence of subclinical ketosis in dairy cows in western Rajasthan.

Materials and Methods

The subclinical ketosis was diagnosed on the basis of plasma BHBA concentration (≥1.2 mmol/L) and NEFA concentration (≥0.7 mmol/L) during post partum transition period, respectively along with the presence of other signs such as decrease in milk yield. Inappetence was not observed all subclinical ketotic animals. The sampling were carried out after approximately 4-5 hrs after feeding because just after feeding BHBA increase. For the present study, 123 dairy cows were selected in 3 districts (Bikaner, Jodhpur and Pali-Marwar) of western Rajasthan.

Results and Discussion

Based on the results of different biomarker (NEFA and BHBA values) of negative energy balance, prevalence for the subclinical ketosis was calculated.

District wise: The prevalence of subclinical ketosis in dairy cows of Bikaner, Jodhpur and Pali-Marwar districts was 25.42 (15/59), 19.51 (8/41) and 26.08 (6/23) per cent, respectively.

Parity-wise prevalence of subclinical ketosis was highest in above 5th parity (32.35 per cent), followed by 3rd to 5th parity (20.22 per cent). The incidences of SCK increased with age. The results of the present investigation were similar to Bihani (2001), Sharma (2006), Kumar (2011) who reported highest prevalence in 3rd and 4th parity. The prevalence of SCK and clinical ketosis were 13.9% and 3.4%, significant difference were observed between regions, but not in parity wise. The mean daily peak milk yield for clinical ketosis, subclinical ketosis and healthy cows in study were 28, 35 and 45 kg, respectively, suggesting a decline ranging from 22 to 38%, compared to the healthy cows (Samiei et al., 2013). Sato et al. (2005) reported 34.7% SCK in Japan and 57.7% in Lithuania (Zilaitis et al., 2007), which were higher than our study.

SCK occurred at 5 days in milk (DIM), when 22.3% of cows had their first SCK-positive test. Peak prevalence of SCK occurred at 5 days in milk (DIM), when 28.9% of cows had a SCK-positive test (McArt et al., 2012). Marjan and Saman (2011) reported the incidence rate of subclinical ketosis was 7.2% (per cent of cows with at least one positive test) in early lactation (0-70th day) period and the peak prevalence of subclinical ketosis occurred during the fourth week of lactation in Fars Province of Iran. The overall prevalence of subclinical ketosis was 6.9 per cent to 14.1 per

*Part of Ph.D. Thesis of first submitted to RAJUVAS, Bikaner-334001 (Raj.) and present address:: SMS (Vet. Sc.), KVK, CAZRI, Pali-Marwar-342 601 (Raj.); 2Asstt. Professor-CVAS, DUVASU, Mathura-281 001 (U.P.)
Table 1: Prevalence rate of subclinical ketosis in crossbred cows in different district of western Rajasthan

<table>
<thead>
<tr>
<th>Districts</th>
<th>No. of cows sampled</th>
<th>No. of SCK (Prevalence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bikaner</td>
<td>59</td>
<td>15 (25.42 %)</td>
</tr>
<tr>
<td>Jodhpur</td>
<td>41</td>
<td>8 (19.51 %)</td>
</tr>
<tr>
<td>Pali-Marwar</td>
<td>23</td>
<td>6 (26.08 %)</td>
</tr>
<tr>
<td>Total</td>
<td>123</td>
<td>29 (23.57 %)</td>
</tr>
</tbody>
</table>

The chi square statistics is 0.5681. The P-value is 0.9665. No significant difference in prevalence of SCK was observed between different districts.

cent in the first two months of lactation (Andersson and Emanuelson, 1985; Nielen et al., 1994; Duffield et al., 1998).

The overall prevalence of ketosis was 9.38 per cent in cows and 2.92 per cent in buffaloes, observed in Tamil Nadu. They further observed low prevalence of ketosis in cows of Erode and Coimbatore districts of Tamil Nadu. They further observed low prevalence of ketosis in cows of Erode and Coimbatore districts of Tamil Nadu (Thirunavukkarasu et al., 2010).

Garro et al. (2013) and Suthar et al. (2013) reported overall prevalence of 21.8 and 10.3 per cent, respectively in the dairy cows, using a threshold of >1.2 mmol/L for blood BHBA. However, our studies having higher prevalence rate as compared to above mentioned study. The major cause of higher prevalence may be non-scientific feeding and management practices adopted in the study area.

Duffield et al. (1998) reported an incidence of 59.00 per cent and 43.00 per cent using a cutoff threshold for BHBA concentration of 1200 and 1400 mmol/L, respectively. Similarly Asl et al. (2011) observed a prevalence of 63.00, 68.00 and 59.00 per cent during the week 2nd, 4th and 6th post-partum, out of which 30.00 per cent of cows were found positive in all of the 2, 4 and 6 weeks post-partum.

McArt et al. (2012) reported a prevalence of 28.70 per cent, 26.30 per cent and 40.80 per cent in cows with parity 1, 2 and above 3, respectively. Higher prevalence reported in their study was due to the bi-weekly sampling (McArt et al., 2012 and Ospina et al., 2010) during the first two months, which resulted in increase in the detection rate of SCK, as the median time from first SCK positive BHBA test to first test <1.2 mmol/L was 5 days, (McArt et al., 2012) which helped them in detecting more number of SCK cases.

Highest prevalence of subclinical ketosis was found in cows from greater than 5th parity in comparison to 3rd, 5th parity. Possible reason for that might be the active haemostatic mechanisms in the young dairy animal to cope up with the negative energy balance during the early lactation period. Similar to the present findings, number of research workers recorded that the prevalence of ketosis increases with the age and peak prevalence was observed between 3rd, 5th lactation (Overby et al., 1974; Erb and Martin, 1978).

Acknowledgments
Authors are highly thankful to Dean, CVAS, RAJUVAS, for providing necessary facility for research.

References
ABSTRACT
A total of 60 cases (32 cows and 6 buffaloes) comprising of 98 quarters were studied under the present investigation. Milk samples from 60 cases (32 cows and 6 buffaloes) comprising of 98 quarters were collected aseptically in sterilized vials. The milk samples were also subjected for some indirect tests viz. strip cup test, white side test, CMT and BTB indicator card method for proper diagnosis of the cases. In addition to this, affected udder was also examined physically. The affected quarters were categorized into 4 groups viz. Group-I 12 cows with 19 affected quarters; 2 buffaloes and 2 quarters, Group II 14 cows and 24 quarters; 2 buffaloes and 3 quarters, Group III (15 cows and 21 quarters; 1 buffaloes and 1 affected quarters and Group IV 13 cows and 26 quarters; 1 buffaloes with 2 affected quarters. The maximum recovery was observed in Group IV which was treated with intramammary preparation of cefoperazone and the cure rate was 85.71% animal-wise and 82.14% quarter-wise on day 3 (post-treatment) whereas cent per cent cure rate both animal-wise and quarter-wise were observed on day 5 (post-treatment) which need one additional intramammary infusion.

Key words: Mastitis, antibiotic sensitivity, cow, buffalo

Introduction
The common intramammary antibiotic therapy has not given valuable results due to lack of optimal absorption and dispersion in the glandular tissue of the lactating cows. In addition, the emergence of resistance by pathogenic bacteria associated with mastitis is the greatest problem. The pattern of drug resistance continues to change in a particular area depending upon various epidemiological factors and indiscriminate use of antibiotics (Nag and Ray, 1982; Chaudhary and Narayan,1984.) So, the current trend necessitates exploring an alternate, suitable, appropriate and supportive therapy by increasing the phagocytic activities of mammary polymorphonuclear and mononuclear cells (Daley and Hayes, 1992). Therefore, the present study was undertaken to treat the affected udder in different combinations.

Materials and Methods
A total of 60 cases (32 cows and 6 buffaloes) comprising of 98 quarters were studied under the present investigation. Milk samples from 60 cases (32 cows and 6 buffaloes) comprising of 98 quarters were collected aseptically in sterilized vials. The samples were streaked on blood agar media for bacteriological culture as per the standard procedure described by Cruickshank et al. (1975) and then in vitro drug sensitivity was done following the method described by Bauer et al. (1966).

The milk samples were also subjected for some indirect tests viz., strip cup test, white side test, CMT and BTB indicator card method for proper diagnosis of the cases. In addition to this, affected udder was also examined physically. The affected quarters were categorized into 4 groups viz., Group-I (cows=12, affected quarters=19;buffaloes=2, affected quarters=2), Group-II (cows=14,affected quarters=24; buffaloes=2, affected quarters=3), Group-III (cows=15, affected quarters=21; buffaloes=1, affected quarters=1) and Group-IV (cows=13, affected quarters=26; buffaloes=1, affected quarters=2. The details of the treatment schedule has been shown in Table 1.

Results and Discussion
The treatment efficacy in 4 different treatments groups in animal-wise and also in quarter-wise was observed during respective common therapeutic duration. The common therapeutic duration in Group I, II and III was observed on day 5 (post-treatment); whereas in Group IV (cefoperazone group) it was observed on day 3 (post-treatment); as well as on day 5 (post treatment), since one additional dose was given on day 3 in which the quarters were not cure. Higher recovery was observed in Group IV animal-wise (85.71%) and quarter-wise (82.14%) on day 3 post-treatment and 100 per cent both animal-wise and quarter-wise on day 5 (post-treatment) as compared to Group I in which per cent recovery was 71.42 per cent and 80.95 per cent, respectively on animal-wise and quarter-wise basis followed by Group III in which per cent recovery animal-wise and quarter-wise was 62.50 per cent and 72.72 per cent, respectively on day 5 post-treatment; whereas in Group II, the pet cent recover both animal-wise and quarter-wise was 56.25 per cent, respectively on day 5 (post-treatment). The details of the above results have been shown in Table 2.

Trisodium citrate was used as supplement therapy along with antibiotics for early recovery from mastitis. Wilson et al. (1986) used cefoperazone as a single dose of 250mg in an oil based suspension infused into bovine quarters suffering from clinical mastitis. Following tri-sodium citrate administration, the citrate content of milk increased and pH dropped to normal. These findings are in agreement with Pal et al. (1994).

Trisodium citrate has several advantages over other remedies
Table 1: Drug dosage regimens for treatment of mastitis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Trade name of drug and their manufacturer</th>
<th>Composition of the drug(s)</th>
<th>Dosage and schedule</th>
<th>Route of administration</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pendistrin-SH (Sarabhai Zydus)</td>
<td>Procaine penicillin (1 lac IU), Streptomycin sulphate (100 mg), Sulfamerazine (500mg) and Hydrocortisone acetate (29 mg) per tube.</td>
<td>1 tube twice daily per affected quarter</td>
<td>Intramammary</td>
<td>5 days</td>
</tr>
<tr>
<td>2</td>
<td>Dicrysticin-S (Sarabhai Zydus)</td>
<td>Procaine penicillin G (15lac), Penicillin G sodium (5lac) and Streptomycin sulphate (2.5g) per vial.</td>
<td>10 mg/kg b.wt. twice daily</td>
<td>Intramuscular</td>
<td>5 days</td>
</tr>
<tr>
<td>2</td>
<td>Mammitel (Intas)</td>
<td>Colistin sulphate (5lacIU) Cloxacillin sodium (200mg) per syringe.</td>
<td>1 tube twice daily per affected quarter</td>
<td>Intramammary</td>
<td>5 days</td>
</tr>
<tr>
<td>3</td>
<td>Intamox (Intas)</td>
<td>Amoxicillin sodium (1.25g)+ Cloxacillin sodium (1.25g) per vial.</td>
<td>8 mg/kg b.wt. twice daily</td>
<td>Intramuscular</td>
<td>5 days</td>
</tr>
<tr>
<td>4</td>
<td>Gentaves (Vesper)</td>
<td>Gentamicin (40 mg/ml) 80 mg+2.0 ml water for injection twice daily</td>
<td>2.5 mg/kg b.wt. twice</td>
<td>Intramuscular</td>
<td>5 days</td>
</tr>
<tr>
<td>4</td>
<td>Mastiwok (Vetoquinol)</td>
<td>Cefoperazone (250 mg) per syringe.</td>
<td>1 tube per affected quarter single infusion</td>
<td>Intramammary</td>
<td>3-5 days</td>
</tr>
</tbody>
</table>

Table 2: Efficacy of therapy of different treatment group in animal-wise and in quarter-wise during common therapeutic duration

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of animal treated</th>
<th>No. of quarters treated</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of animal recovered</td>
</tr>
<tr>
<td>Group-I</td>
<td>14</td>
<td>21</td>
<td>NIL</td>
</tr>
<tr>
<td>Group-II</td>
<td>16</td>
<td>27</td>
<td>NIL</td>
</tr>
<tr>
<td>Group-III</td>
<td>16</td>
<td>22</td>
<td>NIL</td>
</tr>
<tr>
<td>Group-IV</td>
<td>14</td>
<td>28</td>
<td>12 (85.71)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate per cent of animals/quarters recovered.

in mastitis as it replenishes the citrate radical in animal body and is cheap and quarters need not to be treated individually. Mandial et al. (1999) reported the efficacy of Pendistrin-SH (82.35%) and gentamicin (87.80%) which are in agreement with the present study. Moyo et al. (2005) concluded that vitamin E supplementation during the dry and early lactation periods is associated with lower intramammary infection (IMI), somatic cell count (SCC) and clinical mastitis (CM). Colak et al. (2006) concluded that vitamin E supplementation during the transition period benefits the immune system via improving neutrophil activity and consequently reduces the risk for mastitis and retained placenta.

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References
THERAPEUTIC MANAGEMENT OF HYPOTHYROIDISM IN A DACHSHUND BITCH

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Introduction

There are number of factors causing hypothyroidism, which ultimately alter the metabolic status of animal and therefore affecting the efficiency of animal. Hypothyroidism is the natural deficiency of thyroid hormone; it may occur either due to iodine deficiency which is caused by deficient iodine intake (Gupta et al., 2010) or due to impaired production or secretion of thyroid hormone resulting in reduced metabolism. It is one of the most commonly encountered hormonal disorders in dogs with incidence rate at about 1:150 to 1:500 (Chastain and Panacera, 1995). The condition can be produced by immune mediated destruction of thyroid cells, by natural atrophy of the gland, by dietary iodine deficiency or as a congenital problem. Active thyroid hormone is necessary for normal metabolism. Thyroxine is required for normal cellular activity in all cells of the body and thus clinical signs vary depending upon the stage of disease and also the breed of the animal. Though it is very common problem in dogs, many times it poses a challenge to veterinarians.

Case history

A Dachshund, seven-year-old, 21 kg bitch was presented to Teaching Veterinary Clinical Complex, Pantnagar, Uttarakhand with the history of chronic skin affections, along with weight gain, inability to walk properly, weakness, early exertion and tendency to feel comfort in sun for the last 5 months. Pet keeper reported that the dog has been previously treated for skin affections with ciprofloxacin, ivermectin, chlorpheniramine maleate and multivitamin syrup but dog showed no improvement.

Clinical examination

Clinical examination revealed rough brittle hair coat and seborrhoea, symmetrical bilateral alopecia on lateral abdomen, spectacled appearance around the eyes, pale mucous membrane, and generalized obesity. Skin scrappings were found negative for fungal hyphae and mites. Rectal temperature (99.7°F), respiration (17/min), heart rate (96/min) was within the normal range. Haemogram showed anaemia, neutrophilia and lymphocytopenia. There was increased alkaline phosphatase, creatinine kinase and TSH level along with hypercholesterolaemia and low serum thyroxine level.

As the case presented here hypothyroidism is common in middle age dogs i.e. at the age of 4-10 years and mostly dogs of pure breeds like Golden and Labrador Retrievers, Doberman Pinschers, Irish Setters, Dachshund, Cocker Spaniel and Terrier are more susceptible for this condition. There does not appear to be sex predilection, but spayed females appear to be at greater risk (Kahn and Line, 2005).

Clinical signs observed in the present case correlates with signs described by many workers. Typical clinical signs are exercise intolerance; lethargy and weight gain (Bischel et al., 1988). A high proportion of dogs develop dermatological signs (Budsberg et al., 1993) based on duration and severity of the disease and the affected dogs have dry skin having dark pigmentation and alopecia which begins at areas of friction like at the tail region resulting in characteristic “rat tailed appearance” and neck area which progress in advance stage to trunk area. In some breeds like Retrievers nasal alopecia is also observed. In dogs, low level of T4 is highly suggestive of hypothyroidism rather than elevated TSH level (Jonklass and Talbert, 2014). Myxedema, puffy appearance, tragic facial expressions are commonly seen clinical findings. Dogs suffering from hypothyroidism are known as “heat seekers” as there is difficulty in maintaining body temperature. Various reproductive disorders can be seen in intact dogs and bitches (Kahn and Line, 2005). Hypercholesterolaemia is found in about 70% cases and normocytic, normochromic non regenerating anaemia in 30% cases which represent physiological response to lowered basal metabolic rate. Mild to moderate increase in serum activity of ALT, AST, AP and CK may be noted (Scheer, 2010). Neurological abnormalities are uncommon in dogs (Mooney, 2011). Based on clinical signs, haematological and serum biochemistry, the present case was diagnosed as primary hypothyroidism. Haematobiochemical values are tabulated in Table 1.

Treatment

The dog was treated by Levothyroxine sodium @ 20 μg/kg body weight orally, twice daily initially for 15 days along with ointment Dexoderm® topically on skin lesions. Feeding of formulated diet (Royal Canin-Obesity DP34®) was initiated with objective to reduce obesity. After 15 days owner reported improvement in the condition of dog and treatment was continued for next one month. After one month all clinical signs were resolved and treatment was further continued with Levothyroxine sodium @ 20 μg/kg body weight orally once daily as maintenance dose.

Thyroid replacement therapy with levothyroxine sodium which most closely resembles the T4 secretion of thyroid gland is treatment of choice. There are various treatment regimes which suggests that dog should be maintained on levothyroxine

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Dexoderm®- MICRO LAB LTD.- (Ofloxacin 0.75%/w/w, Ornidazole 2%/w/w, Terbinafine 1%/w/w, Clobestasol Propionate 0.05%/w/w)
sodium @ 0.02 mg/kg orally in divided dose, twice daily, until all clinical signs are resolved, before an attempt is made to medicate once daily (Plumb, 1999). In this case, the dog was kept on levothyroxine sodium, twice daily, until clinical signs were resolved followed by once daily medication. Replacement therapy is required for life.

Table 1: Haemato-biochemical values in Dachshund bitch

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (gm%)</td>
<td>9.7 ↓</td>
</tr>
<tr>
<td>TLC (/μl)</td>
<td>7300</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>85</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>6</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>5</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>4</td>
</tr>
<tr>
<td>Alkaline phosphatase (μ/L)</td>
<td>153 ↑</td>
</tr>
<tr>
<td>Creatine kinase (μ/L)</td>
<td>186 ↑</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>302 ↑</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>73</td>
</tr>
<tr>
<td>Serum thyroxine (T4) (nmol/L)</td>
<td>8.2 ↓</td>
</tr>
<tr>
<td>Endogenous thyrotropin (thyroid-stimulating hormone; TSH) (nmol/L)</td>
<td>0.95 ↑</td>
</tr>
</tbody>
</table>

References
MIX INFESTATION OF TRYpanosomiasis AND Theileriosis IN Buffaloes

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Key words: Buffaloes, mix infection, trypanosomiasis and theileriosis

Introduction
Haemoprotozoal diseases are known to cause heavy losses to the livestock industry. Lack of appropriate control strategies for the haemoprotozoal diseases leads to increase in mortality, reduced milk production, lowered animal draft power, this ultimately produces constraint to bovine production and socio-economic development in India (Suryanarayan, 1990). Cattle in India may be infected with a wide variety of vector-borne haemoparasites, most economically important genera are the Trypanosomes, Babesia, Anaplasma and Theileria spp. These haemoparasites can occur either in form of single infection or combination of two or three parasite concurrently, which has been reported in many previous reports (Kamani et al., 2010; Yusufmiaa et al., 2010). The infection of haemoparasite is due to the result of complex interaction between the causative organism, vector, the vertebrate host and the environment (Akande et al., 2010). Trypanosoma evansi is consider as the most common species of trypanosome that causes Trypanosomiasis in India (Pathak and Singh, 2005). Theileriosis occurs due to infection of variety of tick vectors and leads to appearance of infections ranged from clinically inapparent to rapidly fatal one (Taylor et al., 2007).

In bovine, Tropical Theileriosis occurs in Mediterranean countries, Middle East, Indian and China, and which occurs due to infection of T. annulata (Radostits et al., 2007).

History and clinical observations
Two buffaloes having age between 6-7 years reported at TVCC, Mathura with chief complaint of persistent fever (106°F and 105°F) along with enlargement of prescapular lymph node, oedema of the dependent parts of the body, weakness, anorexia, lachrymation, dyspnoea and anaemia. Clinical signs revealed suspicion for haemoprotezoan infection, blood smear examination from peripheral circulation revealed presence of Trypanosoma along with Theileria. Faecal examination was done to rule out any endoparasitic infestation, results of which were negative for presence of any egg or evidence of parasite.

Treatment and Discussion
Parasitic infections are one of the major constraints for profitable dairy industry in tropical and subtropical countries including India. Among the various economically important bovine diseases, vector-borne haemoprotezoan infections such as babesiosis, trypanosomosis and theileriosis are recognized as a cause of severe clinical illness in cattle (Singh and Jyoti, 2012). Mixed infection of haemoprotezoan are not rare in clinical practice, and has been reported previously in many studies (Kumari et al., 2000; Magona and Mayende, 2002). Theileriosis, a tick-transmitted protozoan disease, is a major constraint for cattle production in the tropics and subtropics (Jongejan and Uilenberg, 1994). Trypanosomosis is a complex, debilitating and often fatal disease caused by infection with one or more of the pathogenic tsetse fly-transmitted protozoan parasites of the genus Trypanosoma (Connor and Van den Bossche, 2004). Both the infection can occur either single or concurrent infection of both species. In one study triple infection of haemoprotezoan has been recorded (Nasir et al., 2000), concurrent infections are very challenging for treatment aspect. In present case, treatment was initiated with single injection of diminazene aceturate @ 3.5 mg/kg body weight deep intra muscular along with three dosage of long acting oxytetracycline @ 20 mg/kg b.wt., alternate day, three days later buparvaquone @ 2.5 mg/kg b. wt. was given through deep intra muscular route. Additionally supportive therapy with crystalloid fluid, iron preparations, multivitamin, folic acid and cyanobalamin was also done as it can fasten the recovery rate in Theileria and Trypanosomiasis infected animals. After two weeks of treatment animals were further screened for evidence of infection which was found negative. Case report emphasizes to evaluate concurrent infections in animals with sign and presence of single infections. These infections can be concurrently with gastrointestinal parasitic infestations, therefore, study concludes to rule out endoparasitic infestations in these infections (Magona and Mayende, 2002).

References

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EFFECT OF DROUGHT ON WHITE BLOOD CELL INDICES IN GOATS

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ABSTRACT

The experiment was designed to assess the effect of drought on white blood cell indices in 100 goats 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 overall mean values were 7.967 ± 0.085, 36.900 ± 0.570, 53.090 ± 0.224, 2.760 ± 0.124, 0.710 ± 0.065 and 3.210 ± 0.142, respectively in goat. 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 total leucocyte count (TLC) in goats.

Key words: TLC, DLC

Introduction

Goat is the most prolific ruminants under tropical and sub tropical condition. Although low rain fall and scanty vegetation are common to this area. Generally during drought particularly in western part of Rajasthan the changes are into environment includes scarcity of water and scarcity or no vegetation. The knowledge of blood norms is important in diagnosing many diseases in animals (Vaidya et al., 1970). 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 goats (up to 5 years of age). The effects of sex and age were also determined non 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 in to 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, 1967) from the respective mean value.

Results and Discussion

The overall mean values of various white blood indices i.e. total leucocyte count (TLC) and differential leucocyte count (DLC) and their mean values according to sex and age are presented in Table 1.

The overall mean values of various white blood cell indices viz. WBC (10³/µl), neutrophils (%), Lymphocytes (%), eosinophils (%), basophils (%) and monocytes (%) were 7.967 ± 0.087, 36.900 ± 0.570, 53.090 ± 0.224, 2.760 ± 0.124, 0.710 ± 0.065 and 3.210 ± 0.142, respectively in goats. The ranges were 7.15 to 9.97, 35 to 43, 49 to 57, 1 to 5, 0 to 3 and 0 to 5, respectively in goats.

The overall mean value of total leucocyte obtained in the present investigation were lower than those reported by Parwe et al. (1990), Kataria et al. (1992), Kumar et al. (1997), Wadwa and Prasad (2001) and Singh et al. (2001) in goats.

The overall mean value of total leucocyte obtained in the present investigation was higher than those reported by Khan et al. (2014) and Sandhu et al. (2001) in goats.

The overall mean value of total lymphocyte in the present investigation were higher than those reported by Parwe et al. (1990) but lower than those reported by Kataria et al. (1992), Kumar et al. (1997), Wadwa and Prasad (2001) and Singh et al. (2001) in goats.

The overall mean value of neutrophil in the present investigation was lower than those reported by Bhargava (1980) and more or less corroborated the values recorded by Kataria et al. (1992) and Singh et al. (2001) but higher than those reported by Pyne et al. (1982), Kumar et al. (1997), Wadwa and Prasad (2001), Rastogi and Singh (1990) and Sandhu et al. (2001) in goats.

The overall mean value of eosinophils, basophils and monocytes in the present investigation were lower than those values reported by Rastogi and Singh (1990) and Kataria et al. (1992) but higher than those reported by Wadwa and Prasad (2001) and Singh et al. (2001) in goats.

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 goats and a significant (p≤ 0.05) effect of sex.
and age was observed on the mean values of total leucocytic count.

Selenium and vitamin E appear to be important nutrients in maintaining neutrophils function. Copper deficiency may also adversely affect neutrophil function in drought condition. 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.

References


ASSESSMENT OF MOTION CHARACTERISTICS AND PATH VELOCITIES EXHIBITED BY BARBARI BUCK SPERMATOZOA

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ABSTRACT

Computer assisted semen analysis is being used regularly for studying motility patterns, path velocities and helps in determining semen quality. However, breed specific seminal motility pattern under Indian conditions in goats have not been documented. Therefore, motility and path velocities in fresh ejaculated pure bred Barbari buck semen were studied. Four healthy bucks having similar seminal attributes donated six ejaculates each. The ejaculates were pooled and later extended with standard glycerolated tris extender except egg yolk to final concentration of 50 x 10^6 spermatozoa per ml. Samples which had more than 85% live percentage were selected for sperm kinematics study using CASA system. Curvilinear velocity was found to be 153±3.21 µm/sec while average path velocity was recorded 76.67±0.84 µm/sec. Straight line velocity during study was 69.00±0.40 µm/sec while linearity was recorded to be 87.88±0.78%. Wobble (WOB) during the study was 49.93±0.78% while beat cross frequency was 28.4±1.02 hz. Amplitude of lateral head displacement and Dance (DNC) recorded during the study were 4.65±0.29 µm and 681.58±49.96 µm²/sec. The results recorded during the experiment when compared with those reported by different researchers indicates a breed specific difference in motility characteristic and path velocities of spermatozoa.

Key words: Barbari buck, CASA, semen, spermatozoa

Introduction

Goat has been an integral component of animal husbandry system in India. Goats are mainly reared for milk, meat and hide. Due to their peculiar feeding habits these animals can thrive better in adverse environmental condition with minimum input (Soest, 1996). But due to unawareness about advantages of scientific breeding and rearing, these animals are being reared under traditional rearing system with no scientific know how (Prasad et al., 2013). Furthermore, quality bucks with high growth rate and better genetic potential are being subjected to slaughter and therefore genetic pool is being wasted (Dhanda et al., 2003). These factors result in accumulation and propagation of inferior genotype in a specific area affecting goat husbandry.

So, there is an urgent need to handle situation and formulate strategies that can overcome this aggravating condition. Awareness among goat husbandry practitioners, exchange of superior quality animals among different geographical regions, distribution of superior quality buck and use of artificial insemination in goats are among few important measures that can bring improvement at doorstep of animal-keeper. Use of insemination to upgrade and improve goats breed has an upper hand over rest of the techniques (Kifaro et al., 2007). Monitored insemination not only guarantees use of superior germplasm but helpful in regulating planned breeding. Prerequisite before utilizing semen for insemination or preservation is to evaluate quality in terms of viability, motility, plasma membrane integrity and spermatozoa abnormality. Further, evaluation of semen becomes more important in goat due to persistence of lethal interaction between seminal plasma and egg yolk in semen extender that affect semen quality in terms of viability and spermatozoa motility (Leboeuf et al., 2000). Motility is one of the important seminal attribute that determines timely transport of spermatozoa to site of fertilization and has high correlation with successful conception (Anand et al., 2016). Earlier, motility was evaluated in terms of mass motility and progressive motility through visual observations. But introduction of computer assisted semen analyzer has opened up new opportunity to not only evaluate motility patterns exhibited by spermatozoa but also different path velocities associated with spermatozoa movements that are helpful in determining semen quality. So, in an attempt to evaluate semen quality in term of motility and path velocities in fresh ejaculated semen for better evaluation, experiment was conducted to evaluate the motion characteristics and path velocities exhibited by spermatozoa in fresh ejaculated pure bred Barbari buck semen.

Materials and Methods

The study was conducted to evaluate motion characteristics and path velocities spermatozoa in fresh ejaculated Barbari buck semen. Four healthy bucks of similar age group (2-3 years) and body weight (25-35 kg) were selected as semen donor. Semen collection was made bi weekly from each buck with the help of artificial vagina. A total of 24 ejaculates were collected from four bucks (six ejaculates from each buck). Freshly collected semen after initial evaluation was pooled to eliminate individual variation. Pooled semen was extended with standard glycerolated tris extender except egg yolk (to prevent lethal interactive losses) to final concentration of 50 x 10^6 spermatozoa per ml. Spermatozoa live per cent was evaluated manually using standard protocol (Hancook, 1952). Samples which had more than 85% live were only selected for motion characteristics. Sperm motion characteristics and path velocities were evaluated using CASA negative phase contrast and ×10 objective on a thermostatically warm stage. Settings of CASA system (Biovis CASA2000, Version 4.6, India) designed using algorithm based on size, shape, detection of sperm...
head and classes for motile, immotile, rapid, slow and non-progressive were as follow: Frames/s - 60, number of frames acquired - 61, max velocity (for tracking): V (µm/s) - 150 motility min, curvilinear velocity (VCL) (µm/s) - >25 motility min, average path velocity (µm/s) - >10 motility min, straight-line velocity (µm/s) - >1 min, track length (% of frames) - 51, aspect - 0-99.999, area - 2-20, axis (major) - 4-20, axis (minor) - 2-10, compactness - 0-50, perimeter ratio - 0-99.999, minimum cell size on major axis - 20, minimum cell size on minor axis - 10, magnification - ×10 phase, calibration × (pixels/unit) - 1.905 µ, Y (pixels/unit) - 1.905, size of image - 1280 × 960 pixels. A 10 µl of diluted semen sample was loaded in metallic sperm counting chamber and a range of 3-6 fields were acquired for motility analysis. Statistical analyses were performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The data has been presented as mean ± standard error (SE).

Results and Discussion

The observed mean (±SE) values of motion characteristics and path velocities exhibited by spermatozoa during the study have been presented in Table 1. Curvilinear velocity (VCL) is the total distance travelled by spermatozoa (actual curvilinear trajectory) per unit time. The observed mean (±SE) was found to be 153±3.21 µm/sec. Average path velocity (VAP) is indicative of distance travelled by spermatozoa (average curvilinear trajectory) between first and last tracked points per unit time. The observed mean (±SE) recorded during the study was 76.67 ±0.84 µm/sec. Straight line velocity (VSL) which is indicative of the distance of averaged path of the sperm head trajectory divided by time and is obtained by smoothing the actual curvilinear path. The observed mean (±SE) of VSL recorded during the study was 69.00±2.04 µm/sec. Anand et al. (2016) reported lower values of VCL, VAP and VSL in diluted semen of Sirohi goat while Dorado et al. (2009) recorded higher values in Florida bucks. VCL, VAP and VSL are considered to be the most important characteristics to parameters utilized to evaluate the kinematic characters and have a positive correlation the fertilizing ability of spermatozoa (Jobling et al., 2002). Higher values for these parameters exhibited by spermatozoa are indicative of better conception rate in farm animals. Linearity (Lin %) per cent that reflects linearity of the curvilinear trajectory and is ratio of (VSL/VCP) was recorded to be 44.37±0.95% and straightness (STR%) that represents the straightness of average path is ratio of VSL to VAP was found to be 87.88±0.78%. Linearity is correlated to sperm in vitro migration efficiency, where spermatozoa presenting values of LIN > 50% showed better migration (Cox et al., 2006). Both linearity and ALH seem to be indicators of sperm hyperactivation (Pena et al., 2000). Higher values of linearity per cent is indicative of better capacity of spermatozoa to migrate through female reproductive tract and is indicative of timely transport of spermatozoa to the site of fertilization (Cox et al., 2006). The spermatozoa with higher values of LIN and STR of spermatozoa in semen sample may be thought to have better capacity to overcome barriers in reproductive tract and hence, better conception rate. Values recorded during study were lower than that reported in Florida bucks (Dorado et al., 2007). Wobble (%) measures the oscillation of actual trajectory about its averaged path and is the ratio (VAP/VCL) x 100. The observed mean (±SE) recorded during the study was 49.93±0.78%. Beat cross frequency (BCF) represents number of times the curvilinear trajectory crosses the average path trajectory per second. The observed mean (±SE) recorded during the study was 28.4±1.02 Hz. Amplitude of lateral head displacement (ALH) is the maximum value of the displacement of the sperm head from its average path multiplied by 2 while DNC represents the area swept by the head during its actual movement. The observed mean (±SE) of ALH and DNC recorded during the study were 4.65±0.29 µm and 681.58±49.96 µm²/sec. The results recorded during the experiment when compared with those reported by different researchers indicate a breed specific difference in motility characteristic and path velocities of spermatozoa. The reason for the difference may be the concentration, size of spermatozoa, internal environment and distance to be travelled in reproductive tract or metabolic activity of spermatozoa. Further, competitive studies may be conducted to evaluate the breed specific differences in indigenous goat breeds for better understanding of spermatozoa kinematics and establishing correlation with different factors influencing motility and fertility rate.

Acknowledgments

The authors are thankful to Vice Chancellor, DUVASU and Dean, College of Veterinary Sciences and Animal Husbandry for providing funds and facilities to pursue this research work.

References

GENETIC EVALUATION OF KARAKUL AND MARWARI SHEEP IN ARID ZONE OF RAJASTHAN: BODY WEIGHTS

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ABSTRACT
The comparative performances of Marwari and Karakul breed of sheep have been studied using data (For Marwari 817 and for Karakul 744) collected from C.S.W.R.I., A.R.C., Bikaner over six years. The least-squares means for body weight have been estimated at birth, 3, 6, 9 and 12 months of age to be 3.00±0.02, 13.00±0.01, 17.01±0.08, 20.24±0.10 and 23.40±0.10 kg in Marwari and 3.61±0.02, 12.50±0.12, 17.73±0.11, 20.70±0.14 and 21.73±0.16 kg in Karakul, respectively. The effect of breed was highly (P<0.01) significant for birth, 3, 6, 9 and 12 months weight. Karakul lambs have been found heavier than Marwari lambs at birth weight. The effect of sex of lambs was highly significant (P<0.01) on various body weights. The year of birth had significant effect on body weights from birth to 12 months of age. The interaction between breed and sex have been estimated to be significant (P<0.01) for 9 and 12 months of body weights. The interaction between sex and year have been estimated to be significant for 6, 9 and 12 months body weights. The interactions between breed and year have also been observed to be significant (P<0.01) on body weights. Dam’s weight at lambing was observed to be a significant factor influencing the body weights up to six months of age. Heritability estimates for birth, 3, 6, 9 and 12 months body weight have been estimated as 0.273±0.099, 0.129±0.083, 0.187±0.090, 0.207±0.092 and 0.075±0.077, respectively. The positive and higher phenotypic correlation of weaning weight with weights at subsequent ages suggest that selection may be practiced at an early age. It was concluded that Marwari breed have higher adult body weight in comparison to Karakul breed.

Key words: Marwari, Karakul, sheep, body weight, heritability

Introduction
Sheep contribute animal protein as mutton and provide wool and skin for protection from adverse climate and add to soil fertility by providing valuable manure. Sheep are the backbone of rural economic in arid, semi-arid and hilly regions of our country. The sheep rearing and processing of wool and skin generate rural employment. Rajasthan tops the list of Indian states in sheep population. It is home of to eight sheep breeds viz. Magra, Pugal, Nali, Marwari, Jaisalmeri, Chokla, Malpura and Sonadi. Marwari sheep is well built, sturdy animal having black face, predominant nose, short ear and long legs. Karakul sheep is one of the most important breeds of tropics and subtropics, famous all over the world for the outstanding quality of pelt. Before a breeding plan is implemented, it is necessary to evaluate the factors affecting economic traits. Therefore, the present investigation was an attempt to compare Marwari and Karakul sheep with respect to body weight.

Materials and Methods
The data for the present investigation were collected from the Arid Region Campus, Central Sheep and Wool Research Institute, Bikaner. Data on body weights from birth to 12 months were classified according to breed, sex and year of birth. The least-squares technique using LSMLMW computer programme designed by Harvey (1990) was utilized to study the effect of different factors and to estimate genetic and phenotypic parameters through mixed model approach with non-genetic factors as a fixed effect and sireas a random effect.

Results and Discussion
The body weight at birth, 3, 6, 9 and 12 months of age were 3.00±0.02, 13.00±0.01, 17.01±0.08, 20.24±0.10 and 23.40±0.10 kg in Marwari and 3.61±0.02, 12.50±0.12, 17.73±0.11, 20.70±0.14 and 21.73±0.16 kg in Karakul, respectively. The effect of breed was highly (P<0.01) significant for birth, 3, 6, 9 and 12 months weight. Karakul lambs have been found heavier than Marwari lambs at birth weight. The effect of sex of lambs was highly significant (P<0.01) on various body weights. The year of birth had significant effect on body weights from birth to 12 months of age. The interaction between breed and sex have been estimated to be significant (P<0.01) for 9 and 12 months of body weights. The interaction between sex and year have been estimated to be significant for 6, 9 and 12 months body weights. The interactions between breed and year have also been observed to be significant (P<0.01) on body weights. Dam’s weight at lambing was observed to be a significant factor influencing the body weights up to six months of age. Heritability estimates for birth, 3, 6, 9 and 12 months body weight have been estimated as 0.273±0.099, 0.129±0.083, 0.187±0.090, 0.207±0.092 and 0.075±0.077, respectively. The positive and higher phenotypic correlation of weaning weight with weights at subsequent ages suggest that selection may be practiced at an early age. It was concluded that Marwari breed have higher adult body weight in comparison to Karakul breed.

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Table 1: The least-squares means with standard errors for body weight (kg) at birth, 3, 6, 9 and 12 months of age

<table>
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<tr>
<th>Effect</th>
<th>Birth weight</th>
<th>Three months weight</th>
<th>Six months weight</th>
<th>Nine months weight</th>
<th>Twelve months weight</th>
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</thead>
<tbody>
<tr>
<td>OVERALL</td>
<td>3.30±0.01</td>
<td>12.71±0.07</td>
<td>17.40±0.06</td>
<td>20.47±0.08</td>
<td>22.56±0.10</td>
</tr>
<tr>
<td>BRED</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>MARWARI</td>
<td>3.00±0.02</td>
<td>13.00±0.01</td>
<td>17.01±0.08</td>
<td>20.24±0.10</td>
<td>23.40±0.10</td>
</tr>
<tr>
<td>KARAKUL</td>
<td>3.61±0.02</td>
<td>12.50±0.12</td>
<td>17.73±0.11</td>
<td>20.70±0.14</td>
<td>21.73±0.16</td>
</tr>
<tr>
<td>SEX OF LAMB</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>MALE</td>
<td>3.40±0.02</td>
<td>13.00±0.10</td>
<td>17.72±0.10</td>
<td>21.00±0.13</td>
<td>23.00±0.14</td>
</tr>
<tr>
<td>FEMALE</td>
<td>3.20±0.02</td>
<td>12.50±0.09</td>
<td>17.02±0.10</td>
<td>20.00±0.10</td>
<td>22.19±0.11</td>
</tr>
<tr>
<td>YEAR OF BIRTH</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Yr1</td>
<td>3.32±0.03</td>
<td>12.43±0.15</td>
<td>18.56±0.14</td>
<td>23.19±0.18</td>
<td>26.10±0.20</td>
</tr>
<tr>
<td>Yr2</td>
<td>3.50±0.03</td>
<td>15.00±0.17</td>
<td>19.80±0.17</td>
<td>22.37±0.21</td>
<td>26.36±0.21</td>
</tr>
<tr>
<td>Yr3</td>
<td>3.13±0.03</td>
<td>11.61±0.18</td>
<td>17.08±0.20</td>
<td>20.82±0.23</td>
<td>25.00±0.23</td>
</tr>
<tr>
<td>Yr4</td>
<td>3.45±0.03</td>
<td>13.00±0.16</td>
<td>15.23±0.14</td>
<td>18.14±0.18</td>
<td>21.30±0.28</td>
</tr>
<tr>
<td>Yr5</td>
<td>3.20±0.03</td>
<td>11.32±0.16</td>
<td>17.18±0.17</td>
<td>20.83±0.22</td>
<td>25.16±0.31</td>
</tr>
<tr>
<td>Yr6</td>
<td>3.23±0.04</td>
<td>13.00±0.20</td>
<td>16.42±0.17</td>
<td>17.46±0.14</td>
<td>20.57±0.22</td>
</tr>
<tr>
<td>INTERACTION</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Breed x Sex</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Marwari x Male</td>
<td>3.10±0.02</td>
<td>13.33±0.13</td>
<td>17.36±0.12</td>
<td>22.20±0.14</td>
<td>24.26±0.15</td>
</tr>
<tr>
<td>Marwari x Female</td>
<td>3.00±0.02</td>
<td>12.60±0.12</td>
<td>16.65±0.11</td>
<td>19.29±0.13</td>
<td>22.52±0.14</td>
</tr>
<tr>
<td>Karakul x Male</td>
<td>3.72±0.02</td>
<td>12.61±0.20</td>
<td>18.08±0.17</td>
<td>20.77±0.21</td>
<td>21.62±0.25</td>
</tr>
<tr>
<td>Karakul x Female</td>
<td>3.50±0.02</td>
<td>12.33±0.14</td>
<td>17.40±0.13</td>
<td>20.62±0.16</td>
<td>21.85±0.18</td>
</tr>
<tr>
<td>Sex X Year</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Male x Yr1</td>
<td>3.42±0.03</td>
<td>12.75±0.23</td>
<td>19.21±0.20</td>
<td>24.74±0.28</td>
<td>27.75±0.30</td>
</tr>
<tr>
<td>Male x Yr2</td>
<td>3.61±0.05</td>
<td>15.43±0.26</td>
<td>20.42±0.25</td>
<td>23.09±0.31</td>
<td>26.50±0.32</td>
</tr>
<tr>
<td>Male x Yr3</td>
<td>3.24±0.04</td>
<td>12.00±0.27</td>
<td>17.35±0.26</td>
<td>20.67±0.33</td>
<td>25.22±0.33</td>
</tr>
<tr>
<td>Male x Yr4</td>
<td>3.00±0.04</td>
<td>13.00±0.24</td>
<td>15.40±0.22</td>
<td>18.74±0.27</td>
<td>21.54±0.28</td>
</tr>
<tr>
<td>Male x Yr5</td>
<td>3.30±0.04</td>
<td>11.77±0.23</td>
<td>17.42±0.23</td>
<td>20.88±0.31</td>
<td>20.01±0.43</td>
</tr>
<tr>
<td>Male x Yr6</td>
<td>3.26±0.05</td>
<td>13.17±0.28</td>
<td>16.54±0.25</td>
<td>17.84±0.31</td>
<td>20.56±0.32</td>
</tr>
<tr>
<td>Female x Yr1</td>
<td>3.21±0.04</td>
<td>12.12±0.20</td>
<td>17.90±0.18</td>
<td>21.63±0.23</td>
<td>24.45±0.23</td>
</tr>
<tr>
<td>Female x Yr2</td>
<td>3.40±0.04</td>
<td>15.00±0.22</td>
<td>19.16±0.21</td>
<td>21.74±0.26</td>
<td>22.13±0.21</td>
</tr>
<tr>
<td>Female x Yr3</td>
<td>3.02±0.04</td>
<td>11.40±0.24</td>
<td>16.82±0.22</td>
<td>21.28±0.28</td>
<td>24.57±0.29</td>
</tr>
<tr>
<td>Female x Yr4</td>
<td>3.30±0.04</td>
<td>13.07±0.20</td>
<td>15.08±0.18</td>
<td>17.53±0.22</td>
<td>21.06±0.22</td>
</tr>
<tr>
<td>Female x Yr5</td>
<td>3.10±0.03</td>
<td>11.00±0.20</td>
<td>17.00±0.21</td>
<td>20.77±0.25</td>
<td>20.31±0.31</td>
</tr>
<tr>
<td>Female x Yr6</td>
<td>3.20±0.05</td>
<td>12.64±0.26</td>
<td>16.22±0.24</td>
<td>17.08±0.30</td>
<td>20.60±0.31</td>
</tr>
<tr>
<td>Breed x Year</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Marwari x Yr1</td>
<td>2.84±0.03</td>
<td>12.65±0.20</td>
<td>17.31±0.16</td>
<td>22.85±0.24</td>
<td>25.83±0.25</td>
</tr>
<tr>
<td>Marwari x Yr2</td>
<td>3.16±0.04</td>
<td>15.65±0.21</td>
<td>19.23±0.22</td>
<td>22.82±0.27</td>
<td>24.33±0.27</td>
</tr>
<tr>
<td>Marwari x Yr3</td>
<td>3.00±0.04</td>
<td>12.00±0.21</td>
<td>17.08±0.20</td>
<td>20.12±0.24</td>
<td>24.40±0.24</td>
</tr>
<tr>
<td>Marwari x Yr4</td>
<td>3.00±0.04</td>
<td>14.06±0.20</td>
<td>16.43±0.17</td>
<td>19.20±0.21</td>
<td>21.87±0.21</td>
</tr>
<tr>
<td>Marwari x Yr5</td>
<td>3.03±0.03</td>
<td>12.65±0.17</td>
<td>15.58±0.15</td>
<td>18.62±0.19</td>
<td>22.26±0.20</td>
</tr>
</tbody>
</table>

Note: figures in parenthesis indicate number of observations. * = (P≤0.05), ** = (P≤0.01). Means with superscripts within effect differ significantly from one another.
weights was also significant ($P < 0.01$) whereas, regression of 3 months body weights on 6, 9 and 12 months weights were highly ($P < 0.01$) significant.

Heritability estimates for birth, 3, 6, 9 and 12 months weight were 0.273±0.099, 0.129±0.083, 0.187±0.090, 0.207±0.092 and 0.075±0.077, respectively. Phenotypic correlations of birth weight with 3, 6, 9 and 12 months of body weight were positive. The positive correlation suggested those animals weighing heavier at birth are likely to be heavier at subsequent ages. The genetic correlation of birth weight with 3, 6 and 12 months were positive. There was positive genetic correlation of 3 months body weight with 6, 9 and 12 months body weight. The phenotypic correlation of 3 months body weight was positive and high with 6, 9 and 12 months of body weight. The genetic correlation of 6 months body weight was observed to be positive with 9 and 12 months body weight. The genetic correlation of 6 months body weight was also observed to be positive and high with 9 and 12 months body weight. The positive and high phenotypic and genetic correlation was observed for 9 months body weight with 12 months body weight. The positive and higher phenotypic correlation of weaning weight with weights at subsequent ages suggest that selection may be practiced at early age. It was concluded that Marwari breed have higher adult body weight in comparison to Karakul breed.

**References**

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**Table 2: Heritability’s, phenotypic and genetic correlations among body weights**

<table>
<thead>
<tr>
<th>Traits</th>
<th>Birth weight</th>
<th>Three months weight</th>
<th>Six months weight</th>
<th>Nine months weight</th>
<th>Twelve months weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>0.273±0.099</td>
<td>0.269±0.356</td>
<td>0.015±0.290</td>
<td>-0.137±0.319</td>
<td>0.215±0.361</td>
</tr>
<tr>
<td>Three months weight</td>
<td>0.243</td>
<td>0.129±0.083</td>
<td>0.732±0.156</td>
<td>0.803±0.169</td>
<td>0.474±0.319</td>
</tr>
<tr>
<td>Six months weight</td>
<td>0.197</td>
<td>0.747</td>
<td>0.187±0.090</td>
<td>0.735±0.122</td>
<td>0.225±0.311</td>
</tr>
<tr>
<td>Nine months weight</td>
<td>0.165</td>
<td>0.683</td>
<td>0.814</td>
<td>0.207±0.092</td>
<td>0.627±0.206</td>
</tr>
<tr>
<td>Twelve months weight</td>
<td>0.265</td>
<td>0.681</td>
<td>0.649</td>
<td>0.788</td>
<td>0.075±0.077</td>
</tr>
</tbody>
</table>

*Note: Upper triangle present genetic correlation; Lower triangle present phenotypic correlation; Diagonal present heritability*
GENETIC, PHENOTYPIC AND ENVIROMENTAL TRENDS OF GROWTH AND WOOL TRAITS IN MARWARI SHEEP*1

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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT

The data for the present investigation were analyzed from records (1996-2010) of Marwari sheep research flock maintained with CSWRI, ARC, Bikaner. The phenotypic and environmental trends during over all period (1996-2010) were found positive for all growth and wool traits except greasy fleece yield of first clip, no genetic trends were found for 3 months and 6 months body weight, while positive for other traits (viz. body weight at 12 months of age as 0.09 ± 0.028 kg/year and for greasy fleece yield of first clip as 0.33 ± 0.823 gm/year) except GFY of second clip as -12.01 ± 1.416 gm/year. The reasons for the same were not identifiable at first instance but it is likely that considering selection effects in the model and elimination of some sire effect and period effect confounding/interaction these estimates could be obtained towards true values.

Key words: Marwari sheep, growth, greasy fleece yield (GFY), trend

Introduction

Sheep rearing is major source of livelihood for small and marginal farmers in arid and semi arid areas of Rajasthan. Growth performance and greasy fleece yield of Indian sheep requires immediate attention to meet out ever increasing demand of human population. Marwari is an important sheep breed of hot arid zone of Rajasthan, as it produces medium and coarse quality carpet wool and mutton. Animal breeders are primarily concerned with the genetic improvement of the animal by making suitable selection and breeding policies and their implementation. For a breeding programme, it is pre-requisite to know about the changes occurring in a population to maximize genetic gain. An accurate and early appraisal is essential to study the increase in the genetic and phenotypic merit of individual in the population. Hence, the present study would be aimed to estimating genetic, phenotypic and environmental trends in various economic traits in Marwari sheep.

Materials and Methods

The data for present investigation were obtained from the records of Marwari lambs maintained at the Arid Region Campus of the Central Sheep and Wool Research Institute, Bikaner. The data spread over a period of years 1996 to 2010. Records of 4012 Marwari lambs for 3 months body weight, 3519 records for 6 months body weight and greasy fleece yield clip I and 2315 records for 12 months body weight and greasy fleece yield clip II were taken. Wool traits were classified in to 2 seasons on basis of recording of data on wool production was done at 6 months March-April (clip I) and 12 months Nov-Dec (clip II).

The phenotypic, genetic and environmental trends were estimated by the methods developed by Smith (1962). The phenotypic trend was estimated as the regression of population performance on time. To eliminate the effect of year to year fluctuations in the environment, each record was expressed as deviation from the contemporaries. These records were then used to obtain the genetic trends which were computed as twice the pooled intra sire regression of the performance of the sire progeny on time (–2b(P-S)T). The environmental trend was computed as the difference between the phenotypic and the genetic trends, i.e. (bPT–2(bPT-bST)). Standard errors were estimated for all the trends by using square roots of the variance of the genetic gain.

Results and Discussion

Phenotypic trends: Estimates of phenotypic trends were found positive for all growth traits as 0.16 ± 0.010, 0.34 ± 0.014 and 0.54 ± 0.019 for 3, 6 and 12 months body weight (kg), respectively and for GFY of second clip (g) as 3.13 ± 1.094, which indicate overall improvement in these traits. While estimate for GFY of first clip was negative as -7.96 ± 0.657 (g). Similar results were found by Joshi (2001) in Marwari sheep, Murdia et al. (2003) and Parihar (2012) in Magra sheep, while negative phenotypic trends were found by Dey and Poonia (2006) in Nali flock.

Genetic trends: Positive genetic trends were found for 12 months body weight (kg) and GFY of first clip (gm) as 0.09 ± 0.028 and 0.33 ± 0.823, respectively, no genetic trends were found for 3 and 6 months body weight (kg) as 0.00 ± 0.013 and 0.00 ± 0.019, respectively. Estimate was found to be negative for GFY of second clip as -12.01 ± 1.416. The reasons for the same were not identifiable at first instance but it is likely that considering selection effects in the model and elimination of some sire effect and period effect confounding/interaction these estimates could be obtained towards true values. Positive genetic trends were observed by Mohammadi et al. (2011) in Zandi sheep and Reddy and Naidu (2011) in Nellore sheep.

Environmental trends: Estimates of environmental trends were found positive for all growth traits (body weight at 3, 6 and 12 months of age) and GFY of second clip as 0.16 ± 0.017, 0.34 ± 0.023, 0.45 ± 0.033 and 15.14 ± 1.789, respectively.

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Which indicate proper and healthy management throughout the period, while estimate for GFY of first clip was negative as \(-8.29 \pm 1.053\) (g). Similar results found by Joshi (2001) in Marwari sheep and Parihar (2012) in Magra sheep, while negative environmental trends were observed by Arora et al. (2010) in Malpura sheep.

Acknowledgements
Authors are thankful to the Director, CSWRI; Head, ARC, CSWRI; Vice-chancellor, RAJUVAS and Dean, CVAS; for providing the facilities for the execution of work. The technical help rendered by Mr. Vimal Mehrotra T-7-8 at ARC, CSWRI, Bikaner, during data collection is deeply acknowledged.

Table 1: Estimates of genetic, phenotypic and environmental trends (±S.E.) in different traits during over all period

<table>
<thead>
<tr>
<th>YEAR</th>
<th>1996-2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAIT</td>
<td>Genetic</td>
</tr>
<tr>
<td>3 months weight</td>
<td>0.00 ± 0.013</td>
</tr>
<tr>
<td>6 months weight</td>
<td>0.00 ± 0.019</td>
</tr>
<tr>
<td>12 months weight</td>
<td>0.09 ± 0.028</td>
</tr>
<tr>
<td>Clip 1</td>
<td>0.33 ± 0.823</td>
</tr>
<tr>
<td>Clip 2</td>
<td>-12.01 ± 1.416</td>
</tr>
</tbody>
</table>

References
GENETIC AND NON-GENETIC FACTORS AFFECTING SOME REPRODUCTION TRAITS IN THARPARKAR CATTLE

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ABSTRACT

A total of 1385 lactation records of Tharparkar cattle collected from LRS Chandan, Jaisalmer spreading over a period of 33 years were utilized to assess the effects of period and season of birth/calving on age at first calving, calving interval and service period and also studied effect of parity on calving interval and service period. The least-squares means were 1821.86 ± 37.022 days, 414.16 ± 5.314 days and 122.04 ± 4.264 days, respectively for the above mentioned traits. A mixed model least-squares analysis showed significant effect of sire and period of calving on all the traits. Effect of parity was significant on all traits. Season of calving did not affect all reproductive traits.

Key words: Age at first calving, calving interval, service period, Tharparkar cattle

Introduction
The success of dairy farming largely depends on the level of production and reproduction traits of the animals. There are several genetic and non-genetic factors that influence the phenotypic expression of these traits. In the absence of reliable information regarding these traits, it becomes difficult to estimate genetic parameters of the traits that provide the base to determine the optimum criterion of selection for improving overall performance of the animals. Therefore, this study was carried out to assess the influence of important genetic and non-genetic factors like period of calving, season of calving and parity on reproduction traits in Tharparkar cattle.

Materials and Methods
The data pertaining to various production and reproduction traits of 1385 lactation record by 29 sires, collected from LRS Chandan, Jaisalmer were utilized in the present study. The performance records were distributed over a period of 33 years (1978-2011). The traits under study age at first calving (AFC), calving interval (CI), service period (SP). The entire duration was divided into 4 periods, the specific period were classified as follow P1 (1978-1990), P2 (1991-1997), P3 (1998-2004), P4 (2005-2011), respectively. Further, each calendar year was sub-divided into 3 seasons viz., winter (November to February), summer (March to June) and rainy (July to October). Reproduction performance was studied parity-wise the data were divided in to first, second, third, fourth, fifth, sixth seven, eighth and higher parity were grouped together in eight parity this was so done because there were very few cows completing more than eight lactations. Least-squares analysis (Harvey, 1990) was applied to examine the effect of genetic and non-genetic factors on various reproduction and production traits. The model employed to examine the effect of genetic and non-genetic factors on various reproduction and production traits. Model 2 from Harvey (1990) was used for different traits as follows:

Mathematical model for analysis of age at first calving

\[ Y_{ijklm} = \mu + s_i + B_j + C_k + e_{ijklm} \]

Where,

- \( \mu \) = Overall mean
- \( s_i \) = Random effect attributed to \( i^{th} \) sire
- \( B_j \) = Fixed effect of \( j^{th} \) period of birth (\( j = 1, 2, 3, 4 \))
- \( C_k \) = Fixed effect of \( k^{th} \) season of birth (\( k = 1, 2, 3 \))
- \( e_{ijklm} \) = Residual random error \( i.i.d. \n(0, \sigma^2_e) \)

Mathematical model for analysis of other production and reproduction traits

\[ Y_{ijkl} = \mu + s_i + A_i + B_j + C_k + b(AFC - AFC) + e_{ijkl} \]

Where,

- \( \mu \) = Overall mean
- \( s_i \) = Random effect attributed to \( i^{th} \) sire
- \( A_i \) = Fixed effect of \( i^{th} \) period of calving (\( i = 1, 2, 3, 4 \))
- \( B_j \) = Fixed effect of \( j^{th} \) season of calving (\( j = 1, 2, 3 \))
- \( C_k \) = Fixed effect of \( k^{th} \) parity, (\( k = 1, 2, 3 \))
- \( b(AFC - AFC) \) = The regression of the trait on age at first calving
- \( e_{ijkl} \) = Random error \( i.i.d. \n(0, \sigma^2_e) \)

Results and Discussion
The least-squares means for AFC, CI and SP were...
Factors affecting reproduction traits

Method of least-squares analysis was used to identify the various sources affecting reproduction traits. The sires were treated as a random effect. For age at first calving the period and season of birth were considered as fixed effects while, for other traits the fixed effects considered were period, season of calving, and parity. The age at first calving was used as a covariate in the statistical analysis. The least-squares analysis of means along with their standard errors of reproduction traits is presented in Tables 1.

Genetic factors

Effect of sire on reproduction traits

The least-squares analysis of variance revealed that the sire had a highly significant (≤0.01) influence on all the reproduction traits. Such observations were also reported by Gahlot (1990), Kachwaha (1993), Kalani (1995) and Chand (2011). The results suggested that sire is a significant source of causing variability in the reproduction traits.

Non-genetic factors

Effect of period

The effect of period of birth on age at first calving of Tharparkar heifer was highly significant (P≤0.01) with highest age at first calving in the first period and it was significantly lowest in third period than other periods. Difference in AFC in other three periods was non-significant in the present study. Similar result were also reported by, Vij et al. (1992b), Gahlot (1999) in Tharparkar cattle, Singh and Dubey (2005) in Sahiwal cattle and their crosses and Dhaware et al. (2008) in Khillar cattle.

The calving interval was shortest in second period and longest in first period, in the present study. Effect of period of calving was highly significant (P<0.01) and it was in conformity with the reports of Pannerselvon et al. (1990), Vij et al. (1992b), Gahlot (1993), Yadav et al. (1994a), Singh et al. (1996), Gahlot (1999) and Chand (2011) in Tharparkar cattle, Parra-Bracamonte et al. (2005) in dual purpose cattle in Mexico and Singh and Dubey (2005) in Sahiwal and their crosses for first lactation. But results of present study was contradiction with Parekh and Sahu (1978), Basu et al. (1983) and Parmar et al. (1984) who reported non-significant effect of period in the Tharparkar breed of cattle.

Period of calving was found to be highly significant (P<0.01) in the present study with the longest service period in period first (P1) and smallest in period second (P2). Similar result were also observed by Bhatnagar et al. (1982b), Pannerselvan et al. (1990), Pareek (1991), Vij et al. (1992b), Kachwaha (1993) and Gahlot (1999) in Tharparkar cattle. Singh and Dubey (2005) in Sahiwal and their crosses. Contrary to these results, Basu et al. (1979) and Gahlot et al. (2002) in Tharparkar cattle and Dahiya (2002) in Hariana cattle reported that the effect of period of calving on service period was non-significant. Variation in different periods was probably due to climatic changes, availability of feed across the whole period and managemental practices in the particular period. When environmental and managemental conditions are favourable, reproductive cycle and growth as well as production of the animals will be good, and it is adversely affected if the conditions are unsuitable.

Effect of season of birth

Results of present study revealed that season of birth did not affect the age at first calving in the Tharparkar cattle. Similar reports were also given by Bhatnagar et al. (1982b), Pannerselvan et al. (1990) and Vij et al. (1992b), Gahlot (1999) and Chand (2011) in Tharparkar cattle. Singh and Dubey (2005) in Sahiwal cattle and its crosses and Dhaware et al. (2008) in Khillar cattle also found same result. Contrary to present study, Kachwaha (1993) and Rahumathulla et al. (1994) reported significant effect of season of birth in Tharparkar cattle.

Calving interval was not influenced by season and nearly the same in all the three seasons in the present study. This could probably be due to good management and good pasture availability. Similar results were also reported by Sharma et al. (1972), Basu et al. (1983), Parmar and Johar (1982a), Pannerselvon et al. (1990), Vij et al. (1992b), Singh et al. (1996) and Chand (2011) in Tharparkar cattle, Nehra (2004) in Rathi and Singh and Dubey (2005) in Sahiwal and their crosses for first lactation. Contrary to this, Kachwaha (1993), Rahumathulla et al. (1994), Yadav et al. (1994a), Gahlot (1999) and Hammoud et al. (2010) reported significant effect of season of calving on calving interval.

Service period was not significantly influenced by season of calving. Similar result were found by Pannerselvon et al. (1990), Gahlot et al. (2002) Chand (2011) in Tharparkar cattle. Singh and Dubey (2005) in Sahiwal cattle and their crosses for first lactation also found same trends. As the area of study is located in desert, where fodder availability over the year remains nearly uniform and scanty, which might have resulted in uniform service period. Practices in the farm also support less-variation in service period during the seasons. Whereas, Kachwaha (1993) in Tharparkar cattle and Nehra (2004) in Rathi cattle for pooled lactations found that season of calving significantly affect the service period.

Effect of parity

Analysis of variance revealed a highly significant influence of parity on calving interval. Calving interval decreased from first lactation to second and then remained almost constant. Kachwaha (1993), Yadav et al. (1994a), Singh et al. (1996) and Gahlot (1999) in Tharparkar cattle and Parra-Bracamonte et al. (2005) in dual-purpose cows in Mexico also obtained similar results and support the results of the present study. However, the results of Basu et al. (1983) and Parmar et al. (1984) did not show any effect of parity on successive calving in Tharparkar cattle.

Effect of parity was found to be highly significant (P<0.01) on the service period in the present study. Pareek (1991), Vij et al. (1992b), Kachwaha (1993), Gahlot (1999), Gahlot et al. (2002) and Chand (2011) also made similar observations and corroborate well with the findings of present study in Tharparkar cattle. While, Basu et al. (1979) and Bhatnagar et al. (1982b) in Tharparkar cattle and Nehra (2004) in Rathi cattle did not report significant effect of parity on service period.

Service period was longest in first parity thereafter it decreased up to fourth parity and then slight increase and remained almost constant. This might be due to maturity,
environmental conditions which might also have affected service period over the period in different parities.

**Effect of age at first calving**

The least-squares analysis of variance of data revealed that regression of reproduction traits on age at first calving were highly significant for calving interval (P ≤ 0.01). The regression coefficient was negative but low for calving interval and positive but almost zero for service period. This indicated that age at first calving did not have any effect with the reproduction of the cow except calving interval. This shows that higher age at first calving lowers the calving interval.

Bhatnagar et al. (1982b) and Rahumathulla et al. (1994) reported significant effect of age at first calving on service period and calving interval in Tharparkar cattle. Gahlot (1999) also reported significant effect for all reproductive traits in the same breed.

Whereas, reports of Panneerselvam et al. (1990), Kachwaha (1993), Yadav et al. (1994a) and Chand (2011) showed non-significant effect of age at first calving on service period and agree well with the present findings. Nehra (2004) recorded similar trends for all reproduction traits in Rathi cattle. It could be concluded from the study that the genetic and non-genetic factors (sire, period of calving, season of calving and age at first calving) play an important role in phenotypic expression of the traits under study in Tharparkar cattle.

**References**


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**Table 1: Least-squares means (± SE) of reproduction traits of Tharparkar cattle**

<table>
<thead>
<tr>
<th>Traits / factors</th>
<th>AFC (Days)</th>
<th>CI (Days)</th>
<th>SP (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over all mean (µ)</td>
<td>1821.86±37.022 (201)</td>
<td>414.16±5.314 (151)</td>
<td>122.04±4.264 (944)</td>
</tr>
<tr>
<td>Sire **</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Period **</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>P1</td>
<td>1931.38±73.120 (39)</td>
<td>437.93±9.716 (299)</td>
<td>152.89±8.956 (217)</td>
</tr>
<tr>
<td>P2</td>
<td>1880.72±59.617 (295)</td>
<td>393.65±6.907 (295)</td>
<td>100.51±5.685 (281)</td>
</tr>
<tr>
<td>P3</td>
<td>1630.30±75.942 (242)</td>
<td>412.66±7.793 (242)</td>
<td>120.36±6.781 (210)</td>
</tr>
<tr>
<td>P4</td>
<td>1845.04±81.926 (205)</td>
<td>412.41±9.149 (205)</td>
<td>114.42±8.112 (206)</td>
</tr>
<tr>
<td>Season NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>S1</td>
<td>1771.45±46.867 (86)</td>
<td>413.92±6.970 (415)</td>
<td>121.08±5.264 (334)</td>
</tr>
<tr>
<td>S2</td>
<td>1894.77±50.878 (375)</td>
<td>409.69±6.257 (375)</td>
<td>125.64±5.969 (325)</td>
</tr>
<tr>
<td>S3</td>
<td>1799.36±45.402 (248)</td>
<td>418.88±6.159 (248)</td>
<td>119.42±5.359 (285)</td>
</tr>
<tr>
<td>Parity **</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>L1</td>
<td>478.50±8.874 (179)</td>
<td>174.55±7.936 (145)</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>422.24±5.898 (173)</td>
<td>125.97±6.583 (151)</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>404.42±7.852 (151)</td>
<td>112.41±6.781 (134)</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>387.42±6.468 (126)</td>
<td>96.62±7.376 (116)</td>
<td></td>
</tr>
<tr>
<td>L5</td>
<td>400.61±6.810 (105)</td>
<td>115.59±7.810 (109)</td>
<td></td>
</tr>
<tr>
<td>L6</td>
<td>397.72±9.689 (85)</td>
<td>113.53±8.791 (94)</td>
<td></td>
</tr>
<tr>
<td>L7</td>
<td>398.96±9.090 (129)</td>
<td>114.53±7.800 (114)</td>
<td></td>
</tr>
<tr>
<td>L8</td>
<td>423.44±10.767 (93)</td>
<td>123.15±9.794 (81)</td>
<td></td>
</tr>
<tr>
<td>AFC</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>-0.02±0.009</td>
<td>0.01±0.009</td>
<td></td>
</tr>
</tbody>
</table>

No. of observations are given in parenthesis. Figures with different superscripts differ significantly **Highly significant (P<0.01), * Significant (P<0.05), NS Non-significant.
NUTRIENTS INTAKE AND DIGESTIBILITY IN ADULT INDIGENOUS DONKEYS IN ARID REGION OF RAJASTHAN

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ABSTRACT

Four large white indigenous donkeys weighing 216.5 kg were selected for studying the digestibility and nutrients intake by the donkeys. The animals were kept separately in individual stalls at Equine Production Campus, Bikaner. The feeding trial was conducted for one month with five days of digestibility trial. The animals were fed 3.6 kg (soaked) concentrate mixture (40% gram, 30% oats and 27% wheat bran from Hafed), green oats (2 kg) and sewanhay (4.5 kg). Feed intake and feed left was recorded on daily basis throughout the feeding trial while, feed given, feed left and faeces voided were recorded daily during the digestibility trial. The initial average body weight and final body weight was 216.5 kg and 217.25 kg, respectively. It was observed that the average DMI/100 kg body weight (kg), CPI (g), EEI (g), CFI (g), NFEI (g), DM Digestibility (%), CP Digestibility (%), EE Digestibility (%), CF Digestibility (%) and NFE Digestibility (%) were 2.34±0.04, 458.83±20.26, 89.83±2.96, 484.77±21.52, 549.47±24.09, 53.57±0.83, 59.03±1.99, 79.13±0.79, 56.38±2.13 and 87.09±0.62, respectively. During the trial there was slight increase in body weight and body condition which indicates that the feed given to the animals was sufficient enough to maintain their body weight and for breeding.

Key words: Donkey, maintenance, digestibility, ration, energy, feed-intake

Introduction

Donkeys play a pivotal role in supporting rural and urban transport and serve as an under utilized energy source in agriculture, the more so with the resource-poor farmers in India. Very little attention was paid to feeding requirements of donkeys and that they are often left to agricultural byproducts. Despite the negligence in their feeding, the donkeys graze on available low quality roughages and can maintain fairly good body condition. At present, there are no published feeding guidelines/standards specific to donkeys. But, the recommendations suggest feed 0.75 of horse energy needs per kg of body weight. Donkeys have greater digestive efficiency than horses and ponies and have actually been compared to small ruminants in their capabilities to digest and utilize fibre. Thus, feeding them on the horse’s recommendations results in excess energy and as a consequence they become obese. The aim of the study was to determine the dry matter intake and nutrient digestibility at maintenance by mature donkeys.

Materials and Methods

The experiment was carried out between the month of July, 2015 at Equine Production Campus (ICAR-NRCE), Jorbeer, Bikaner, Rajasthan, India. Four large white indigenous donkeys were chosen for studying the digestibility and nutrients intake by the donkeys. A feeding trial of 30 days period was conducted. The donkeys were housed separately with fixed cement manger for feeding and water trough for drinking water arrangement. The concentrate mixture (Table 1) was prepared and supplied by HAFED (Haryana State Co-operative Supply and Marketing Federation Limited) and composition of the mixture was oats 40%, gram 30%, wheat bran 27%, mineral mixture and salt 3%. The concentrates mixture was given at 9.00h, green oats provided at 13.00 h and Sewan hay (Lasiurus scindicus) at 15.00 h. The animals were fed with concentrate mixture (1.44 kg from Hafed), green oats (2 kg) and Sewan hay (4.5 kg) (Table 1). Potable fresh water was made available to the donkeys throughout the day. The experimental animals were weighed before starting and at the ending of the experiment (Table 2). A digestibility trial of 5 days collection period was conducted before finishing the trial. Feed samples, oats and faeces voided during 24 hours duration were properly collected, weighed and measured separately for individual donkeys. All the samples were further processed for proximate analysis AOAC (1995) and fibre analysis were done (Van Soest, 1963).

Results and Discussion

In the present experiment, dry matter intake per 100 kg body weight by the donkeys was 2.34 kg (Table 3). As per the recommendations for donkeys, adult donkeys of 200 kg body weight kept on forage-based diet may consume 2.5 kg of dry matter and may receive approximately 9.43 Mcal/d (NRC, 2007). Earlier reports of Pearson et al. (1992), Ramachandra et al. (1995) and Jagjiwan Ram et al. (2003) observed per cent DM intake in donkeys was 2.38, 1.96 and 4.14 kg/day, respectively. However, for the idle donkeys Jagjiwan Ram et al. (2013) reported per cent DM intake of 1.93 kg, whereas Pearson (2005) reported DMI of 1.9-2.5% of body weight. The DM intake in the present experiment is well accord with NRC, (2007), which is 2.34% of the body weight.

The digestibility co-efficient was 53.57 (DM), 59.03 (CP), 79.13 (EE), 56.38 (CF) and 87.09 (NFE) (Table 4) for the donkeys at maintenance. Digestion pattern of fibre in donkeys in present study is in agreement to earlier reports by Izraelly et al. (1989a), Tisserand et al. (1991), Pearson et al. (2001) and Cuddeford et al. (1995). The digestibility co-efficient observed at the present investigation is lower than
reported by Pagan (2013). This lower digestibility may also be
due to the fact that the donkeys in present study fetched more
DM (2.34% of b.wt), thus, the transit time in the gut was less
which may have reduced the digestibility (Frape, 2004). Many
scientists doubted donkeys efficiency in digesting fibre for being
monogastric animal, and thought only ruminants have the
ability to digest fibre. In the present study, dry matter digestibility
was better than horses (Bala et al., 2015b).

Crude protein requirements for the donkeys range from
3.8 to 7.4 per cent (Mueller et al., 1994). In the present study
protein intake through feed was 458.83 g and 59.03% crude
protein digestibility. Cuddedorf et al. (1995) reported an overall
mean CP digestibility of 52% with 67 part of lucerne. However,
Pearson et al. (1992) reported that CP digestibility at 67 and
33% combination of lucerne and oat straw diet was 66%.
Donkeys are normally maintained on forages with low protein
content. Field research by Smith and Wood (2008) has shown
that the donkey’s requirement for crude protein is approximately
40 g/100 kg body weight per day. The donkeys have evolved to
thrive on highly fibrous, poor-quality foodstuffs, and are grazers
as well as browsers. According to Burden (2011), they have
different nutrient requirements with significantly lower energy
and protein needs, when compared with horses. The donkey
is different from horse in its ability to thrive on highly fibrous
feeds (Burden et al., 2013). A donkey needs less feed than a
horse or pony of similar height and weight (David and Wood,
2008). Pearson et al. (2001) further reported very high CP
digestibility in donkeys fed with lucerne at ad libitum
during rest. However NRC (2007) stated that there is lack of information
on protein requirements for donkeys. Izraely et al. (1989a) fed
donkeys a low protein diet (3% CP) without loss in body weight
and reported that donkeys compensated lower protein in the
low quality diet by increasing its intake rate. They further stated
that, donkeys were able to recycle urea at the rate of 75.5%
of the entry rate and which was accomplished by decreasing
renal urea filtration rate, increasing re-absorption, increasing
nitrogenous waste retention there by allowing recycling nitrogen
in to the gut. Izraely et al. (1989b) also stated that irrespective of
diet, donkeys can maintain their body weight if they are not at
work.

In fact, the available literature suggested that the donkey
is most efficient animal to utilize fibre effectively (Wolter and
Velendia, 1970; Tisserand et al., 1991) among non-ruminants.
Van Soest (1994) compared the digestibility of cellulose and
hemicellulose in different species of non-ruminants and
reported that the donkey digested 54-58% hemicellulose and
39-48% cellulose and emerged best among the non-
ruminants. Donkeys have very high fibre digestibility and
caecum and colon are the main site of fibre digestion (Banerjee,
1998). Butterworth et al. (1987) also found fibre digestion by
donkeys to be less efficient than in ruminants. It appears,
therefore, that the energy digestibility of the donkey is achieved
by its capacity to utilize readily digestible food components
efficiently (soluble carbohydrates, proteins, etc.). These said
nutrients are absorbed in the small intestine before
fermentation takes place (Hintz et al., 1978) and thus, yield
more energy than in ruminants. Whether fed on lucerne hay or
wheat straw, the daily gain in digestible energy in the donkey
exceeds that of the Bedouin goat as a result of its consistently
high gross energy intake even when fed a high-fibre diet.

Mean CF digestibility (%) observed by idle large white
donkeys at maintenance was 56.20 (Jagiwan et al., 2012).
Fonnesbeck (1968) reports lucerne hay digestibility 54.2% for
crude fibre and 46.8% for ADF in donkeys. The donkey, even
when fed wheat straw (i.e. a very low quality agro-industrial by-
product), demonstrated 50.9% and 42.0% digestibility of NDF
and ADF, respectively (Izraely et al., 1989a). Donkeys fed fibrous
forage require 80 to 95 KJ digestible energy per kg body weight
per day (Wood et al., 2005), which is significantly lower than
the requirements for a similar-sized pony or cattle (Bala et al.,
2015b). The daily dry matter intake of the donkey is significantly
lower than that of a horse. Janis (1976) and Demment and
Van Soest (1985) described two possible different strategies
for meeting energy requirements for herbivores maintained
on a diet high in fibre and low in soluble carbohydrates and
protein. Ruminants accomplish this by retaining the digesta
for a longer period of time in the gut, where as equines increase
their feed intake with no change in the digesta retention time.
Equines have a shorter digesta retention time than ruminants
regardless of diet (Hoppe, 1984).

In the present experiment, along with sewan (Lassarius
sindicus) hay (straw and green oats, concentrate mixture was
supplemented to the donkeys. Donkeys being monogastric
animal, have fairly better ability to digest fibre. They allow coarse
feed residues of low digestibility to pass rapidly through the
gastrointestinal tract, while passage of the smaller more easily
digestible particles is delayed (Bjornhag, 1989). Retentive
mechanisms have been described as the most important factor
determining intake and digestion of feed (Demment and Van
Soest, 1985).

References
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222-243, South Africa: The Science Press.
Table 1: Proximate composition of the feed offered to the donkeys under trial

<table>
<thead>
<tr>
<th>Feed</th>
<th>Concentrate mixture</th>
<th>Sewa hay</th>
<th>Oats green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>40.02±0.18</td>
<td>92.92±0.21</td>
<td>17.22±0.59</td>
</tr>
<tr>
<td>Crude protein (%) DM</td>
<td>15.44±0.26</td>
<td>4.61±0.17</td>
<td>8.9±0.43</td>
</tr>
<tr>
<td>Ether extract (%) DM</td>
<td>2.51±0.17</td>
<td>0.75±0.11</td>
<td>2.5±0.18</td>
</tr>
<tr>
<td>Crude fibre (%) DM</td>
<td>9.87±0.33</td>
<td>35.3±1.08</td>
<td>33.1±2.37</td>
</tr>
<tr>
<td>NFE(%) of DM</td>
<td>68.75±1.87</td>
<td>51.53±0.93</td>
<td>46.16±1.02</td>
</tr>
</tbody>
</table>

Table 2: Initial and final body weight of the Donkeys under trial.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Animal number</th>
<th>Initial b.wt</th>
<th>Final b.wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>222</td>
<td>220</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>214</td>
<td>215</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>210</td>
<td>212</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>220</td>
<td>222</td>
</tr>
<tr>
<td>Average body weight</td>
<td>216.5</td>
<td>217.25</td>
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</tr>
</tbody>
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Table 3: Nutrients intake by the donkeys

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake (kg)/% body weight</td>
<td>2.34±0.04</td>
</tr>
<tr>
<td>Crude protein intake (g)</td>
<td>458.83±20.26</td>
</tr>
<tr>
<td>Ether extract intake (g)</td>
<td>89.83±2.96</td>
</tr>
<tr>
<td>Crude fibre intake (g)</td>
<td>484.77±21.52</td>
</tr>
<tr>
<td>Nitrogen free extract intake (g)</td>
<td>549.47±24.09</td>
</tr>
</tbody>
</table>

Table 4: Digestibility of the nutrients in donkeys

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Digestibility co-efficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>53.57±0.83</td>
</tr>
<tr>
<td>Crude protein</td>
<td>59.03±1.99</td>
</tr>
<tr>
<td>Ether extracts</td>
<td>79.13±0.79</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>56.38±2.13</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>87.09±0.62</td>
</tr>
</tbody>
</table>
NUTRIENT BALANCE AND ECONOMICS OF YOUNG CALVES RAISED ON LACTOBACILLI SUPPLEMENTED MILK REPLACER

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Palampur-176 062, Himachal Pradesh, India

ABSTRACT
Eighteen, day-old Jersey calves divided into three groups viz. T₁, T₂ and T₃ which were fed on milk and milk replacer up to three months of age. Group T₁ was standard (control), however group T₂ and T₃ were supplemented with *L. acidophilus* (leopard excreta) and *L. plantarum* (carrot) @ 6.8 x 10⁶ cells/litre of milk, respectively. The retained calcium (g/head/day) significantly (P<0.05) higher in T₁ (14.39 ± 0.43) and T₃ (14.28 ± 0.46) as compared to control (T₂, 13.00 ± 0.48). The retained calcium (% basis) was 56.60 ± 1.78, 72.98 ± 1.40 and 72.58 ± 1.34 in T₁, T₂ and T₃ treatments, respectively. The retained phosphorus (g/head/day) was non-significant under different groups (5.98 ± 0.33, 7.25 ± 0.41 and 6.76 ± 0.43) in T₁, T₂ and T₃ treatments, respectively. The retained phosphorus (% basis) was 56.07 ± 1.86, 64.79 ± 2.05 and 61.97 ± 2.73 in T₁, T₂ and T₃ treatments, respectively. The retention of nitrogen (g/head/day) was 20.48 ± 1.02, 23.04 ± 0.32 and 23.81 ± 0.68 in T₁, T₂ and T₃ treatments, respectively. It was significantly (P<0.05) higher in T₂ and T₃ as compared to T₁. The differences in T₂ and T₃ treatments were non-significant. As far as economic is concerned of three months old calves under different treatments, the feed cost per kg of body weight gain was 129.63 ± 1.28, 87.17 ± 1.49 and 93.97 ± 1.33 in T₁, T₂ and T₃ treatments, respectively. While comparing with standard (control T₁), treatment T₂ and T₃ had 32.18 and 27.50 per cent lower cost of feed per kg gain in body weight, respectively. Result revealed that supplementation of probiotics significantly influence the balance of nutrients viz. calcium, phosphorus, nitrogen and also reduce the feed cost in per kg body weight gain of three months old Jersey calves.

Key words: Milk replacer, *L. acidophilus*, leopard excreta, *L. plantarum*, carrot

Introduction
The development of the rumen of new born calves is largely influenced by the consumption of dry feed and the products of its digestion in the rumen. Early introduction of dry feed and its intake by new born dairy calves are beneficial to their health and reduce rearing cost. It is highly desirable to obtain rapid development of ruminal function in the calf (Morrill, 1984) as prolonged milk feeding was found to delay the asset of the typical ruminal microflora (Langemann and Allen,1959). Calves can be raised successfully even at substantially reduced milk feeding provided that care must be taken in selecting the ingredients for milk replacer formulation (Razdan et al.,1965; Nagine et al., 1969; Dave et al., 1971). The lactobacillus population in rumen was directly affected by diet (Frizzo et al., 2010; 2011). The interactions between nutrition and microbial flora are complex. Bacteria in gut may effect digestion, absorption and the products of bacterial metabolism may provide nutrient or affect the health of the calves. Hence feeding of probiotics to calves will improve their health and dry matter intake at their early stage of life. Probiotics are mostly lactic acid bacteria which are indigenous (natural inhabitants) of the body. During the last decade, the intestinal microbial flora balance has progressively been recognized as one of the main factor to be manipulated in order to obtain the best growth performance in dairy calves. These microbial flora represent an ecological system which is essential to animal health. So lactobacilli (probiotics) are incorporated into diet with the purpose to improve animal health and its overall performance. The positive effect of probiotics (*L. acidophilus*) was observed (Mudgal and Bhagal, 2010) in pre-ruminant buffalo calves and it diminishes with age due to development of rumen.

Dietary effect of different feed supplements on anatomical (Tomat et al., 1962; McGavinnd Morrill,1976; and physiological; Sutton et al., 1963; Young et al., 1965) development of the rumen are reviewed by various scientists. However, Studies on ruminal microbial development in calves especially as influenced by lactobacilli supplementation with milk replacer are limited. Thus, recent research has lead to development of an early weaning programme involving the use of a pre starter ration fortified with lactobacilli to stimulate dry feed consumption in calves and our objective was to compare the ruminal microbial development of new born calves raised under early weaning or conventional weaning programme when fed the milk replacer along with different lactobacilli strains. Milk replacer feeding has been developed as a cost effective way of feeding calves instead of using whole milk which can be spared for human consumption.

Materials and Methods
Eighteen, day-old Jersey calves were selected and randomly distributed into three equal groups. Group T₁, T₂ and
The phosphorus balance in three months old female calves is found to be positive in lactobacillus supplemented groups (T<sub>2</sub> and T<sub>3</sub>) as compared to control (T<sub>1</sub>). Lactobacillus acidophilus supplemented group shown slightly higher retained phosphorus than Lactobacillus plantarum supplemented group. The result of present investigation is in accordance with the findings of Zhang et al. (2015) reported that apparent digestibility of total phosphorus was significantly greater in probiotics fed 8 weeks old holisten calves. Tripathi (2002) and Bhupal (1999) who reported higher retention of phosphorus with the supplementation of combination of microbes.

Nitrogen balance in three months old female calves is found to be positive in lactobacillus supplemented groups (T<sub>2</sub> and T<sub>3</sub>) as compared to control (milk with milk replacer, T<sub>1</sub>). The nitrogen balance in three months old female calves was almost same in all treatments (32.12 ± 0.70, 33.08 ± 0.50 and 33.75 ± 0.77) in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. The nitrogen intake (g/head/day) in microbial fed groups viz. T<sub>2</sub> and T<sub>3</sub> was significantly (P<0.05) higher in T<sub>3</sub> (30.62 ± 1.80 in T<sub>1</sub>) as compared to T<sub>2</sub> (6.96 ± 0.49) and T<sub>3</sub> (6.77 ± 0.45). The excretion of nitrogen through urine (g/head/day) was non-significant (3.32 ± 0.35, 3.08 ± 0.41 and 3.17 ± 0.48) in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> treatments, respectively. However, the excretion of nitrogen through faeces in 3 months old calves (% basis) was 25.87 ± 1.23, 20.97 ± 1.31 and 19.88 ± 1.25 in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> treatments, respectively. It was significantly (P<0.05) higher in T<sub>1</sub> as compared to T<sub>2</sub> and T<sub>3</sub>. The excretion of nitrogen through urine (% basis) was non- significant (10.38 ± 1.19, 9.31 ± 1.20 and 9.36 ± 1.34) in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> treatments, respectively. The retention of nitrogen (g/head/day) was 20.98 ± 1.02, 23.04 ± 0.32 and 23.81 ± 0.68 in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> treatments, respectively. It was significantly (P<0.05) higher in T<sub>2</sub> and T<sub>3</sub> as compared to T<sub>1</sub>. The differences in T<sub>2</sub> and T<sub>3</sub> treatments were non-significant. Similar pattern was followed in retention of nitrogen (% basis) in three months old calves. Moreover, result revealed that the balance of nutrients viz. calcium, phosphorus and nitrogen in three months old female calves was significantly (P<0.05) higher in microbial fed viz. L. acidophilus and L. plantarum groups.

The nitrogen balance in three months old female calves is found to be positive in lactobacillus supplemented groups (T<sub>2</sub> and T<sub>3</sub>) as compared to control (milk with milk replacer, T<sub>1</sub>). All microbial supplemented treatments support better maintenance of calves. The excretion of nitrogen through faeces is significantly (P<0.05) higher in control group where as the differences in excretion of nitrogen through urine are non-significant in all the treatments including control. The result of present investigation is in accordance with the findings of Tripathi et al. (2015) reported that the apparent digestibility of crude protein was significantly (P<0.05) higher at 8 weeks old holisten calves fed on oral probiotics.

The average gain in body weight was 17.58 ± 0.62, 26.17 ± 1.40 and 24.25 ± 1.09 kg in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. When it was compared with T<sub>1</sub>, it was significantly (P<0.05) higher in T<sub>2</sub> and T<sub>3</sub>. The differences in T<sub>2</sub> and T<sub>3</sub> treatment were non-significant. The gain/day (g) was 195.37 ± 6.92, 290.74 ± 15.56 and 269.44 ± 12.07 in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. It was significantly (P<0.05) higher...
in \( T_2 \) as compared to \( T_1 \) and \( T_3 \). Feed cost per kg of body weight gain was significantly lower in microbial fed calves under treatment \( T_2 \) (\( L. \) acidophilus) and \( T_3 \) (\( L. \) plantarum) as compared to standard control. While comparing with standard control (\( T_1 \), treatment \( T_2 \) and \( T_3 \) had 32.18 and 27.50 per cent lower cost of feed per kg gain in body weight, respectively. Economics of rearing of day-old female calves upto three months of age under different treatments have been presented in Table 4, respectively. The result of present study is positively correlated with Zhang et al. (2015) observed that Holeisten calves fed on oral administration of probiotics showed that FCR was improved in \( L. \) acidophilus-fed group similarly Devchand et al. (2013) match the result in the cost of feeding per kg body weight gain in Mehasana buffalo calves was lower in probiotics supplemented group than control. Jatkauskas and Vrontiakienne (2010) reported that the body weight and daily weight gain were significantly improved and average feed conversion rate was improved by 12.9% in the probiotic fed calves. Tripathi (2002), who reported that cost/kg body weight gain is lowest in \( S. \) cerevisiae (yeast) as compared to control. The other findings supporting the present investigation was significantly (\( P<0.05 \)) higher in \( T_2 \) and \( T_3 \) as compared to \( T_1 \). The overall results revealed that the calves fed \( L. \) acidophilus \( (T_2) \) and \( L. \) plantarum \( (T_3) \) have shown significantly (\( P<0.05 \)) higher body weight gain, improved dry matter intake along with better FCR as compared to milk replacer fed control group \( (T_1) \). These results are in agreement with the findings of Bhumal (1999) who reported significantly (\( P<0.05 \)) higher DMI (kg) in lactobacillus fed groups as compared to control. The total cost of feeding up to 90 days of age was Rs. 2278.82, 2281.13 and 2278.82 in \( T_1 \), \( T_2 \) and \( T_3 \) treatments, respectively. It was almost similar in all the treatments. The feed cost/kg body weight gain was 129.63 ± 1.28, 87.17 ± 1.49 and 93.97 ± 1.33 in \( T_1 \), \( T_2 \) and \( T_3 \) treatments, respectively. It was significantly (\( P<0.05 \)) higher reported by Abe et al. (1995) and Cruywagen et al. (1996) who reported that lactobacilli fed calves resulted in improved body weight gain. Wolter et al. (1987) also reported that combination of probiotics enhances growth performances. However, Choudhary et al. (2007) reported that no further improvement in the performance of male cross calves by supplementation of lactic acid producing bacteria. Frizzo et al. (2008) showed no significant effect on calf rearing due to the excellent health status of the animal. They recommended the advantages of using probiotics be more easily detected in the farms having high morbidity and mortality rates mainly produced by diarrhoea syndrome.

The result of the present experiment indicated that

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_1 )</td>
<td>( T_2 )</td>
<td>( T_3 )</td>
</tr>
<tr>
<td>Intake (g/head/day)</td>
<td>19.50±0.27</td>
<td>19.70±0.31</td>
<td>19.68±0.33</td>
</tr>
<tr>
<td>Excretion (g/head/day)</td>
<td>5.95±0.31</td>
<td>4.74±0.29</td>
<td>4.86±0.25</td>
</tr>
<tr>
<td>In Faces</td>
<td>0.54±0.04</td>
<td>0.58±0.03</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>Excretion (%)</td>
<td>30.62±1.80</td>
<td>24.10±1.52</td>
<td>24.78±1.41</td>
</tr>
<tr>
<td>In Faces</td>
<td>2.79±0.20</td>
<td>2.92±0.15</td>
<td>2.73±0.13</td>
</tr>
<tr>
<td>Retained (g/head/day)</td>
<td>13.00±0.48</td>
<td>14.39±0.43</td>
<td>14.28±0.46</td>
</tr>
<tr>
<td>Retained %</td>
<td>66.60±1.78</td>
<td>72.98±1.40</td>
<td>72.58±1.34</td>
</tr>
</tbody>
</table>
supplementation of *L. acidophilus* in the diet of calves have positive influence on nutrient balance viz. calcium, phosphorus and nitrogen retention and reduce the feed cost per kg body weight gain.

**References**


Nagine, O.P. et al. (1969) *Nutritional and physiological studies on cattle (Bovines) in relation to economics conference on intensive approach to animal production*, Bombay, Maharashtra, 12th to 15th may 1969.


**Table 4: Comparative economics of feeding of day old calf up to three months of age under different treatments**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T&lt;sub&gt;1&lt;/sub&gt;</th>
<th>T&lt;sub&gt;2&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. birth wt. (kg)</td>
<td>18.67 ± 0.67</td>
<td>19.33 ± 0.71</td>
<td>18.67 ± 0.49</td>
<td>NS</td>
</tr>
<tr>
<td>Av. wt. at three months of age (kg)</td>
<td>36.25 ± 1.27</td>
<td>45.50 ± 2.09</td>
<td>42.58 ± 1.49</td>
<td>NS</td>
</tr>
<tr>
<td>Metabolic body size (W&lt;sup&gt;0.75&lt;/sup&gt;)</td>
<td>14.79 ± 0.39</td>
<td>17.53 ± 0.60</td>
<td>16.66 ± 0.44</td>
<td>2.53</td>
</tr>
<tr>
<td>Gain in body wt. (kg)</td>
<td>17.58 ± 0.62</td>
<td>26.17 ± 1.40</td>
<td>24.25 ± 1.09</td>
<td>5.66</td>
</tr>
<tr>
<td>Av. daily body wt. gain (g/d)</td>
<td>195.37 ± 6.92</td>
<td>290.74 ± 15.56</td>
<td>269.44 ± 12.07</td>
<td>62.92</td>
</tr>
<tr>
<td>Milk fed (lit)</td>
<td>107.34</td>
<td>107.50</td>
<td>107.34</td>
<td>--</td>
</tr>
<tr>
<td>*Milk replacer fed (kg)</td>
<td>23.90</td>
<td>23.90</td>
<td>23.90</td>
<td>--</td>
</tr>
<tr>
<td>Cost of milk (Rs. 14.00/lit)</td>
<td>1502.76</td>
<td>1505.00</td>
<td>1502.76</td>
<td>--</td>
</tr>
<tr>
<td>Cost of milk replacer (Rs. 30.00/kg)</td>
<td>717.00</td>
<td>717.00</td>
<td>717.00</td>
<td>--</td>
</tr>
<tr>
<td>Cost of culture (Rs. 0.45/ml)</td>
<td>59.06</td>
<td>59.13</td>
<td>59.06</td>
<td>-</td>
</tr>
<tr>
<td>Total cost of calf feeding (Rs.)</td>
<td>2278.82</td>
<td>2281.13</td>
<td>2278.82</td>
<td>--</td>
</tr>
<tr>
<td>Feed cost/kg body wt. gain (Rs)</td>
<td>129.63 ± 1.28</td>
<td>87.17 ± 1.49</td>
<td>93.97 ± 1.33</td>
<td>16.35</td>
</tr>
</tbody>
</table>

*Milk replacer fed up to 90 days of age
EFFECT OF SHATAVARI SUPPLEMENTATION ON FAT PERCENTAGE AND FAT YIELD IN THE MILK OF KANKREJ COWS

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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT

The present study was conducted to investigate the effect of Asparagus racemosus (Shatavari), supplementation during postpartum period on production performance of lactating Kankrej cows. A total of 16 lactating Kankrej cows on the basis of their parity, milk yield and body weight were grouped in to 4 homogenous treatment groups of 4 each. Out of these, one group served as control fed as per NRC (1982) wherein no supplementation was given. Cows in treatment groups T1, T2 and T3, respectively, were subjected to shatavari supplementation @ 50, 100 and 150 g per day. During the supplementation period the fat % was found significantly higher (p<0.01) in T3 group than the control and T1 group however, there was no significant difference between control, T1 and T2 group. Also there was no significant difference between T2 and T3 group. The fat yield was significantly higher in T1, T2, T3 group than control but there was no significant difference between the different treatment groups. Therefore, it can be inferred that shatavari supplementation is beneficial and it could serve as potential management tool to improve fat percentage and fat yieldin milk in lactating cows.

Key words: Shatavari (Asparagus racemosus), fat percentage, fat yield, Kankrej cow

Introduction

India is the leading dairy nation of the world. Livestock rearing is the main source of livelihood for the small scale marginal farmers and landless farmers. Economy of rajasthan depend more on livestock rearing than agriculture due to arid climate and frequently occurring drought. Kankrej is the heaviest dual purpose breed of indian cattle adapted well to hot arid conditions and is a ideal dairy animal for arid regions due to heat resistance and disease resistance. Milk fat is the fatty portion of milk consisting largely of the glycerides of oleic, steric and palmitic acids. In india milk with high butter fat is preferred and price of milk in market is also decided according to fat content. So fat is an important parameter for the dairy economics. Herbal feeds are considered safe as they have no adverse side effects and residual effects on the health of the consumers. So in present scenario the use of herbal feed supplements have a great role in improving the quality of milk and economics of milk production.

Materials and Methods

Sixty healthy Kankrej cows were selected from the institute herd with similar body weight, parity and milk yield. All the animals were grouped into four similar groups control, T1, T2 and T3 groups with four animals in each group. All animals were kept in similar conditions in the loose housing system for the 60 days trial period. Cows in all groups were fed a control diet as per NRC (1982) wherein no supplementation was given. Cows in treatment groups T1, T2 and T3 groups were supplemented with shatavari during supplementation period @ 50, 100 and 150 g per day. The overall mean of fat yield was found to be 0.26 ± 0.19, 0.29 ± 0.12 and 0.32 ± 0.10 in T1, T2 and T3 groups, respectively. The statistical analysis of data revealed that the fat yield was significantly higher (p<0.01) in T3 group than the control and T1 group however, there was no significant difference between control, T1 and T2 group. Also there was no significant difference between T2 and T3 group.

Results and Discussion

Fat percentage

The overall mean of weekly fat % were found to be 4.44 ± 0.19, 4.58 ± 0.12 and 4.98 ±0.10 in T1, T2 and T3 groups, respectively and 4.16 ± 0.15 in control group. The fat % was 6.3, 6.7, 19.71 % higher in T1, T2 and T3 groups, respectively than control. The statistical analysis of data revealed that the fat % was found significantly higher (p<0.01) in T3 group than the control and T1 group however, there was no significant difference between control, T1 and T2 group. Also there was no significant difference between T2 and T3 group.

The results are similar to Sridhar and Bhagat (2007) who reported that Galactin Vet Bolus improved fat% in dairy cows. Similar results were also reported by Santosh (2009) who reported significant improvement in fat% of cows supplemented with shatavari during supplementation period and post supplementation period. However, the results are contradictory with the findings of Berhane (2000) and Berhane and Singh (2002) who observed no significant improvement in milk fat percent on shatavari supplemented in freshly calved cross bred cows.

Fat yield

The overall mean of fat yield was found to be 0.26 ± 0.19, 0.29 ± 0.01, 0.32 ± 0.32 in T1, T2 and T3 groups, respectively and 0.19 ± 0.01 in control group. The data revealed that the fat yield was significantly higher in T1, T2, T3 group than control but there was no significant difference between the different treatment groups.

References

### Table 1: Mean (±SE) of weekly milk fat percentage in different treatment groups

<table>
<thead>
<tr>
<th>WEEK</th>
<th>Milk Fat %</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.07 ± 0.21</td>
<td>4.1 ±0.28</td>
<td>4.27 ±0.25</td>
<td>4.5 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.2 ±0.38</td>
<td>4.52 ± 0.15</td>
<td>4.62 ±0.39</td>
<td>4.925 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.25 ±0.20</td>
<td>4.32±0.39</td>
<td>4.55 ±0.32</td>
<td>5.2 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.17± 0.33</td>
<td>4.62± 0.26</td>
<td>4.95 ± 0.48</td>
<td>5.12 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.97± 0.08</td>
<td>4.15± 0.48</td>
<td>4.05 ± 0.17</td>
<td>4.95 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.05 ± 0.29</td>
<td>4.7 ±0.38</td>
<td>4.77 ± 0.24</td>
<td>5.05 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.23 ±0.27</td>
<td>4.42 ±0.20</td>
<td>4.55 ± 0.05</td>
<td>5.17 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.37 ±0.16</td>
<td>4.72±0.04</td>
<td>4.97 ±0.08</td>
<td>5.05 -0.11</td>
<td></td>
</tr>
<tr>
<td>Mean ±SE</td>
<td>4.16±0.15</td>
<td>4.44±0.19</td>
<td>4.58±0.12</td>
<td>4.96 ±0.10</td>
<td></td>
</tr>
</tbody>
</table>

Note: means bearing different superscripts within a row differ significantly (p<0.01)


### Table 2: Mean (±SE) of weekly milk fat yield (kg/cow/day) in different treatment groups

<table>
<thead>
<tr>
<th>Week</th>
<th>Milk Fat Yield (kg/cow/day)</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.18 ±0.01</td>
<td>0.19 ±0.01</td>
<td>0.22 ± 0.01</td>
<td>0.24 ±0.01</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.18 ±0.01</td>
<td>0.24 ±0.01</td>
<td>0.27 ±0.03</td>
<td>0.29 ±0.01</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.19 ±0.01</td>
<td>0.25 ±0.02</td>
<td>0.29 ± 0.02</td>
<td>0.34 ±0.03</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.19 ±0.01</td>
<td>0.28 ±0.021</td>
<td>0.33 ± 0.03</td>
<td>0.33 ±0.02</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.18 ±0.01</td>
<td>0.26 ±0.03</td>
<td>0.27 ±0.01</td>
<td>0.32 ±0.02</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.18 ±0.01</td>
<td>0.29 ±0.03</td>
<td>0.32 ± 0.02</td>
<td>0.33 ±0.01</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.19 ±0.01</td>
<td>0.28 ±0.01</td>
<td>0.31 ±0.01</td>
<td>0.35 ±0.02</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.20 ±0.01</td>
<td>0.31 ±0.00</td>
<td>0.34 ± 0.01</td>
<td>0.34 ±0.00</td>
<td></td>
</tr>
<tr>
<td>Overall mean</td>
<td>0.19±0.01</td>
<td>0.26±0.01</td>
<td>0.29±0.01</td>
<td>0.32±0.02</td>
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</tr>
</tbody>
</table>

Note: means bearing different superscripts within a row differ significantly (p<0.01)

PROTEIN PARAMETERS IN MILK OF KANKREJ COW SUPPLEMENTED WITH HERB JIWANTI (LEPTADENIA RETICULATA)*

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ABSTRACT

An experiment was conducted to investigate the effect of Leptadenia reticulata (Jiwanti) supplementation during postpartum period on protein content of milk of lactating Kankrej cows. A total of 16 lactating kankrej cows on the basis of their milk yield, parity and body weight were grouped into 4 homogenous treatment groups of 4 each. Out of these, one group served as control wherein no supplementation was given. Cows in treatment groups T1, T2 and T3, were subjected to Jiwanti supplementation @ 50, 100 and 150 g per day, respectively along with control diet. During supplementation there was no significant (p<0.05) difference observed between T1, T2 and T3 as compared to control group in protein percentage and protein yield. In post supplementation period protein percentage was non significantly different in treatment groups and control while in protein yield there was a significant difference between T3, control and T3, T1. The statistical analysis reveals that jiwanti supplementation did not change protein percentage and yield in milk of Kankrej cattle during supplementation period but it exerts its effect on protein yield in post supplementation period. It means Jiwanti takes time for improvement in protein yield through improving milk yield and it has no detrimental effect on milk protein and beneficial in lactating kankrej cows.

Key words: Milk protein, herb supplement, jiwanti (leptadenia reticulata), Kankrej

Introduction

Galactogogue not only improves milk production but also solve the problem of letting down of milk in milch animals. Some herbal galactogogue also improve milk composition by increasing protein, lactose and fat percentage in milk. Realizing this a herbal galactogogue has been identified viz., Jiwanti (Leptadenia reticulata) which in turns may improve the productivity of indigenous cattle. Leptadenia reticulata belongs to family Asclepiadaceae commonly known as Jiwanti. The bark, leaves and whole plant can improve decreased milk flow in ruminants. The whole plant is also used to stimulant heat and prevents abortion (Chauhan et al., 2010).

Milk and in particular milk proteins not only supply to the body amino acids necessary for the maintenance and growth of body protein, but also give rise, during food digestion, to a myriad of protein fragments and large and small peptides that have distinct biological functions (Ward and German, 2004).

Keeping in view the paucity of research work on protein parameters of milk, Present study was aimed to study the effect of Jiwanti on Protein parameter of milk of Kankrej cow is proposed.

Materials and Methods

Necessary permission of Institutional animal ethics committee was taken before initiation of experiment. Present study was conducted on Kankrej cows maintained at livestock research station, Kodemdesar, College of veterinary and animal science, Rajasthan University of Veterinary and Animal Sciences, Bikaner from June to August 2014. The climate of the farm is hot desert climate with very little rainfall and extreme temperatures. In summer temperatures can exceed up to 45°C, and during the winter it may decline below freezing. All groups of the animals were reared under similar climatic conditions. A total of 16 cows were selected on the basis of their parity, body weight, and milk yield and were grouped in 4 homogenous groups of 4 each. Out of these, one group served as control wherein no supplementation was given. Cows in treatment groups T1, T2 and T3 were subjected to jiwanti supplementation @ 50 g, 100 g and 150 g per day, respectively.

In a 10 weeks experiment, 60 days of supplementation including first week as adaptation period and the last 10 days were used as post experimental period to determine post supplementation effect of herb. All the animals were fed as per NRC (1982) during supplementation period. During supplementation period cows were fed ad lib (at least 15% in excess of requirement) available green fodders (maize) and wheat straw with concentrates based on their body weight, maintenance and gestation requirements. Concentrates were fed independent of fodders and were fed in two installments during milking. Along with concentrate experimental feed were given according to Table 1.

During post supplementation period Jiwanti was not given and feed offered was the same as during supplementation.

Animals were kept loosen in an open paddock of Kodemdesar farm throughout the day and night except milking time. The experimental shelter was an individual tying system in a row during milking and feeding of experimental animals. Concentrate and experimental feed fed at milking time in separate manger. Milking was done in milking barn. Milking of cows was mainly done by hand milking by the milkers twice daily at 5:00 AM and 4:00 PM at milking parlour. The individual milk yields were recorded in kg at each milking by digital weighing machine.

*Part of M.V.Sc Thesis and corresponding author; 1Professor; 2Professor and Head, Dept. of Livestock Production and Management; 3Ph.D Scholar, Dept. of Biotechnology; 4M.V.Sc Scholar, Dept. of Animal Nutrition.
Table 1: Feeding Schedule

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplementation period</th>
<th>Post supplementation period</th>
<th>No. of cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Roughage and concentrate as per NRC (1982)</td>
<td>Roughage and concentrate as per NRC (1982)</td>
<td>4</td>
</tr>
<tr>
<td>T1</td>
<td>Control diet + Jiwanti @ 50 g/cow</td>
<td>Roughage and concentrate as per NRC (1982)</td>
<td>4</td>
</tr>
<tr>
<td>T2</td>
<td>Control diet + Jiwanti @ 100 g/cow</td>
<td>Roughage and concentrate as per NRC (1982)</td>
<td>4</td>
</tr>
<tr>
<td>T3</td>
<td>Control diet + Jiwanti @ 150 g/cow</td>
<td>Roughage and concentrate as per NRC (1982)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2: Mean (±SE) of milk protein yield (kg/cow/day) in different treatment groups

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.18 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>0.18 ± 0.37</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.18 ± 0.04</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.14 ± 0.00</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.14 ± 0.00</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.14 ± 0.00</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>0.15 ± 0.00</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.15 ± 0.00</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>8</td>
<td>0.15 ± 0.00</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.02</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.15 ± 0.00</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.02</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>P.S.</td>
<td>0.15 ± 0.00</td>
<td>0.15 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>

Note: Means bearing different superscripts within a row differ significantly (p<0.05); P.S. - Post Supplementation Mean ± SE

Table 3: Mean (±SE) of milk protein yield (kg/cow/day) in different treatment groups

<table>
<thead>
<tr>
<th>Week</th>
<th>Protein Yield (kg/cow/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>4</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>5</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>6</td>
<td>0.15 ± 0.00</td>
</tr>
<tr>
<td>7</td>
<td>0.15 ± 0.00</td>
</tr>
<tr>
<td>8</td>
<td>0.15 ± 0.00</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.15 ± 0.00</td>
</tr>
<tr>
<td>P.S.</td>
<td>0.15 ± 0.00</td>
</tr>
</tbody>
</table>

Note: Means bearing different superscripts within a row differ significantly (p<0.05); P.S. - Post Supplementation Mean ± SE

Protein parameters

(i) Weekly milk protein% - Milk protein% was calculated weekly for individual cow.

(ii) Weekly yield (kg) of milk protein (PRY) - The weekly yield of milk protein was calculated by multiplying milk yield (MY) with protein % and divided by 100.

PRY = MY x Protein %/100

Milk Protein were determined weekly by using automatic milk analyzing device i.e. MILKOTESTER (Model: MASTER LM2/LM2 P2) manufactured by Milkotester Pvt. Ltd., 49, Hristo Botev Street, 4470 BELOVO, Bulgaria.

Statistical analysis

Collected data was subjected to statistical analysis by adopting appropriate methods of analysis of variance as described by Snedecor and Cochran (2004). Wherever, the variance ratio (F-values) were found significant at 5 per cent and 1 per cent levels of probability, the significance of mean differences were tested by Duncan’s New Multiple Range Test (Duncan’s Range Test) as modified by Kramer (1957).

Results and Discussion

Data on protein percentage has been presented in Table 2. Data reveal that during supplementation period protein percentage in T1 group was non significantly higher than T2, T3 and control. Several factors like milk yield, seasonal variation and availability of rumen protected protein are reported to affect milk protein content. In post supplementation period protein percentage was non significantly higher in T3 followed by control, T2 and then T1. Data reveal that jiwanti supplementation does not change the protein percentage in milk. Similar results reported by Berhane (2000) and Berhane and Singh (2002) observed no change in milk protein per cent in shatavari supplemented crossbred cows. Mech et al. (2008) also found that protein % did not vary significantly in milk on use of herbal preparation.

The protein yield data has been presented in the Table 3. The statistical analysis of data revealed that during supplementation period, protein yield was higher in T1 (15.78% higher than control) followed by T2, T3 and control. In post supplementation period there was a significant difference between T3, control (T3 was 23.52% higher than control) and T2, T1 (T3 was 24.34% higher than control). Data reveal that jiwanti supplementation does not change protein yield significantly during supplementation but it exerts its effect on protein yield in post supplementation period. It means Jiwanti takes time for improvement in protein yield and it has no detrimental effect on milk protein.

References


NRC (1982) Nutrients requirements of ruminants in developing countries. 1st ed. International feedstuffs institute, Utah state university, UMC 46, Logan, Utah 84322 USA.


EFFECT OF FEEDING PROBIOTICS AND VIRGINIAMYCIN ON BODY WEIGHT GAIN OF BROILER CHICKS

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Department of Livestock Production and Management, College of Veterinary and Animal Science
Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT

A total of 270 day-old broiler chicks of Cobb strain were randomly distributed into six treatment groups of 45 chicks with three replicates having 15 chicks each. They were fed probiotics @ 1 g/10 kg and virginiamycin @ 1 g/kg alone, and in all possible combinations at the same dose rate as the feed supplement up to 42 days of age to evaluate their effect on body weight gain. The results obtained at sixth week of age revealed highest mean body weight in group T6 (1625.26 ± 46.98 g) followed by group T5, T2, T1, T3 and T4 groups having mean body weight of (1610.81 ± 47.33 g), (1608.86 ± 48.09 g), (1603.33 ± 49.30 g), (1584.57 ± 47.52 g) and (1551.36 ± 47.52 g), respectively. Mean body weight of all the six groups did not differ significantly among themselves but feed additives as growth promoter improved the body weight of chicks as compared to the control group. The maximum cumulative mean body weight gain was found in T6 group (1498.28 ± 45.70 g) and minimum cumulative mean body weight gain was observed in T1 (1421.87 ± 46.23 g). Intermediate body weight gain were found in T5 (1484.84 ± 46.25 g), T2 (1481.70 ± 46.79 g), T3 (1474.85 ± 47.96 g) and T4 (1458.50 ± 46.24 g). The differences in the cumulative mean body weight gain of all the groups were found to be non-significant. The findings of present study suggest that group of chicks fed virginiamycin (T5) attained numerically more body weight than control and other treatment groups.

Key words: Body weight, probiotics, virginiamycin, broilers, body weight gain

Introduction

The poultry industry is one of the most important contributors for the growth of rural economy in our country. Recent trend in poultry development have revealed that this industry has a tremendous potential in improving the nutritional status of million of people.

Profit margin in poultry is greatly affected by its cost of production. Nutrition is far the most important factor which plays a significant role in broiler production by sharing about 70 to 75 per cent of total cost production. Rising prices of feed has certainly reduced the profitable nature of broiler farming. Hence, economic poultry farming is the need of the hour. In this regard efforts have been made to minimize the input costs by the use of feed additives (Swain and Johri, 2000).

Feed additives are commonly described as non-nutrient substances that accelerate growth, efficiency of feed utilization, or beneficial for health or metabolism of the animals (Church and Pond, 1988). The additives that hold great promise in the feeding of poultry comprise of antibiotics, coccidiostats, antioxidants, enzymes, hormones, probiotics, buffers, organic acids, mould inhibitors, herbal products, synthetic micronutrients etc.

A probiotic, by the generally accepted definition, is defined as a live microbial supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotic is a culture of specific live micro-organisms primarily lactobacillus species. During the last few years the research workers have documented the results of incorporating probiotics into poultry diets and have found variable results.

Virginiamycin used as feed additive antibiotic, is non-therapeutic and not absorbed from gastro intestinal tract. This additive do not act directly on chicken, rather produce favourable effect on growth rate, feed efficiency and mortality by inhibiting undesirable micro-organisms in the digestive tract. Enhanced utilization of sulphur amino-acid, phosphorus and manganese has also been reported for birds consuming virginiamycin (Belay and Teeter, 1994). In poultry industry, a 3-5 per cent increase in growth and feed conversion efficiency is a typical effect of antibiotics used in feed at prophylactic levels (Thomke and Elwinger, 1998).

Hence, the present experiment has been designed to study the effect of supplementation of different feed additives in broiler ration on the performance of broiler chicks.

Materials and Methods

A total of 270 day-old Cobb strain were used for the experiment. These chicks were randomly divided in to six treatment groups of 45 chicks and each group was further divided in to three replicates having 15 chicks each. The chicks were reared under identical standard managemental practices like brooding, feeding, watering, lighting, health care etc. The birds were offered basal diet both starter ration for 0-3 weeks and finisher ration for 4-6 weeks. The first feeding group (T1) was kept as the control and no additive was supplemented to the starter and basal ration. The second group (T2) virginiamycin @ 1 g/kg feed (0.002 per cent); the third group (T3) probiotics @ 1 g/10 kg feed; the fourth group (T4) a combination of virginiamycin and probiotics was incorporated with ration at the same rates as in treatment groups alone; the fifth group (T5) virginiamycin 0-3 weeks and probiotics from 4 to 6 weeks; the sixth group (T6) probiotics 0 to 3 weeks and virginiamycin from 4 to 6 weeks. Feed and fresh water were offered ad libitum to each group throughout...
from the difference in body weight attained at the end and at the start of the period. The data were subjected to statistical analysis mixed model least square and maximum likelihood computer programme PC-I version of Harvey (1987).

**Results and Discussion**

The results of six weeks study have been presented in Tables 2 and 3. The mean body weight was maximum influenced by the treatment group T and followed by T, T, T, T, and finally in T group. The differences in mean body weight of chicks in different treatment group were found similar but feed additives as growth promoter improved the body weight of chicks as compared to the control group. Body weight gain revealed that the effect of various treatments was significant (P<0.05) in week II. During I, III, IV, V and VI weeks effect of treatment was insignificant. The maximum cumulative mean body weight gain was found in T group (1498.28 ± 45.70 g) and minimum cumulative mean body weight gain was observed in T (1421.87 ± 46.23 g). Intermediate body weight gain were found in T (1484.84 ± 46.25 g), T (1481.70 ± 46.79 g), T (1474.85 ± 47.96 g) and T (1458.50 ± 46.24 g). The overall effect of feed additives on cumulative weight gain (week I - VI) was also to be non significant. In the present study, mean body weight and body weight gain was highest in group T, but there was no significant difference from the control group (T). Similarly body weight and body weight gain improved non significantly in groups T, T and T as compared to the control group. Similarly Dash et al. (1990); Khan (1997) and Iafigliola et al. (2000); Ahmad (2011) recorded non significantly increased body weight gain of chicks fed vitaminamin in broiler ration. In the present study, the effect of probiotic on body weight and body weight gain was found to be non significant. However, the effect of probiotic on body weight and weight gain was more but not significant as compared to the control group (T). These findings are in accordance with Panda et al. (1999); Ladukar et al. (2001) and Hasan et al. (2007); Falaki et al. (2011) who observed no significant effect of probiotic on body weight.

**References**


Harvey, W.R. (1987) *User's guide for LSMLMW PC-1 version mixed model least square and maximum likelihood programme. IOWA, State University, IOWA, USA.*


---

### Table 1: Composition of basal ration fed to the chicks

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter ration (parts per 100)</th>
<th>Finisher ration (parts per 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Rice polish</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Soya DOC</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10</td>
<td>07</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>02</td>
<td>02</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Calculated composition:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>22.26</td>
<td>19.76</td>
</tr>
<tr>
<td>Energy (Kcal/Kg)</td>
<td>2868.71</td>
<td>2941.62</td>
</tr>
</tbody>
</table>

### Table 2: Least squares means with respective standard errors for body weight (g/chick) at different weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age in weeks</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>52.93±0.60</td>
<td>125.33</td>
<td>231.11</td>
<td>422.21</td>
<td>741.22</td>
<td>1135.18</td>
<td>1584.57</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>52.67±0.60</td>
<td>127.02</td>
<td>250.17</td>
<td>456.11</td>
<td>789.09</td>
<td>1192.76</td>
<td>1625.26</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>53.07±0.60</td>
<td>127.02</td>
<td>234.88</td>
<td>434.56</td>
<td>747.68</td>
<td>1142.38</td>
<td>1608.86</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>51.87±0.60</td>
<td>126.20</td>
<td>253.91</td>
<td>446.45</td>
<td>740.46</td>
<td>1150.86</td>
<td>1610.81</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>52.53±0.60</td>
<td>129.87</td>
<td>247.38</td>
<td>436.00</td>
<td>740.80</td>
<td>1116.94</td>
<td>1551.36</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>53.96±0.60</td>
<td>129.16</td>
<td>240.64</td>
<td>412.68</td>
<td>721.10</td>
<td>1141.32</td>
<td>1603.33</td>
</tr>
</tbody>
</table>

### Table 3: Least squares means with respective standard errors for body weight gain (g/chick) at different weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age in weeks</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>0 - VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>72.40±2.52</td>
<td>105.78</td>
<td>189.32</td>
<td>319.03</td>
<td>393.92</td>
<td>449.37</td>
<td>1468.50</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>74.36±2.52</td>
<td>123.16</td>
<td>205.94</td>
<td>323.95</td>
<td>412.71</td>
<td>432.52</td>
<td>1498.28</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>74.40±2.52</td>
<td>107.16</td>
<td>199.71</td>
<td>311.25</td>
<td>395.69</td>
<td>461.20</td>
<td>1481.70</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>74.33±2.52</td>
<td>127.52</td>
<td>202.92</td>
<td>344.05</td>
<td>410.22</td>
<td>458.87</td>
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<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>77.33±2.52</td>
<td>117.51</td>
<td>188.62</td>
<td>307.51</td>
<td>376.24</td>
<td>434.46</td>
<td>1421.87</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>75.20±2.52</td>
<td>112.14</td>
<td>171.70</td>
<td>306.07</td>
<td>407.54</td>
<td>461.99</td>
<td>1474.85</td>
<td></td>
</tr>
</tbody>
</table>

Mean values in each column having same superscript do not differ significantly (P<0.05).

the experimental period.

1. Mineral mixture supplied: Each gram contained 3% moisture, 30% calcium, 9% phosphorus, 0.4% manganese, 0.01% iodine, 0.4% zinc, 0.05% fluorine, 500 ppm copper, 2000 ppm iron, 20% sodium chloride.

2. Vitamin premix supplied: The following: Vit. A, 500,000 I.U.; Vit D<sub>3</sub>, 100,000 I.U.; Vit B<sub>1</sub>, 0.1 g; Vit E 75 I.U.; Vit. K 0.1 g; calcium panthenoate 0.25 g; nicotinamide 1.0 g; Vit. B<sub>6</sub>, 0.6 mg; choline chloride 15 g; calcium 75 mg; manganese 2.75 g; iodine 0.1 g; iron 0.75 g; zinc 12.5 g; copper 0.2 g; cobalt 0.045 g was added at the rate of 500 g in 100 kg of feed.

The various criteria fixed and the methods used for evaluating them are weekly body weight (g). Individual body weight of all the chicks was recorded on the day of their procurement and thereafter regularly at weekly interval up to six weeks of age. The weekly live weight gain was calculated
EFFECT OF FEEDING OF BAKERY WASTE ON PERFORMANCE OF BROILER CHICKS IN HOT ARID ZONE OF RAJASTHAN

S. Kumar, R.S. Choudhary, S.C. Goswami, S. Meel, R.S. Gadhwal, D.S. Manohar, J. Saini and N. Mitharwal
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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

ABSTRACT

Forty unsexed day-old, commercial broiler chicks were purchased from Sandhu Poultry farm, Bikaner. These chicks were weighed individually and uniformly distributed as 20 chicks in two groups. Each group was divided into two replicates with 10 chicks in each. The birds were offered feed and water ad libitum. The chicks were fed with starter mash which contained crude protein 22.03% and metabolizable energy 2910.70 Kcal/kg up to 3 weeks of age. For next 3 weeks i.e. from 4 to 6 weeks of age with finisher mash which contained crude protein 20.14% and metabolizable energy 3009.01 Kcal/kg. Group T1 (control group) was fed standard broiler mash. In group T2, maize was replaced by bakery waste at 20% level in feed. Growth, feed consumption and feed conversion ratio of broilers in group T2 (basal diet + bakery waste at 20 % level in feed) was significantly higher than that recorded on control diet. Present study suggested that supplementation of bakery waste was effective in improving performance in broiler chickens.

Key words: Bakery waste, broiler, performance, feed conversion ratio

Introduction

The poultry industry in the country has grown rapidly because of its early assured returns, short generation interval and limited land requirements. Today, it is a highly specialized, complex competitive business, characterized by phenomenal growth and has become one of the fastest growing segment of the animal science industry in India.

Bakery waste, a by-product of wheat processing industry have been identified as a promising feed resource for their use in the ration of poultry and can be used as a energy source as it has about 12% C.P, 0.65% C.F. and 4.2 M Cal/kg. D.E (Waldroup et al., 1982 and Gonzalez et al., 1982). The availability of biomass is also quite appreciable at present and is expected to increase further as is indicated by the flourishing of the bakery industry. Considering this, a study on utilization of bakery wastes in the poultry ration as an energy supplement to replace high priced energy source like maize is planned.

Though, the efforts made regarding the utilization of non conventional feed resources in poultry ration can definitely help us to relieve the situation to some extent but for efficient utilization of alternative feed ingredients indicts as well as to maximize availability and utilization of available nutrients and to improve the growth and production performance of livestock and poultry for maximum profitability. These efforts are required to be combined with suitable feed technology and the use of appropriate feed additives in poultry feeding appears to be very promising and positive.

Keeping in view, the above facts, the present study was undertaken to study the effect of feeding multi-enzymes on broiler performance in arid zone of Rajasthan.

Materials and Methods

Forty unsexed day-old, commercial broiler chicks were purchased from Sandhu Poultry farm, Bikaner. These chicks were weighed individually and uniformly distributed into 2 treatment groups with 20 chicks in each group. Each group was divided into two replicates with 10 chicks in each. The birds were offered feed and water ad libitum. The chicks were fed with starter mash which contained crude protein 22.03% and metabolizable energy 2910.70 Kcal / kg up to three weeks of age. For next 3 weeks i.e. from 4 to 6 weeks of age with finisher mash which contained crude protein 20.14% and metabolizable energy 3009.01 Kcal/kg. Group T1 (control group) was fed standard broiler mash. In group T2, bakery waste at 20% level was added in basal diet. Adequate and identical floor, feeding and watering space were provided to chicks of both groups throughout the experiment. Earthen vessels were used to provide water. The detailed composition of the basal ration (both starter and finisher) used for feeding the chicks was presented in Table 1. The data obtained in this experiment were analyzed using conventional statistical procedure as suggested by Snedecor and Cochran (2004) and significance of mean differences was tested by Duncan’s new multiple range test.

Results and Discussion

The parameters studied were average weekly live body weight, weekly gain in body weight, weekly feed consumption and feed conversion ratio.

Body weight

At first week of age, more mean b.wt. was observed in T2 group (92.70 ± 1.41 g) as compared to T1 group (91.55 ± 1.51 g). During second week of age, the mean body weight of chicks was higher in T2 group (170.64 ± 3.50 g) than group T1 (166.02 ± 2.93 g). The response of body weight in third week of age was similar to first two weeks i.e. the mean body weight of chicks of T2 group (331.84 ± 8.81 g) was higher than control group T1 (309.97 ± 8.34 g). At fourth week of age, significantly higher body weight was found in T2 group (529.60 ± 14.07 g) than that was observed in control group T1 (520.37 ± 14.34 g). At five weeks of age, same trend as that of week fourth was observed, the mean body weight of T2 group (807.47 ± 22.54 g) differs significantly from control group T1 (757.02 ± 19.45 g). Like week four and fifth the body weight at sixth week of age revealed significantly higher body weight in group T2 (1144.89 ± 40.73 g) than that was found in control group T1 (1071.13 ± 33.04 g). The results of study are in agreement

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with the reports of Rao et al. (2000), Hosamani et al. (2001).

Body weight gain

The means for body weight gain of broiler chicks at week first indicated that group T₂ chicks fed bakery waste had higher body weight gain (38.15 ± 1.37 g) than control group T₁ (37.45±1.74 g). The means for body weight gain of broiler chicks at week II was found to be more in T₂ group (78.07±4.03 g) than control group T₁ (74.43±3.39 g). Similarly, at week III, the mean body weight gain of chicks was significantly higher in group T₂ (161.21±8.87 g) as compared to control group T₁ (143.94±7.54 g). Likewise, at fourth, week of age significantly higher average body weight gain was attained by chicks of treatment T₂ group (197.76±14.12 g) in comparison to control group T₁ (207.37±16.31 g). At 5th week, on comparing the mean b.wt. gain of two groups it was found that group T₂ group (277.86 ± 24.76 g) attained significantly higher b.wt. gain than group T₁ (236.64±23.97 g). Week sixth followed the same pattern as that of groups II, III and IV. Significantly higher mean b.wt. gain was attained by chicks of T₂ group (337.42±38.28 g) than in control group T₁ (314.10±38.70 g). These finding are in accordance to that of Abdullatif et al. (2004) and Vaishnav (2009).

Feed consumption

During 1st week, average feed consumption by the broiler chicks in control group T₁ (83.63±9.80 g) was less than that in T₂ group (77.44 ± 5.16 g). During week 2nd, higher feed intake was observed in control group T₁ (153.42±8.23 g) as compared to group T₂ (151.10±9.04 g). The mean feed consumption during week III of the experiment was recorded to be significantly higher in group T₂ (324.43±16.00 g) than in control group T₁ (313.08±19.45 g). During week fourth, the average feed consumption was found to be significantly higher in group T₂ (395.52±24.25 g) as compared to group T₁ (435.49 ± 36.62 g). Mean feed intake during week fifth revealed that the feed consumption in group T₂ (552.99±16.53 g) was higher than in group T₁ (483.35±73.22 g). The mean feed consumption during week sixth of the experiment was recorded to be significantly higher in group T₂ (704.54 ± 82.49 g) in comparison to control group T₁ (648.63±73.27 g). The overall feed consumption throughout the experimental period (first to sixth weeks) revealed that feed intake was statistically higher for group T₂ (2199.49±99.83 g) than control group T₁ (2145.14±133.78 g). These finding are in accordance to Abdullatif et al. (2004), Gujrathi (2009) who observed improved effect of multi-enzymes on feed intake of broiler chicks.

Feed conversion ratio

The effect of multi enzyme on feed conversion ratio was found to be significant (P<0.01) at the overall level (1st to 6th weeks). The means feed conversion ratio at the starter phase (I-III weeks) was significantly better in T₂ group (1.98±0.07) than control group T₁ (2.13±0.03). The feed conversion efficiency at this stage revealed that bakery waste helped in efficient utilization of feed which resulted in significantly lower feed conversion ratio over control. At finisher phase (week fourth-sixth) the mean feed conversion ratio was better for group T₂ (2.03±0.04) than control group T₁ (2.06±0.02). The overall feed conversion efficiency (week first to sixth) showed almost similar trend to that of starter and finisher phase, in which best feed conversion efficiency was observed in T₂ group (2.01±0.05) in comparison to control group T₁ (2.08±0.02). The results of significantly better feed conversion ratio due to incorporation of bakery waste are similar to those of, Abdullatif et al. (2004) and Afzalzadeh et al. (2007) who reported that feed conversion ratio

References

EFFECT OF *OCIMUM SANCTUM* EXTRACT ON STORAGE AND MICROBIAL QUALITY OF VACUUM PACKED CHICKEN NUGGETS

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**ABSTRACT**

The present study was undertaken to assess the antioxidant properties of *Ocimum sanctum* in enhancing the shelf-life of vacuum packed chicken nuggets. Meat products are very vulnerable to spoilage due to excessive fats and protein contents. The study was conducted on chicken nuggets fortified with 1, 2, and 3% extracts of *Ocimum sanctum* along with control to explore the potency of *Ocimum sanctum* on oxidative stability and storage quality of vacuum packed chicken nuggets on 0, 15th, 30th and 45th days. Extracts of *Ocimum sanctum* in desired percentage were prepared and incorporated in vacuum packed chicken nuggets. The chicken nuggets fortified with 3% of *Ocimum sanctum* were adjudged to the best among all, based on sensory attributes and were found safe for consumption till 45 days of refrigerated storage (4±1°C) on the basis of pH, moisture, free fatty acid (FFA), thiobarbituric acid reacting substance (TBARS), microbiological profile, and sensory evaluation.

**Key words:** *Ocimum sanctum* extract, storage quality, vacuum packed chicken nuggets, free fatty acid (FFA), thiobarbituric acid reacting substance (TBARS)

**Introduction**

Today, consumer demands for safe, natural and high quality foods. The preference of consumer towards natural or organic food compels the food industry to include natural antioxidant in meat products to impart oxidative stability (Camo et al., 2007). The herbal extract can act as a potent natural antioxidant which can be used in different meat products. The addition of such extracts not only improves the sensory characteristics but also enhances self-life of meat products (Wojdylo et al., 2007).

Most of the diseases that are encountered today are lifestyle diseases which also includes degenerative diseases. The occurrence of these diseases is due to excessive formation of pro-oxidants in the body. To counter these pro-oxidants, the antioxidant are also secreted at cellular level but due to several reasons like genetic factors, dietary habit, physiological status, work load and environment pollution etc. There is excessive production of pro-oxidants at cellular level which leads to oxidative damage of the cell resulting in various lifestyle diseases (cancer, cardiovascular diseases, atherosclerosis, Alzheimer and Parkinson).

Therefore, in today’s prospective natural antioxidants are essential dietary requirements and development of value added meat products with incorporation of herbal extract as antioxidants helps in combating oxidative stress.

*Ocimum sanctum* Linn. (Family: Labiatae), locally known as ’Tulsi’ in Hindi and ‘Holy Basil’ in English, is a herb which is found throughout India. The Indian herb *Ocimum sanctum* may serve as dietary antioxidant with various modes of action viz. anti-microbial agents, anti lipolytic agents etc. Therefore, the extract of *Ocimum sanctum* can elongate the self-life of meat product when incorporated at standardized and optimized level in emulsion based meat product. The extract of *Ocimum sanctum* also acts as antioxidant when consumed by humans and protect them from oxidative damage and age old cognitive decline (Deka et al., 2009). The aim of present study was to develop *Ocimum sanctum* extract incorporated vacuum packed chicken nuggets and to evaluate for its quality parameters during refrigeration storage.

**Materials and Methods**

**Preparation of *Ocimum sanctum* extract**

Fresh *Ocimum sanctum* sp. plants were collected and botanically authenticated. The leaves were separated and dried at 40°C for 6 hours in hot air oven. Dried leaves were grinded into fine powder form, which was dissolved in 80% ethanol aqueous solution for four days by changing solution daily. The extract was filtered and evaporated to 10% dryness v/v using rotatory evaporator (Hannan et al., 2008).

**Preparation of chicken nuggets**

Lean meat from broiler was cut and minced in mincer. The common salt, vegetable oil, refined wheat flour (maida), nitrite, sodium hexapolyphosphate, spice mixture and condiment mixture were added as per formulation. Meat emulsion for chicken nuggets was prepared in Sirman Bowl Chopper. Crushed ice was added and blended continuously for about 1.5 minutes. Refined vegetable oil, spice mixture, condiments and other ingredients were added and again mixed for 1.5 to 2 minutes to get the desired emulsion. Chicken nuggets were moulded in rectangular stainless steel boxes. The weighed quantity of batter or emulsion was stuffed in mould with parchment paper pre-smeared with refined soybean oil to avoid sticking. Mould was covered with lid and tied properly. The moulds were subjected to steam cooking for about 30 minutes in pressure cooker. The boxes were allowed to cool at room temperature after removal from pressure cooker. The brick shaped chicken nuggets so obtained were sliced and...
cut into pieces to get smaller nuggets. The formulation of chicken nuggets in (%) was standardized, optimized and used for preparation of chicken nuggets from broiler meat was lean meat- 68.6, added water- 9.1, vegetable oil - 8.9, condiment mixture - 4.9, refined wheat flour- 4.1, spice mixture - 1.9, table salt-1.6, monosodium glutamate-0.4, sodium tripolyphosphate- 0.4, sodium nitrite - 100 ppm, 1, 2, and 3% Ocimum sanctum extracts was added in chicken nuggets (wt/wt.). In control chicken nuggets Ocimum sanctum extracts was not added. The nuggets were cooled and packed under vacuum packaging. These were stored in refrigerator (4±1°C) for evaluation of phisio-chemical and sensory parameter on 0, 15th, 30th and 45th day.

Analytical procedures

pH

The pH of cooked nuggets was measured soon after its preparation by the method of Keller et al. (1974). The pH of the suspension was recorded by immersing combined glass electrode of digital pH meter.

Moisture contents

Ten gram of mashed sample was transferred in pre-weighted flat bottom aluminum moisture cup, which was transferred to hot air oven at 101±1 °C and kept for 18-18 hrs. Dried sample was then placed in desiccator having silica gel as desiccant. After 1 hr., the cup containing dried sample was weighed. Moisture content was calculated by applying the following formula:

\[
\text{Moisture (\%) = } \frac{W_2 - W_3}{W_2 - W_1} \times 100
\]

Where, \(W_1=\) Weight of empty cup
\(W_2=\) weight of cup + sample
\(W_3=\) Weight of cup+ dried sample.

Thiobarbituricacidreacting substance (TBARS)

It was determined using the method of Witte et al. (1970). 10 g of chicken nuggets sample was blended finely with 50 ml of 20% Trichloro acetic acid (TCA) in a waring blender for 2 minutes and the resulting slurry was allowed to stand for 10 minutes. The extract was filtered through Whatman filter paper No. 42 and in a test tube 3 ml of this extract was mixed with equal volume of 0.1% (w/v) TBA reagent and blank sample was prepared by mixing 3 ml 20% TCA with equal volume of 0.1% thiobarbituric acid (TBA) reagent. The contents of each test tube were thoroughly mixed and boiled for 35 minutes in boiling water bath and were then allowed to cool down. The absorbance was measured at 532 nm by spectrophotometer and TBA value was calculated by comparing the absorbance of test sample with a standard graph prepared by using known concentrations of malondialdehyde. For preparing standard graph, 0.3055 gm. of 1, 1, 3, 3 tetraethoxypropane (TEP) was dissolved in 100 ml of 95% absolute alcohol to obtain a concentration of 1mg malonaldehyde/ml and was used as stock solution. To prepare working standard solution of TEP, 0.3 ml of stock solution was diluted to a volume of 100 ml by distilled water. The diluted solution contained 3 μg/ml of malonaldehyde and from that solution a standard graph was prepared by using different concentration of malondialdehyde.

Free fatty acid (FFA)

For the determination of free fatty acids, the method described by Koniecko (1979) was followed. Exactly 5 g nuggets blended for two minute with 30 ml of chloroform in presence of about 5g of anhydrous sodium sulphate. The mixture was filtered through Whatman No.1 filter paper into a 150 ml conical flask. About 2 to 3 drops of 0.2% phenolphthalein indicator was added to the chloroform extract, which was titrated against 0.1N alcoholic potassium hydroxide and consumed volume during titration was recorded. Percent free fatty acid was calculated as under and was also taken as absolute value in lipid profile.

\[
\text{FFA (\% Oleic acid) = } \frac{0.1 \times \text{ml} \times 0.1 \text{ N alcoholic KOH} \times 0.282}{\text{Weight of sample}} \times 100
\]

Microbiological profile

Total plate count, psychrotrophic count, coliform count and yeast and mould count in the sample were determined by method described by American Public Health Association (1984). Ready made media (Hi-Media) were used for the analysis.

Sensory evaluation

The sensory evalution of fresh and stored sample was carried for different attributes viz. appearance, flavour, juiciness, texture and the overall acceptability of fresh and stored samples using 8 point descriptive scale (Seman et al., 1987) where 8 denotes “extremely desirable” and 1 denotes “extremely undesirable”. Seven members of the panel replicated the experiment thrice (n =21). Panelists were seated in a room free of noise and odours and suitably illuminated. Coded samples for sensory evaluation were prepared.

Statistical analysis

The results were analyzed statistically as per Snedecor and Cochran (2004). In significant effects, least significant differences were calculated at appropriate level of significance for pair wise comparison of treatment means.

Experimental design

The ethanolic-aqueous Ocimum sanctum extract was added in standardized formulation of chicken nuggets substituting proportionately (wt./wt.) at the level of 1, 2 and 3%. The products were vacuum packed and evaluated based on physio-chemical, sensory and microbiological profile on 0, 15th, 30th and 45th day kept under refrigeration storage at (4±1°C).

Results and Discussion

Physio-chemical parameters

The changes in physio-chemical profile of Ocimum sanctum extract fortified vacuum packed chicken nuggets at refrigeration temperature (4±1°C) are given in Table1.

pH

The pH of Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets was recorded as significantly low (p<0.05) as compared to control (Table1). The pH increased significantly on successive storage days irrespective
of levels of incorporation of Ocimum sanctum sp. extract in vacuum packed chicken nuggets including control. However, the inclination in pH level was significantly low in treated product as compared to control. It may be due to the fact that Ocimum sanctum sp. extract contains urolic acid, apigenin, and luteolin which are proton donors and acidic in nature. The poultry meat recorded lower pH when fed on diet incorporated with Ocimum sanctum sp. powder (Lanjewar et al., 2000). The acidic pH of Ocimum sanctum sp. extract helpful in treatment of wilt disease of tomato plant reported by Murthy et al. (2014). Rojas and Brewer (2008) have reported similar findings on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork.

Moisture

The moisture level recorded in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets and control was comparable as the extract prepared contained similar moisture levels as present in meat emulsion. However, the moisture level observed in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets and control decreased significantly (p<0.05) on successive refrigeration storage days. This fact is supported by the finding of Lanjewar et al. (2000); Shan et al. (2005) and Wojdylo et al. (2007). They worked on Indian holy basil (Ocimum sanctum) sp., oregano (Origanum vulgare L.), rosemary (Rosmarinus officinalis L.) and sage (Salvia officinalis L.) in various emulsion based meat products.

Thiobarbituric acid reacting substances (TBARS)

The TBARS value is quantitative indication of lipid peroxidation in meat products. In order to know the rate of lipid peroxidation, the malondialdehyde content was evaluated by assaying meat product during storage. The TBARS values decreased significantly (p<0.05) with increased in the level of incorporation of Ocimum sanctum sp. extract while it was found to be significantly (p<0.05) increased on successive storage days in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets including control Table 1. The TBARS value indicated that control vacuum packed chicken nugget was not suitable for consumption on 45th day of refrigeration storage whereas all levels of Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets were found to be suitable for consumption even on 45th day. This was an indicative of the fact that Ocimum sanctum sp. extract had preventive effect on lipid peroxidation and hence enhance its shelf-life. This was due to the fact that mainly urolic acid, apigenin, and luteolin present in Ocimum sanctum sp. acted as anti-lipolytic factor due to which the shelf-life of meat product was enhanced. This finding was in congruence with the finding of Ayo et al. (2005) who worked on properties of water, ethanol and methanol extract of Ocimum sanctum sp. The plant extract had positive effect on lipid oxidation by reducing the production of 2-TBA and malondialdehyde formation in herbal extract incorporated meat product during refrigeration storage. Fasseas (2007) reported that extract of various herbal plants causes reduction in TBA value and lipid oxidation. Rojas and Brewer (2008) had reported on similar line who worked on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork. Tanabe et al. (2002) also supported our present finding who reported the incorporation of phenols and flavonoids in pork product reduced production of malondialdehyde and lowered TBA value.

Free fatty acid (FFA)

The FFA value is quantitative indication of lipolysis in meat products. The FFA value of Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets recorded significantly low (p<0.05) in comparison to control on 0’ day (Table 1). This was an indicative of the fact that Ocimum sanctum sp. extract had preventive effect on lipolysis and hence enhance its shelf-life. The FFA value had increased significantly (p<0.05) on successive refrigeration storage days in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets including control (Table 1). This also suggested that Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets had better shelf life than control. It was due to the fact that urolic acid, apigenin, and luteolin present in extract of Ocimum sanctum sp. has anti-lipolytic activity due to which free fatty acid production was less. It is supported by the work of Yanpallewar et al. (2004) who worked on Ocimum sanctum sp. antioxidant and neuro-protective effect of Ocimum sanctum on transient cerebral ischaemia and long-term cerebral hypoperfusion. The present finding is also supported by the finding of Djenane et al. (2003) who concluded that surface application of extract of various herbs had positive effect on oxidative stability of beef steaks packed in vacuum and modified atmospheric packaging. Rojas and Brewer (2008) had reported on similar line who worked on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork. The present finding is also in parallel with finding of Simitzis et al. (2008) who reported dietary natural antioxidants obtained from different herbs had positive effect on oxidative stability by producing fewer amounts of free fatty acids during refrigeration storage.

Microbiological characters

The changes in microbiological profile of Ocimum sanctum sp. extract fortified vacuum packed chicken nuggets at refrigeration temperature (4±1°C) are presented in Table 2.

Total plate count

The total plate count was low in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets than control on 0’ day (Table 2). This was indicative of anti-microbial nature of Ocimum sanctum sp. extract. The total plate count was increased on successive refrigeration storage days in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets including control (Table 2). The TPC value of control vacuum packed chicken nuggets was indicative that the product was not suitable for human consumption on 45th day of storage. The TPC value of Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets was found to be in the range of 3 log cfu/gm which was indicative of fact that Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets were suitable for consumption even on 45th day of refrigeration storage. The herbal extract had affected microbial cell by various antimicrobial mechanisms. It may disrupt enzyme system; disrupt genetic material of bacteria
attacking on phospholipid bilayer cellular membrane and forming fatty acid hydro-peroxidase (Arques et al., 2008 and Burt et al., 2007). The present finding was also supported by Ceylan and Fung, (2004) who reported significantly decline in microbial load with incorporation of herbal extract in various meat products. The herbal extract had antimicrobial activity and when incorporated in meat product could elongate its shelf-life during refrigeration storage. The urolic acid, apigenin, and luteolin active principal content in Ocimum sanctum sp. extract was having broad spectrum antimicrobial activity (Singh et al., 2012). Rojas and Brewer (2008) had reported on similar line who worked on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork.

Psychrotrophic count
Psychrotrophic counts were not detected till 15th day of refrigeration storage (Table 2). The psychrotrophic were found to be lower on 30th and 45th day of refrigeration storage in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets as compared to control. Ocimum sanctum sp. extract incorporated vacuum packed chicken nugget was found to be suitable for human consumption even on 45th day of refrigeration storage (Table 2). It may be due the fact that the principle component urolic acid, apigenin, and luteolin present in Ocimum sanctum sp. extract had significant antimicrobial effect at refrigeration temperature. The apigenin, and luteolin interacted with phospholipid bilayer of microbial cell wall and cell membrane and disrupted it. It also defunct electron transport system, ion gradient and other enzyme dependent cellular mechanism of psychrotropic bacteria (Burt, 2007). The present result was also supported by finding of Rota et al. (2008) who suggested psychrotropic antimicrobial effect of essential oils and extracts of herbal plants.

Coliform count
The coliform were not detected at any day of refrigeration storage in any of the product (Table 2). It may be due to the fact that strict hygienic condition was followed during meat product processing. It may also be due to antimicrobial effect against coliform by urolic acid, apigenin, and luteolin present in Ocimum sanctum sp. extract. Our present finding was supported by reports of Ben Sassie, et al., (2008); Graumann and Holley (2008), Ibrahim et al. (2008) and Winward et al. (2008) who concluded that the extract and essential oil obtained from various herbs had significant antimicrobial effect against almost all coliforms.

Yeast and molds count
The yeast and molds count was not detected till 15th day of storage but it was appeared in all product from 30th day onward (Table 2). The yeast and mold count of Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets was significantly lower than control on 30th and 45th day of storage (Table 2). It was also indicative of the fact that control vacuum packed chicken nugget was not suitable but Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets was found to be suitable for human consumption even on 45th day of refrigeration storage. The appearance of yeast and moulds on 30th day may be due to the fact that yeast and moulds requires incubation period of approximately 10 days. Moreover, these extract possess natural fungicidal effect against food borne fungi (Fisher and Phillips, 2008; Daferere et al., 2000 and Majhenic et al., 2007). Our finding was also supported by Razzaghi-Abyaneh et al. (2008) and El. Seedi et al. (2008) who reported that ethanolic extract of Ocimum sanctum sp. and other herbs significantly reduces yeast and moulds count in various meat product. It was also effective against mycotoxin (Friedman, 2007; Musyimi et al., 2008; Kong et al., 2007 and Lopez et al., 2007). Sanchez-Escalante et al. (2003) reported similarly while working on beef patties packed in modified atmosphere.

Sensory parameters
All the sensory attributes viz. color and appearance, flavour, texture, juiciness and overall acceptability was found to be lower on successive refrigeration storage in Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets including control (Table 3). The colour and appearance, flavour and juiciness of 3% Ocimum sanctum sp. extract incorporated chicken nuggets was found to be significantly higher than 1 and 2% Ocimum sanctum sp. extract incorporated and control vacuum packed chicken nuggets. The texture value of Ocimum sanctum sp. extract incorporated and control chicken nuggets were comparable with each other. The Overall acceptability of 3% incorporated Ocimum sanctum sp. extract in vacuum packed chicken nugget was higher than 1 and 2% Ocimum sanctum sp. extract incorporated and control vacuum packed chicken nuggets.

The vacuum packed control chicken nuggets have been quickly deteriorated on all parameters of sensory attributes as compared to Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets. The Ocimum sanctum sp. extract incorporated vacuum packed chicken nuggets was found to be acceptable on the basis of sensory attributes even on 45th day of refrigeration storage but the control vacuum packed chicken nuggets was rejected on 45th day of refrigeration storage.

The herbal extract has positive effect by inhibiting discoloration and off-odour formation in different meat product during refrigeration storage (Djenane et al., 2003; Nerin et al., 2006 and Camo et al., 2008). Natural antioxidant can positively affect colour and appearance parameter and maintained the original colour of product for longer duration during refrigeration storage (Djenane et al., 2003; Carpenter et al., 2007; Chouliara et al., 2007; Nerin et al., 2006 and Simitzis et al., 2008). The herbal extract can act as a very good flavoring agent. It can also act as a binding agent. Sanchez-Escalante et al. (2003) reported similarly while working on beef patties packed in modified atmosphere. The sensory character can also be enhanced with herbal extract incorporation in various meat products (Chaves et al., 2008).

Conclusion
The ethanolic: aqueous (80:20) extract of Ocimum sanctum sp. was used in preparation of value added vacuum packed chicken nuggets. The developed product exhibited significant (p<0.05) anti microbial, anti lipolytic and anti oxidant activity. The incorporation of Ocimum sanctum sp. extract (3%) in vacuum packed chicken nuggets has enhanced sensory scores as well as shelf life for a period of 45 days in refrigerated (4±1°C) condition without any marked loss of physio-chemical,
Table 1: Mean±SE values of physico-chemical characteristics of vacuum packed chicken nuggets treated with different levels of *Ocimum sanctum* extract at refrigeration temperature

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 Day</th>
<th>15th-DAY</th>
<th>30th-DAY</th>
<th>45th-0 DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0%)</td>
<td>5.69±0.006</td>
<td>5.96±0.007</td>
<td>6.25±0.010</td>
<td>6.71±0.006</td>
</tr>
<tr>
<td><em>O. sanctum</em> (1%)</td>
<td>5.66±0.015</td>
<td>5.85±0.008</td>
<td>6.10±0.009</td>
<td>6.47±0.009</td>
</tr>
<tr>
<td><em>O. sanctum</em> (2%)</td>
<td>5.63±0.012</td>
<td>5.70±0.009</td>
<td>6.00±0.006</td>
<td>6.34±0.007</td>
</tr>
<tr>
<td><em>O. sanctum</em> (3%)</td>
<td>5.58±0.016</td>
<td>5.61±0.026</td>
<td>5.83±0.014</td>
<td>6.27±0.008</td>
</tr>
</tbody>
</table>

Mean±SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05). n=6 for each treatment

Table 2: Mean±SE values of microbiological characteristics of vacuum packed chicken nuggets treated with different levels of *Ocimum sanctum* extract at refrigeration temperature

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 Day</th>
<th>15th-DAY</th>
<th>30th-DAY</th>
<th>45th-0 DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0%)</td>
<td>ND</td>
<td>ND</td>
<td>1.49±0.0102</td>
<td>2.64±0.0152</td>
</tr>
<tr>
<td><em>O. sanctum</em> (1%)</td>
<td>ND</td>
<td>ND</td>
<td>1.24±0.0122</td>
<td>2.16±0.0122</td>
</tr>
<tr>
<td><em>O. sanctum</em> (2%)</td>
<td>ND</td>
<td>ND</td>
<td>0.88±0.0102</td>
<td>1.74±0.0112</td>
</tr>
<tr>
<td><em>O. sanctum</em> (3%)</td>
<td>ND</td>
<td>ND</td>
<td>0.60±0.0012</td>
<td>1.11±0.0112</td>
</tr>
</tbody>
</table>

Mean±SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05). n=6 for each treatment
Table 3: Mean±SE values of sensory attributes of vacuum packed chicken nuggets treated with different levels of Ocimum sanctum extract at refrigeration temperature

<table>
<thead>
<tr>
<th>Treatments</th>
<th>STORAGe PERIOD(DAYS)</th>
<th>0 Day</th>
<th>15th-DAY</th>
<th>30th-DAY</th>
<th>45th-0 DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour and appearance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0%)</td>
<td>6.53±0.095</td>
<td></td>
<td></td>
<td></td>
<td>3.97±0.109</td>
</tr>
<tr>
<td>O. sanctum (1%)</td>
<td>6.74±0.130</td>
<td></td>
<td></td>
<td></td>
<td>4.09±0.106</td>
</tr>
<tr>
<td>O. sanctum (2%)</td>
<td>6.78±0.082</td>
<td></td>
<td></td>
<td></td>
<td>4.17±0.071</td>
</tr>
<tr>
<td>O. sanctum (3%)</td>
<td>7.18±0.099</td>
<td></td>
<td></td>
<td></td>
<td>4.86±0.075</td>
</tr>
<tr>
<td>Flavour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0%)</td>
<td>5.96±0.115</td>
<td></td>
<td></td>
<td></td>
<td>4.13±0.089</td>
</tr>
<tr>
<td>O. sanctum (1%)</td>
<td>6.22±0.126</td>
<td></td>
<td></td>
<td></td>
<td>4.22±0.075</td>
</tr>
<tr>
<td>O. sanctum (2%)</td>
<td>6.74±0.113</td>
<td></td>
<td></td>
<td></td>
<td>5.01±0.134</td>
</tr>
<tr>
<td>O. sanctum (3%)</td>
<td>7.35±0.133</td>
<td></td>
<td></td>
<td></td>
<td>5.84±0.131</td>
</tr>
<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0%)</td>
<td>7.41±0.089</td>
<td></td>
<td>6.40±0.108</td>
<td></td>
<td>4.62±0.101</td>
</tr>
<tr>
<td>O. sanctum (1%)</td>
<td>7.43±0.112</td>
<td></td>
<td>6.87±0.136</td>
<td></td>
<td>4.67±0.133</td>
</tr>
<tr>
<td>O. sanctum (2%)</td>
<td>7.52±0.113</td>
<td></td>
<td>6.98±0.127</td>
<td></td>
<td>4.78±0.132</td>
</tr>
<tr>
<td>O. sanctum (3%)</td>
<td>7.37±0.079</td>
<td></td>
<td>6.79±0.108</td>
<td></td>
<td>4.65±0.064</td>
</tr>
<tr>
<td>Juiciness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0%)</td>
<td>6.81±0.103</td>
<td></td>
<td>6.34±0.094</td>
<td>5.58±0.124</td>
<td>4.89±0.094</td>
</tr>
<tr>
<td>O. sanctum (1%)</td>
<td>7.05±0.084</td>
<td></td>
<td>6.55±0.105</td>
<td>5.86±0.114</td>
<td>5.12±0.076</td>
</tr>
<tr>
<td>O. sanctum (2%)</td>
<td>7.14±0.113</td>
<td></td>
<td>6.67±0.094</td>
<td>5.98±0.096</td>
<td>5.23±0.103</td>
</tr>
<tr>
<td>O. sanctum (3%)</td>
<td>7.46±0.081</td>
<td></td>
<td>6.98±0.091</td>
<td>6.42±0.102</td>
<td>5.50±0.051</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0%)</td>
<td>6.86±0.106</td>
<td></td>
<td>6.22±0.072</td>
<td>5.41±0.142</td>
<td>3.96±0.118</td>
</tr>
<tr>
<td>O. sanctum (1%)</td>
<td>6.98±0.117</td>
<td></td>
<td>6.34±0.078</td>
<td>5.53±0.110</td>
<td>4.13±0.064</td>
</tr>
<tr>
<td>O. sanctum (2%)</td>
<td>7.27±0.066</td>
<td></td>
<td>6.72±0.115</td>
<td>5.90±0.084</td>
<td>4.55±0.117</td>
</tr>
<tr>
<td>O. sanctum (3%)</td>
<td>7.58±0.092</td>
<td></td>
<td>7.17±0.072</td>
<td>6.32±0.091</td>
<td>5.01±0.093</td>
</tr>
</tbody>
</table>

Means ± SE with different superscripts in a row (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05). Mean values are on 8 point descriptive scale where 1—extremely poor and 8—extremely desirable. n=21 for each treatment.

microbial and sensory quality. The result revealed the possible application of Ocimum sanctum sp. extract (3%) as a natural source of anti oxidant in development of vacuum packed chicken meat product with potential health benefits.

References

EFFECT OF STORAGE CONDITION ON SENSORY AND MICROBIAL CHARACTERISTICS OF AEROBICALLY PACKAGED LOW FAT MILK NUGGETS PREPARED WITH SKIM MILK COAGULUM AND BARNYARD MILLET FLOUR

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ABSTRACT
The present study was carried out to determine the physico-chemical, microbiological, sensory properties and storage stability of developed low fat milk nuggets. Two groups of low fat milk nuggets were prepared. First group known as control having skim milk coagulum, refined wheat flour, spices and condiments as ingredients in its formulation while other group known as treatment group additionally contained optimum level of Barnyard millet flour. Developed products were aerobically packaged in low density polyethylene bag and kept under refrigeration (4±1ºC). Storage stability of product was evaluated on 0, 3rd, 6th, 9th and 12th day of storage for physico-chemical, microbiological and sensory parameters. pH value of both groups tended to decrease significantly (p<0.01) over the storage period and pH value of control group was significantly (p<0.01) lower than that of treatment group over the storage period. TBARS values of both the groups increased significantly (p<0.01) with advancement of storage period. Total plate count, psychrophillic and yeast and mold counts of both the groups increased significantly (p<0.01) with the advancement of storage period. Overall acceptability of low fat milk nuggets of treatment group was comparable to that of control ones on even on 12th day of storage.

Key words: Milk nuggets, skim milk, coagulum, barnyard millet flour (BMF), physico-chemical analysis, sensory evaluation

Introduction
Composite dairy food offers multiple benefits in terms of variety, nutrition, health and economy. Developed low fat milk nuggets is a kind of composite dairy food based on skim milk coagulum, optimum level of Barnyard millet flour, refined wheat flour, salt, sugar, spices and condiments. Consumers are now more quality conscious. These quality requirements are reflected in labeling requirement rules of low fat food regulations as use by date or best before. Shelf life study is integral activity of product development process to ensure the safety and quality of new food product. Shelf life may be defined as the period following the manufacture during which the product meets the consumer expectations. Microbiological changes are of major importance for highly perishable food product with short shelf life and sensory and physico-chemical changes for medium to long shelf life product (McGinn, 1982). Intrinsic factors, extrinsic factors and their interplay are key determinant of the shelf life of the product. Shelf life is influenced by the formulation of the product, nature of the raw materials and packaging interventions. Packaging can enhance the shelf life of food products (Rozbeh et al.,1993). Present study was undertaken to assess the storage stability of two groups of low fat milk nuggets, aerobically packaged in low density polyethylene and kept in refrigerated condition (4±1ºC).

Materials and Methods
Milk for pursuing this study was procured from Dairy Technology Section of Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P). The milk procured was combination of cow and buffalo milk as per daily production at dairy farm and was pasteurized. The skim milk used in this study was prepared by separating cream by a hand driven centrifugal cream separator. Other ingredients like refined wheat flour, spices, finger millet flour and LDPE bags were procured from local market of Bareilly. All the chemicals and reagents used were of analytical grade and were obtained from standard firms (Qualigens, Merck and Hi Media). Ingredients of formulation of control in decreasing order were coagulum from skim milk, binders, refined wheat flour, spice mix, salt, condiments. In formulation of treatment group skim milk coagulum was replaced with Barnyard millet flour at optimum level and rest of the ingredient was same as control group. Dough was prepared in mixer with prior standardization of mixing time and batter consistency. Dough was placed in mould and steam cooked for 20 minutes followed by cooling, cutting and packaging.

Both groups of low fat milk nuggets was aerobically packaged in low density polyethylene bag and stored at refrigeration temperature of 4±1ºC. Storage stability of product was evaluated at 0, 3rd, 6th, 9th and 12th day of storage for physico-chemical, microbiological and sensory parameters.

Observations for pH, thiobarbituric acid reacting substances (TBARS) value, total plate count, psychrophillic count, coliform counts, yeast and mould count and sensory analysis was made. pH value of the product was determined by the method described by Trout et al. (1992). TBARS value of the product was determined by the method described by Tarladgis et al. (1960).

All the microbiological parameters were determined following the methods described by APHA (2001). Readymade media (Hi-Media, India) were used for the analysis. Sensory evaluation of low fat Milk nuggets was conducted by the method used by (Rajkumar et al., 2010) using nine point descriptive scale where 9=excellent and 1=extremely poor. The experienced panel consisting of scientists and post graduate

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students of the Division of Livestock Products Technology, IVRI, Izatnagar evaluated the samples. The panelists were briefed with the nature of the experiments without disclosing the identity of the samples and were requested to rate them on nine point descriptive scale on the sensory evaluation proforma for different attributes. The milk nuggets were evaluated for appearance, flavour, juiciness, body and texture, and overall acceptability. The product was warmed for 10-15 seconds and served to the panelists. Plain potable water was provided to rinse the mouth in between the testing of each samples.

Statistical analysis
Data generated from various trials under each experiment were pooled and compiled and analyzed as per the standard statistical methods (Snedecor and Cochran, 2004) and interpreted. Means and standard error were computed for each parameter. The data were subjected to analysis of variance.

Results and Discussion
Physico-chemical characteristics
The mean value of physico-chemical parameters for control and treatment (BMF incorporated) products during storage period is presented in Table 1.

pH
The average scores for pH of both the products indicated a decreasing trend with the advancement in storage period. This could be related to the growth of lactic acid bacteria and production of lactic acid causing decrease in pH during the storage. Further, there was a significantly lower (p<0.05) pH value observed for control product than treatment on 9th and twelfth day of storage.

The Studies by Pal (1989) and Sanyal (1997) also showed declining trend of pH in reduced fat. Days of storage significantly (p<0.01) affected pH, TBARS value, total plate count, psychrophilic count and yeast and mould count. Further, treatment X storage days interaction significantly (p<0.01) affected pH and psychrophilic count of low fat milk nugget during storage.

TBARS
There was a significant (p<0.05) linear increase in TBARS values with increase in the storage period in both the products. However, the values remained well below the threshold value of 1 mg malonaldehyde/kg of product on 12th day of storage. The initial value of TBARS on 0 day in control product was 0.16±0.00, which increased to 0.66±0.03 mg malonaldehyde/kg on 12th day of storage. In BMF incorporated milk nuggets the initial value was 0.17±0.00 and this increased to 0.63±0.09 mg malonaldehyde/kg on 12th day of storage. Further, the concentration of TBARS in both types of nuggets showed no significant (p>0.05) difference between them during entire period of storage except on 9th day. The increase in TBARS value on storage might be attributed to increased lipid oxidation and production of volatile metabolites during storage (Joc and AHN DU, 1999).

Microbiological parameters

Total Plate Count (TPC)
The average total plate count (log cfu/g) of both the products indicated a significant (p<0.01) linear increasing trend and increased from initial values of 1.08±0.09 and 1.05±0.16 log cfu/g in control and BMF incorporated milk nuggets respectively to 4.39±0.21 and 4.29±0.14 log cfu/g, respectively after 12 days during storage of the products. However, these values are very well within the permissible limits for milk products prescribed by Rossetti et al. (2008), which was 2.8×10^2 cfu/ml. No significant difference (p<0.05) was observed for total plate count between control and BMF incorporated milk nuggets during entire storage period.

Psychrophilic count
Psychrophiles were not detected up to 6th day of storage. It started appearing from 9th day onward. On day 9th, the mean value for psychrophilic count was 1.18±0.02 log cfu/g and 1.76±0.08 log cfu/g in control and BMF milk nuggets, respectively. There was no significant difference observed in mean value of psychrophilic count of 9th and 12th day of storage for the control nuggets. However, in BMF incorporated products it was significantly (p<0.05) higher on 12th day than the 9th day's count. The appearance of psychrophiles after a long gap might be due to the sufficient heat treatment during product processing, which drastically injured and killed the psychrophilic population in milk nuggets reducing the number of surviving and resistant ones to a non countable limit. Further, during the storage period, the count was significantly (p<0.05) higher in BMF incorporated product than the control.

Coliform count
The non detection of coliforms throughout the period of storage in both control and BMF incorporated milk nuggets could be because of the destruction of these bacteria during cooking at high temperature for sufficient time (far above their thermal death point of 57°C and hygienic practices followed during the preparation and packaging of products.

Yeast and mould count
Yeast and moulds were not detected up to 6th day of storage. These started appearing from 9th day onward. On day 9th, the mean value for yeast and mould count was 1.21±0.05 log cfu/g and 1.15±0.04 log cfu/g in control and BMF milk nuggets, respectively. In both the products, mean value on 12th day was significantly (p<0.05) higher than the 9th day's count. But these values were within the permissible limits prescribed by Rossetti et al. (2008), which was 8.0 ×10^3 cfu/ml. Further, there was no significant (p>0.05) difference between the two products during entire storage period.

Sensory attributes
Mean±SE values of sensory characteristics during storage are presented in Table 2. ANOVA revealed that days of storage significantly (p<0.01) affected appearance, flavour, juiciness, body and texture and overall acceptability and treatment x storage days interaction significantly (p<0.01) affected appearance, flavour, juiciness and body and texture (p<0.05) of low fat milk nuggets during storage.

The appearance scores of both types of milk nuggets showed a gradual decreasing trend with increase in storage period. The initial mean score on 0 day in control milk nuggets was 7.90±0.11, which decreased to 6.95±0.11 on 12th day of storage. In BMF milk nuggets the initial value was 7.57±0.12
and this decreased to 6.53±0.12 on 12th day of storage. The decrease in appearance scores could be due to some pigment and lipid oxidation resulting in non enzymatic browning as well as surface dehydration in aerobic packaging. Overall treatment means represented significant (p<0.05) difference between the products on 3rd and 12th day of storage.

The average scores for flavour of both the products indicated decreasing trend and decreased from an initial scores of 7.81±0.12 and 7.45±0.16 in control and treatment products, respectively to 7.03±0.14 and 6.50±0.10, respectively after 12 days during storage. The flavour score was comparable up to 3rd day of storage but thereafter it decreased significantly (p<0.05) on 6th day and 12th day of storage in control products. The flavour score in treatment product remained comparable (p<0.05) on 6th day and thereafter, it decreased significantly up to 9th day of storage but thereafter it decreased significantly (p<0.05) on 12th day of storage. The score was significantly higher in control product than treatment product on initial day (p<0.05) on 12th day of storage.

Table 1: Changes in physico-chemical properties of aerobically packaged selected low fat milk nuggets during storage at refrigerated temperature ((4±1°C) (Mean±SE)

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.26±0.00a</td>
<td>6.15±0.01a</td>
<td>6.13±0.01a</td>
<td>5.98±0.01a</td>
<td>5.87±0.00b</td>
</tr>
<tr>
<td>Product 2</td>
<td>6.27±0.00a</td>
<td>6.13±0.01a</td>
<td>6.12±0.00a</td>
<td>6.07±0.01A</td>
<td>6.01±0.01A</td>
</tr>
<tr>
<td>TBARS (mg malondehyde/kg)</td>
<td>0.16±0.00a</td>
<td>0.31±0.01A</td>
<td>0.46±0.01b</td>
<td>0.61±0.02a</td>
<td>0.66±0.03a</td>
</tr>
<tr>
<td>Product 2</td>
<td>0.17±0.00a</td>
<td>0.28±0.01a</td>
<td>0.43±0.01b</td>
<td>0.55±0.01b</td>
<td>0.63±0.09b</td>
</tr>
<tr>
<td>Total plate count (log cfu/g)</td>
<td>1.08±0.09a</td>
<td>2.62±0.07b</td>
<td>3.31±0.03a</td>
<td>3.82±0.10b</td>
<td>4.39±0.21c</td>
</tr>
<tr>
<td>Product 2</td>
<td>1.05±0.16b</td>
<td>2.54±0.01a</td>
<td>3.51±0.11b</td>
<td>3.62±0.26b</td>
<td>4.29±0.14b</td>
</tr>
<tr>
<td>Psychrophillic count (log cfu/g)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Product 2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Yeast and Mould count (log cfu/g)</td>
<td>1.18±0.02a</td>
<td>1.87±0.04b</td>
<td>1.76±0.08a</td>
<td>2.44±0.05a</td>
<td></td>
</tr>
<tr>
<td>Product 2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Coliform count (log cfu/g)</td>
<td>2.44±0.05a</td>
<td>2.85±0.05b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product 2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Mean±S.E. with different superscripts row wise (lower case) and column wise (upper case) differ significantly (p<0.05); n=6 for each treatment at each storage period; Product 1- Control; Product 2- Treatment (BMF incorporated) low fat milk nuggets

Table 2: Changes in sensory attributes of aerobically packaged selected low fat milk nuggets during storage at refrigerated temperature ((4±1°C) (Mean±SE)

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>7.90±0.11a</td>
<td>7.71±0.13b</td>
<td>7.22±0.11a</td>
<td>7.22±0.11b</td>
<td>6.95±0.11ab</td>
</tr>
<tr>
<td>Product 2</td>
<td>7.57±0.12a</td>
<td>7.35±0.15a</td>
<td>7.40±0.21a</td>
<td>7.30±0.12a</td>
<td>6.53±0.12ab</td>
</tr>
<tr>
<td>Flavour</td>
<td>7.81±0.12ab</td>
<td>7.66±0.12a</td>
<td>7.34±0.11a</td>
<td>7.34±0.10a</td>
<td>7.03±0.14a</td>
</tr>
<tr>
<td>Product 2</td>
<td>7.45±0.16ab</td>
<td>7.42±0.14a</td>
<td>7.33±0.08a</td>
<td>7.23±0.11a</td>
<td>6.5±0.10ab</td>
</tr>
<tr>
<td>Juiciness</td>
<td>7.70±0.12a</td>
<td>7.64±0.12a</td>
<td>7.39±0.11a</td>
<td>7.39±0.11a</td>
<td>6.8±0.08ab</td>
</tr>
<tr>
<td>Product 2</td>
<td>7.32±0.10a</td>
<td>7.32±0.17a</td>
<td>7.29±0.15a</td>
<td>7.25±0.12a</td>
<td>6.37±0.13ab</td>
</tr>
<tr>
<td>Body and texture</td>
<td>7.91±0.15a</td>
<td>7.73±0.13b</td>
<td>7.39±0.12a</td>
<td>7.39±0.06b</td>
<td>6.95±0.11a</td>
</tr>
<tr>
<td>Product 2</td>
<td>7.54±0.10a</td>
<td>7.53±0.17a</td>
<td>7.51±0.20a</td>
<td>7.37±0.12a</td>
<td>6.37±0.16ab</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.81±0.09a</td>
<td>7.65±0.12a</td>
<td>7.46±0.11a</td>
<td>7.46±0.11a</td>
<td>6.88±0.13ab</td>
</tr>
<tr>
<td>Product 2</td>
<td>7.70±0.16a</td>
<td>7.50±0.14a</td>
<td>7.42±0.12a</td>
<td>7.27±0.10a</td>
<td>6.51±0.06ab</td>
</tr>
</tbody>
</table>

*Mean±S.E. with different superscripts row wise and column wise differ significantly (p<0.05); n=21 for each treatment at each storage period; Product 1- Control; Product 2- Treatment (BMF incorporated) low fat milk nuggets
trend and decreased from an initial scores of 7.70±0.12 and 7.32±0.10, respectively to 6.80±0.08 and 6.37±0.13, respectively after 12 days during storage of the products. However, in both the products, the score declined significantly (p<0.05) on 12th day of storage. Juiciness scores of control milk nuggets were non significantly (p>0.05) higher than that of treatment milk nuggets throughout the storage period and significantly (p<0.05) higher on 12th day of storage. This could be related to the marginally higher moisture content of control than treatment (BMF incorporated) milk nuggets.

In both the products, mean score for body and texture showed a declining trend and the score decreased from 7.91±0.15 and 7.54±0.10 to 6.95±0.11 and 6.37±0.16, respectively in control and BMF incorporated milk nuggets. Texture scores of control milk nuggets declined significantly (p<0.05) on 6th day onward but there was no significant (p>0.05) difference noticed between 6th and 9th day of storage. Texture score of 12th day was significantly (p<0.05) lower than the scores of 0, 3rd, 6th and 9th day of storage. The decrease in texture scores might be due to protein oxidation and microbial action on proteins during the refrigerated storage. In treatment products, no significant (p>0.05) difference in score was observed up to 9th day but on 12th day it was significantly (p<0.05) lower. Further, significant (p<0.05) difference was observed in the score of 3rd day between the two products.

Mean overall acceptability scores showed a declining trend and decreased from initial score of 7.81±0.09 to 6.88±0.13 on 12th day of storage in control product and from 7.70±0.16 to 6.51±0.06 in treatment products. In control product, the decline was significant on 12th day and the scores were not significantly different up to 9th day of storage. However, in treatment product, the score decreased significantly on 6th day onwards. This sensory attribute reflects the cumulative effect of all other attributes, but mainly attributed to loss of flavour and reduced appearance scores. Further, there was significant difference in mean score of 12th day between the two types of product.

Acknowledgements

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References


A STUDY ON FARMER’S VIEW ON DELIVERY OF VETERINARY SERVICES IN TERMS OF AVAILABILITY, EFFICIENCY AND SATISFACTION

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ABSTRACT

The present study is conducted in Tonk district of Rajasthan in order to ascertain the status of livestock services delivery system of state department of animal husbandry (SDAH) in Rajasthan. A total of 120 livestock farmers were selected as respondent from 8 selected villages of 4 tehsils of the district while secondary data were collected from annual reports and other occasional reports of SDAH. Majority of the livestock farmers had medium availability for SDAH livestock services followed by high and low availability. Majority of the livestock farmer's rated SDAH livestock services as average in terms of their effectiveness followed by excellent and poor. Majority of the livestock farmers belonged to medium level of satisfaction towards SDAH livestock services followed by low and high satisfaction categories.

Key words: SDAH, effectiveness, availability, satisfaction

Introduction

Livestock have been an integral component of India’s agricultural and rural economy since time immemorial. In India almost sixty per cent of the population involved in agriculture which accounts for only 18-20% of our country’s Gross Domestic Product (GDP) (M. Jothilakshmi, 2011). Livestock sector grew at an annual rate of 5.3% during 1980s, 3.9% during 1990s and 3.6% during 2000s. Distribution of livestock is more equitable compared to that of land. In 2003, marginal farm households (<1.0 hectare of land) who comprised 48% of the rural households controlled more than half of country’s cattle and buffalo, two-thirds of small ruminants (goat, sheep) and pigs as well as poultry as against their share of 24% in land. Livestock contributed 16% to the income of small farm households as against an average of 14% for all rural households (GOI, 2012). It has been supporting as a livelihood options during crop market failures and droughts. The livestock sector has potential to create employment in rural areas with at least investments as compared to other sectors (GOR, 2012). Besides, the sector will also come under significant pressure of increasing globalization of agri-food markets. Demand for livestock products is growing primarily expected to originate due to human population growth, increasing urbanization and rising income (Ahuja et al., 2008). These developments present enormous opportunities to boost rural income and accelerate the pace of poverty reduction through promoting livestock sub-sector. However, this requires a policy regime that would facilitate sustainable growth in livestock productivity and competitiveness, for which livestock services are critical. In turn government supports livestock rearing community and their livestock by promoting Animal health, production, marketing and extension services. Comparing made to the contribution to the national economy, the support to this sector was meagre. The support in terms of investment, subsidies from the governments and rough estimate of public spending on this sector comprises about 5% of the value of its output (Parthasarathy and Birthal, 2002; CALPI, 2005). Availability, effective and satisfied delivery of animal health and production services have been traditionally funded, managed and delivered by the public sector with significant subsidies or on free basis (Kumar et al., 2006). Farmers need the quality services of technical personnel on which they depend for adopting most of the improved technologies in dairy farming. So, present study was carried out to assess satisfaction of the farmers about veterinary services provided by department of animal husbandry through veterinary personnel.

Research methodology

The present study was carried out in Rajasthan state followed an ex-post facto and exploratory research designs. Multistage random sampling was adopted for this study. The study was conducted in the state of Rajasthan as it is one of the important agricultural states in the country with high livestock population. Tonk district was selected randomly out of 33 districts for the study. A total of four tehsils in Tonk district were selected randomly. A total of 8 villages at the rate of two from each of the selected tehsils were selected randomly. Out of all the selected tehsils, a total of 120 livestock farmers at the rate of 15 livestock farmers from each of the village were
selected for the study. Data were collected personally from respondents with the help of structured interview schedule developed for the study. A pretested closed structured interview schedule was used for the farmers. Data collection was done by personal interview method, the collected data were coded, tabulated, classified and further categorized for systematic statistical analysis. The descriptive statistical tools like mean, SD, frequency, percentage and correlation coefficient were used for data analysis. The results were interpreted accordingly.

Results and Discussion

(A) Livestock farmer’s view on availability of SDAH livestock services

It is evident from the Table 1 that 50.00 per cent of farmers were found in medium category of availability of SDAH livestock services followed by high and low category of services, 39.20 per cent and 10.80 per cent, respectively. The data showed that majority of farmers belonged to medium level of availability of veterinary services, as in-time veterinary services are not available at every village due to larger area of coverage under each hospital. The livestock farmers rated various services in terms of timely availability, availability of medicines, vaccines and other required infrastructural facilities for carrying out various services and distance to the veterinary institution. The results of the availability were fairly satisfactory because majority of the respondents belonged to medium category. But, the results clearly indicate the need to take measures by the concerned authorities to increase the availability of livestock services to the farmers.

Service wise availability of SDAH livestock services for the livestock farmers:

The results of the availability of various SDAH livestock services revealed in Table 2 that service wise analysis revealed that, majority of the livestock farmers indicated the services like artificial insemination (75.00%), pregnancy diagnosis (75.80%), post-mortem (46.70%), deworming (76.70%), medical treatment (73.30%), minor surgical treatment (42.50%), gynaecological and obstetrical treatment (60.80%), livestock advisory service (57.50%) and issue of post-mortem certificate (46.70%) as always available; laboratory diagnosis (50.80%), sometimes available; and radiography (79.20%), major surgical treatment (83.30%), distribution of fodder seedlings (100%), issue of health certificate (50.80%) and supply of SDAH publications (99.20%) as never available to them.

(B) Livestock farmer’s perceived effectiveness SDAH livestock services

Distribution of livestock farmers according to their perceived effectiveness of SDAH livestock services

It is evident from Table 3, that majority of the livestock farmers rated SDAH livestock services as average (50.8%) in terms of their effectiveness followed by 44.2 per cent as excellent and 5 per cent as poor.

The livestock farmers had rated the various livestock services of SDAH based on the frequency of positive results obtained from each service. The indication of average effectiveness by majority of the livestock farmers clearly suggests the need for improving the quality of the SDAH livestock services. The results of the study were in line with that of Davidson and Ahmed (2002), Ravikumar (2007) who reported that majority of the farmers perceived the services of Department of Agriculture as average.

Service wise perceived effectiveness of livestock services

The results from Table 4 clearly indicated of the perceived effectiveness on various SDAH livestock services that, majority of the livestock farmers rated the services like issue of post-mortem (41.70), as above average; artificial insemination (68.30%), pregnancy diagnosis (44.20%), deworming (55.40%), medical treatment (53.30%), minor surgical treatment (45.80%), gynaecological and obstetrical treatment (77.50%) livestock advisory service (70.80%) issue of health certificate (57.50%) as average; laboratory diagnosis (51.70%), as below average; and radiography (70.80%) major surgical treatment (47.50%) and distribution of fodder seedlings (95.00%) and supply of publications (72.50%) as poor in their effectiveness.

(C) Livestock farmer’s satisfaction towards SDAH livestock services

Distribution of farmers based on level of satisfaction regarding veterinary services: It is clear from the Table 5 that 80.00 per cent of farmers were found in medium category of satisfaction followed by low and high category of satisfaction, 13.30 per cent and 6.70 per cent, respectively. The data showed that majority of farmers belonged in low to medium level of satisfaction towards veterinary services, as in-time veterinary services are not available at every village due to large area of coverage under each hospital. Majority of the livestock farmers belonged to medium level of satisfaction towards the various aspects of SDAH livestock services like competency of the SDAH personnel in providing various services, behaviour of the service providers and recovery of animals after treatment. The study reveals that there is lot of scope for improvement in SDAH livestock service delivery so as to make the end-users more satisfied with them.

Data in the Table 6 clearly depict that, of the provided veterinary services majority of the livestock farmers were satisfied with the services like pregnancy diagnosis (95.80%), post-mortem (80.80%), deworming (96.70%), vaccination (90.00%), medical treatment (98.30%), minor surgical treatment (65.80%), gynaecological and obstetrical treatment (95.00%) livestock advisory service (70.80%), issue of health certificate (50.00%) and issue of post-mortem certificate (87.50%).

References

Table 2: Livestock farmer’s perceived availability of various SDAH livestock services

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of services</th>
<th>Livestock farmers (N= 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Always</td>
</tr>
<tr>
<td>A.</td>
<td>Breeding services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Artificial insemination</td>
<td>75.00</td>
</tr>
<tr>
<td>2.</td>
<td>Pregnancy diagnosis</td>
<td>75.80</td>
</tr>
<tr>
<td>B.</td>
<td>Diagnostic services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Laboratory diagnosis</td>
<td>2.50</td>
</tr>
<tr>
<td>2.</td>
<td>Minor surgical treatment</td>
<td>46.70</td>
</tr>
<tr>
<td>3.</td>
<td>Major surgical treatment</td>
<td>0.00</td>
</tr>
<tr>
<td>C.</td>
<td>Prophylactic services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Deworming</td>
<td>76.70</td>
</tr>
<tr>
<td>2.</td>
<td>Vaccination</td>
<td>68.30</td>
</tr>
<tr>
<td>D.</td>
<td>Curative services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Medical treatment</td>
<td>73.30</td>
</tr>
<tr>
<td>2.</td>
<td>Minor surgical treatment</td>
<td>42.50</td>
</tr>
<tr>
<td>3.</td>
<td>Major surgical treatment</td>
<td>2.50</td>
</tr>
<tr>
<td>4.</td>
<td>Gynecological &amp; Obstetrical treatment</td>
<td>60.80</td>
</tr>
<tr>
<td>E.</td>
<td>Miscellaneous services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Livestock advisory services</td>
<td>57.50</td>
</tr>
<tr>
<td>2.</td>
<td>Distribution of fodder seedlings</td>
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</tr>
<tr>
<td>3.</td>
<td>Supply of publications</td>
<td>0.00</td>
</tr>
<tr>
<td>4.</td>
<td>Issue of health certificates</td>
<td>32.50</td>
</tr>
<tr>
<td>5.</td>
<td>Issue of post-mortem certificates</td>
<td>44.20</td>
</tr>
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</table>

Table 3: Distribution of livestock farmers according to their perceived effectiveness on livestock services of SDAH

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Category</th>
<th>Livestock farmers (N=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>1.</td>
<td>Poor (14-20)</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Average (20-26)</td>
<td>61</td>
</tr>
<tr>
<td>3.</td>
<td>Excellent (26-32)</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 4: Service wise perceived effectiveness of the livestock farmers

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of services</th>
<th>Livestock farmers (N = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Excellent</td>
</tr>
<tr>
<td>A.</td>
<td>Breeding services</td>
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</tr>
<tr>
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<td>Artificial insemination</td>
<td>5.00</td>
</tr>
<tr>
<td>2.</td>
<td>Pregnancy diagnosis</td>
<td>7.50</td>
</tr>
<tr>
<td>B.</td>
<td>Diagnostic services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Laboratory diagnosis</td>
<td>0.00</td>
</tr>
<tr>
<td>2.</td>
<td>Post-mortem</td>
<td>13.30</td>
</tr>
<tr>
<td>3.</td>
<td>Radiography</td>
<td>0.00</td>
</tr>
<tr>
<td>C.</td>
<td>Prophylactic services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Deworming</td>
<td>21.50</td>
</tr>
<tr>
<td>2.</td>
<td>Vaccination</td>
<td>19.20</td>
</tr>
<tr>
<td>D.</td>
<td>Curative services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Medical treatment</td>
<td>15.80</td>
</tr>
<tr>
<td>2.</td>
<td>Minor surgical treatment</td>
<td>6.70</td>
</tr>
<tr>
<td>3.</td>
<td>Major surgical treatment</td>
<td>0.80</td>
</tr>
<tr>
<td>4.</td>
<td>Gynecological &amp; Obstetrical treatment</td>
<td>5.80</td>
</tr>
<tr>
<td>E.</td>
<td>Miscellaneous services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Livestock advisory services</td>
<td>0.80</td>
</tr>
<tr>
<td>2.</td>
<td>Distribution of fodder seedlings</td>
<td>0.00</td>
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<td>3.</td>
<td>Supply of publications</td>
<td>0.00</td>
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<tr>
<td>4.</td>
<td>Issue of health certificates</td>
<td>5.80</td>
</tr>
<tr>
<td>5.</td>
<td>Issue of post-mortem certificates</td>
<td>9.20</td>
</tr>
</tbody>
</table>

Table 5: Distribution of livestock farmers according to their satisfaction towards livestock services of SDAH

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Category</th>
<th>Livestock farmers (N=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>1.</td>
<td>Low satisfaction (3-7)</td>
<td>16</td>
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<tr>
<td>2.</td>
<td>Medium satisfaction (7-11)</td>
<td>96</td>
</tr>
<tr>
<td>3.</td>
<td>High satisfaction (11-15)</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 6: Service wise level of satisfaction towards livestock services

<table>
<thead>
<tr>
<th>S. No.</th>
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</tr>
</thead>
<tbody>
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<td></td>
<td>Satisfied</td>
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<tr>
<td>A.</td>
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<tr>
<td>1.</td>
<td>Artificial insemination</td>
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<td>2.</td>
<td>Pregnancy diagnosis</td>
<td>95.80</td>
</tr>
<tr>
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<td>Diagnostic services</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Laboratory diagnosis</td>
<td>22.30</td>
</tr>
<tr>
<td>2.</td>
<td>Post-mortem</td>
<td>80.80</td>
</tr>
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<td>3.</td>
<td>Radiography</td>
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<tr>
<td>C.</td>
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<td>1.</td>
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<tr>
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<td>Vaccination</td>
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<td>4.</td>
<td>Gynecological &amp; Obstetrical treatment</td>
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<td>E.</td>
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</tr>
<tr>
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<td>Livestock advisory services</td>
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<tr>
<td>2.</td>
<td>Distribution of fodder seedlings</td>
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</tr>
<tr>
<td>3.</td>
<td>Supply of publications</td>
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</tr>
<tr>
<td>4.</td>
<td>Issue of health certificates</td>
<td>50.00</td>
</tr>
<tr>
<td>5.</td>
<td>Issue of post-mortem certificates</td>
<td>87.50</td>
</tr>
</tbody>
</table>

Note: Figures indicate the percentage


GOI (2013) National Livestock Policy, Ministry of Agriculture, Department of Animal Husbandry, Dairying & Fisheries


http://www.fao.org/wairdocs/lead/ae761e/ae761e00.htm#top.


N.G. Hegde, working paper for discussion of the sub-group for xii five year plan.

Data collected from 20 PPR affected farm households (HHs) of Jaganpur village of Auraiya district of Uttar Pradesh where outbreak of *Peste des petits ruminants* (PPR) disease was reported. The findings from the household survey indicated that out of total households (210) in the village, about 85% households (HHs) reared goats with an average flock size of five goats. However, study HHs reared 266 goats with an average flock size of 13. Majority of the HHs (70%) reared goats under extensive management system. The average age of the respondents was 40 years and average family size was of 6. About 35% goat farmers were landless and most of goat farmers belonged to schedule cast social group. The contribution of goats to family income was about 17%. The overall incidence rate was 57.52% however, the rate was found slightly higher in young stock than adults. Similarly, mortality rate in goats in study households was 42%. It was high due to delay in diagnosis and unavailability of veterinary services during the outbreak, as revealed by the goat farmers. Economic loss due to mortality was estimated to be Rs. 12320 per HH. Total morbidity loss due to reduction in yield, weight loss and reduction in market value was Rs.1567. The opportunity cost which include expenses on veterinary care, extra labour and other charges was estimated to be Rs. 269. The total economic loss due to disease was Rs. 14156.00 (Rs.1064.36 per goat). Unavailability of vaccines, medicines, timely veterinary services, high cost medicines and poor knowledge of diseases and their symptoms were to be found major constraints. Study suggests that there is need to organize capacity strengthening programmes on important goat diseases and their vaccination schedule.

**Key words:** PPR, mortality, opportunity cost, constraints

**Introduction**

*Peste des petits ruminants* (PPR) is an acute febrile viral disease of small ruminants (Singh et al., 2004). This disease is endemic in India and considered to be a major constrain in the development of goat industry due to high morbidity (50-90%) and case fatality (50-85%) rates (PDADMAS, 2011). Up to 90% of the sheep and goats on a farm may die within few days which have a severe economic impact (Anderson and McKay, 1994; Shaila et al., 1996). Soni et al. (2013) also found young animals are more susceptible than adult. The estimated value of economic losses due to PPR alone at country level was 1800 million Indian rupees every year in India (Balamurugan, 2011). The present study focuses on post outbreak assessment of economic losses due to PPR in Jaganpur village of Auraiya district where outbreak occurred.

**Materials and Methods**

This sample study was conducted in the Jaganpur village of Auraiya district of Uttar Pradesh, where PPR outbreak was reported during 2012-13. Out of 70 PPR affected households (HHs), 20 HHs (29% of PPR affected households) were selected randomly to study socio-economic profile of the goat rearing households, production system, disease incidence, and mortality, direct and indirect losses which also include opportunity cost incurred due to disease. Though the goat rearing households was small however, the goat population in these households was sizable in number. The collected data were tabulated, classified and further categorized for systematic and suitable analysis. Morbidity rate was measured as proportion of number of infected goats to population of goats. Similarly, mortality rate was calculated as a proportion of number of goats died to population of goats in study households. The case fatality rates measured as proportion of number of goat died due to disease to total number of goats infected. Garrett's ranking technique has been used to analyse the goat farmers' perception about the major constraints in goat production.

**Results and Discussion**

Village Jaganpur of Auraiya district has 210 households. Out of which 85% of households were having goats. The goat population in the village was 950 with an average flock size of 5.34 goats. 370 goats were infected with PPR in the village. As per respondents, about 70% goat owning households were found to affect with PPR. The majority of goats in the village were reared under extensive and semi-intensive management system with other livestock. The socio-economic attributes of goat farmers indicated that the average age of goat farmers was about 40 years. It was also noted that the majority of goat farmers were educated up to middle and high school level (70%). The average family size of the goat farmers was 6 members. The major source of income was wages, followed by agriculture. Goat rearing contributes about 17% to total family income. However, this income often fluctuates due to various diseases in goat production. Composition of goat flock mainly constituted with adult (55%) and kids/young stock (45%). The average flock size of study households was 13 goats.

It could be observed from Table 2 that overall morbidity, mortality and case fatality rate across the functional categories of goats was 57.5%, 42.1% and 73.2%, respectively. These findings are in agreement with Thornbore and Sinha (2009); Hossain et al. (1996). The average economic loss per study household was 1800 million Indian rupees every year in India (Singh et al., 2004). The average economic loss per household was Rs. 269. The total economic loss due to disease was Rs. 14156.00 (Rs.1064.36 per goat). Unavailability of vaccines, medicines, timely veterinary services, high cost medicines and poor knowledge of diseases and their symptoms were to be found major constraints. Study suggests that there is need to organize capacity strengthening programmes on important goat diseases and their vaccination schedule.

Accepted: 03.08.2016

**ABSTRACT**

**ECONOMIC LOSSES DUE TO PESTE DES PETITS RUMINANTS (PPR) DISEASE IN GOATS: A POST OUTBREAK SAMPLE STUDY IN AURAIYA DISTRICT OF UTTAR PRADESH**

A.K. Dixit, Vijay Kumar, Ashok Kumar, Braj Mohan and B. Rai

ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura-281 122, Uttar Pradesh, India
Epidemiology and economic losses in the study households (Rs./HH)

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Value</th>
</tr>
</thead>
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<td><strong>Epidemiology</strong></td>
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</tr>
<tr>
<td>Morbidity (%)</td>
<td>57.5</td>
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<tr>
<td>Mortality (%)</td>
<td>42.1</td>
</tr>
<tr>
<td>Case fatality (%)</td>
<td>73.2</td>
</tr>
<tr>
<td><strong>Economic Losses (Rs./HH)</strong></td>
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</tr>
<tr>
<td>Mortality loss</td>
<td>12,320.00 (926.32)</td>
</tr>
<tr>
<td>Morbidity loss:</td>
<td></td>
</tr>
<tr>
<td>Milk loss due to reduction in yield</td>
<td>374.42 (28.15)</td>
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<tr>
<td>Losses due to weight loss, reduction in market value etc.</td>
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<td>Total morbidity losses</td>
<td>1567.1 (117.83)</td>
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<td>Expenses on veterinary care on survive goats</td>
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<td>Extra labour charges</td>
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<tr>
<td>Other charges</td>
<td>41.00 (3.08)</td>
</tr>
<tr>
<td>Total opportunity cost</td>
<td>268.94 (20.22)</td>
</tr>
<tr>
<td><strong>Total losses due to disease</strong></td>
<td>14156.04 (1064.36)</td>
</tr>
</tbody>
</table>

(Figures in parentheses indicate per animal losses)

Table 2: Constraints in goat production

<table>
<thead>
<tr>
<th>Constraints</th>
<th>Mean Scores</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unavailability of vaccines</td>
<td>12.19</td>
<td>I</td>
</tr>
<tr>
<td>Unavailability of medicines</td>
<td>10.48</td>
<td>II</td>
</tr>
<tr>
<td>Lack of veterinary services</td>
<td>9.12</td>
<td>III</td>
</tr>
<tr>
<td>High cost of services and medicines</td>
<td>8.88</td>
<td>IV</td>
</tr>
<tr>
<td>Poor knowledge of diseases and their symptoms</td>
<td>7.23</td>
<td>V</td>
</tr>
<tr>
<td>Poor knowledge of home remedies/Ayurveda/Herbal medicines</td>
<td>5.30</td>
<td>VI</td>
</tr>
<tr>
<td>Lack of transportation and other infrastructure</td>
<td>4.27</td>
<td>VII</td>
</tr>
</tbody>
</table>

The household was estimated to be Rs. 14156.04. Out of which Rs. 12320 was loss due to mortality of affected goats. This loss accounted for 87% of total economic loss, possibly due to high rate of case fatality (73%). Moreover, no vaccination against PPR before outbreak, delay in diagnosis and unavailability of veterinary services during the outbreak were also the important reason for high magnitude of mortality loss. The high severity of the disease was reported by the goat farmers first time as they recalled their memories. The morbidity loss was estimated to be Rs. 1567.10. This includes loss due to reduction in milk yield and reduction in market value due to weight loss of Rs. 374.42 and Rs.1192.68, respectively. The reduction in market value due to body weight loss alone constituted about 9% of total economic losses due to PPR outbreak. The average opportunity cost born by the household was Rs.268.94. This includes expenses on veterinary care on survived goats (Rs.164.5), extra labour charges (Rs.63.44) and other charges (Rs.41.00). Considering average flock size of study household is 13.3 goats, the loss per goat due to PPR disease was estimated to be Rs.1064.36.

Unavailability of vaccines, medicines and lack of veterinary services were found to be the most important constraints in goat production with their mean scores of 12.19, 10.48 and 9.12, respectively (Table 2). Knowledge gap on goat diseases, home remedies with herbal medicines and poor infrastructure like transportation and market were the other constraints in goat farming as revealed by the goat farmers.

The sample study has estimated the economic losses loss due to outbreak of *peste des peste ruminants* disease in goat. Though the number of study goat farmer is small but the population of goats reared by these farmers were good in number. The major losses accrued due to mortality followed by reduction in market value of animals. The magnitude of losses seems to be large because of post outbreak estimates. Studies done in the past revealed that if the annual preventive goat health calendar been adopted, losses could have been reduced by about 70% (Kumar *et al.*, 2002). There is need to made goat farmers aware about important diseases in goat and their vaccination schedule.

**Acknowledgements**

The authors are thankful to the, Director, ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura (Uttar Pradesh) for providing the facilities to pursue this work.

**References**


CUSTOMS AND BELIEFS OF TRIBALS ASSOCIATED WITH ANIMAL HUSBANDRY IN BANSWARA DISTRICT OF RAJASTHAN

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ABSTRACT

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The study documents different customs and beliefs of tribals associated with different livestock management practices. A total of 120 tribal families were selected from 8 selected villages of 2 tehsils namely Bagidora and Kushalgarh in Banswara district of Rajasthan. Data were collected through personal interview and detailed discussion with respondents. Livestock play the central and identifying role in various rituals, customs, traditions and festivals. Livestock rearing has influenced the culture life of tribals to a great extent. Tribals tie a thread/metal ring (tanti) around the neck of diseased animal in the name of their local God Kallaj. They have belief that this thread will help to recovery of their animal from diseases. There is tradition to collect holy water from “Gomukh” at the temple of “Ghotiaambamata”. This water is used for washing the ulcers of FMD with the belief of quick recovery of their animal from disease.

Key words: Tribals, customs, belief, animal husbandry, Rajasthan

Introduction
The richness and variety of tribal life is a most valuable heritage of the great Indian tradition. The tribal population in India is the second largest in the world which contributed 8.6 per cent (10.4 crores) of the total population (121.01 crores) in the year 2011. The tribals are predominantly rural (89.90%), poorest social group and also overwhelmingly illiterate. Tribal communities live in about 15 per cent of the country’s areas in various ecological and geo-climatic conditions ranging from plains and forests to hills and inaccessible areas. Tribal groups are at different stages of social, economic and educational development. While, some tribal communities have been adopted a mainstream way of life (GOI, 2010). District of Banswara has highest tribal population in the state having 14.86 per cent of the total tribal population of the state (Anonymous, 2011). Bheel and Meena are the main tribal communities in the district (Salvi, 2012). For centuries, the tribal groups have remained outside the realm of the general development process due to their habitat in forests and hilly tracts. Even when they migrate into villages, they often live apart from the village (Maharatna,2005). Tribals are people living in isolation with their traditional values, customs, beliefs and myth intact. Tribals have rich heritage along with their innocent life style. As they are living in hilly areas and forests, they have some peculiar characters like indications of primitive traits, distinctive culture and shyness of contact with other communities, geographical isolation and backwardness etc. So, for their development central and state governments are implementing different programmes and schemes since 1951. After the Ministry of Tribal Affairs were constituted in 1999, there is more focus on development of Schedule Tribes in Indian society. The main obstacles to economic development of Schedule Tribes are destruction of forests, illiteracy, indebtedness, lack of awareness about the schemes for them, strict follow of traditions and customs and in-effective implementation of schemes (Subramanyachary, 2013). Hence, there should be more commitment by both central and state government and local bodies to develop schedule tribes in the society.

The customs, rituals, art, ceremonial functions, folkways, traditions, etc. of any specific culture contributes to the development of particular pattern of life (Parikhand Patel, 1989). An attempt was made to document some of the customs and beliefs of tribals associated with animal husbandry based on observation, interaction and discussion with some of old tribals, key communicators and family members.

Materials and Methods

The present study was conducted in Banswara districts of Rajasthan. District was selected purposively on the basis of highest tribal population in the state. Out of total five tehsils in the district, Bagidora and Kushalgarh were selected purposively due to the existence of large number of tribal families. The district is predominantly inhabited mainly by Bhils, Bhil Meenas, Damor, Charpopas, Ninamas etc. A village wise comprehensive list of number of tribal families residing in different villages was prepared with help of panchayatsamities, village patwari and local tribals. Four villages selected from Bagidora tehsil and another four villages from Kushalgarh tehsil. Thus, a total of eight villages have been selected from two selected tehsils of district. Total 15 tribal families residing in selected village, owning large number of livestock were select randomly from each village. Thus, a total of 120 tribal families were selected randomly from eight selected villages. Information was obtained through personal observation, consultation with tribal family members, interaction dialogue, and detailed discussion with key informants, aged persons and housewives.

Results and Discussion

The paper provides an overview of some of customs and beliefs as reported by of tribals associated with animal husbandry are as follows.

1. Tribals of study area have custom of giving livestock as “dowary” in marriage of their daughters. Camel is generally

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given as “dowry” by Raikas during marriage of their daughter (Rajput and Tripathi, 2006).

2. Tribals considered livestock as status of symbol. According to them, tribal have more livestock has more prestige in their society. Keeping/owing of large camel herd is considered as matter of reputation and high prestige in society (Kohler-Rollefsion, 1994; Taper, 1985; Khanna, 1998).

3. Diwali is considered as festival of prosperity. On the occasion of “Diwali” (Kartic purnuma), all the villagers gather at common place in the village along with their livestock. One of the tribal among them lying in middle of ground, all of the other tribes perceived as this particular lying person possess divine power of god. All the livestock of the village run together crossing the lying person. If all animals crossed without touching the lying person, it considered as man have divine power of god. There is a belief that, if white coloured cow/cows reach to another corner of ground first then other animals. They considered that, this season will be prosperous for livestock vice versa if black cow reach another corner first then season will not prosperous for livestock.

4. There is custom among the tribals of study area to purchase a male kid and rear it upto maturity age and after cutting the edge of ear the animal left free in village in the name of local God. They believe that by doing so their God will bless them and fulfill their needs.

5. If animal does not conceive after all efforts, the tribals pray to their local God that, if their animal will conceive, they will prepare sweet (Prasadi) and/or ghee from milk produced by such animals after calving. This Prasadi sweet was distributed among family member and villagers. Raika pastoralists also prepare kheer (sweet rice boiled with milk) from first milk of camel after parturition. It is firstly offered to their local God Pabuji and Bhomiaji. After that, prasad (kheer) is distributed among family members. Raika believe that Pabuji and Bhomiaji provide them strength to take care of camel (Rajput and Tripathi, 2006).

6. Tribals tie a thread/metal ring (tanti) around the neck of diseased animal in the name of their local God Kallaji. They have belief that this ring will be help to recovery of their animal from diseases. Raikas believe that if a tanti (cotton thread) is tied around the neck of camel bitten by snake or scorpion, in their name of God Nakat Banna, the life of camel will definitely be saved (Rajput and Tripathi, 2006). The farmers believe that by tying dora (magical thread) around the necks, animals can overcome the many diseases (Rihan and Kumar, 1980). Lodha tribe of West Bengal believe that tying of Suthlisan (one kind of jute) round the neck of goat helps recovery from sores and abscess at mouth and Santal tribe paint lime on the horn of cattle and tie kari (snail) for protection from evil eye (Dana, 1998).

7. On the next day of Diwali (Goverdhan pooja), tribals decorate their bullock with mahendi and flowers. They worship their bullock and provide special ration ad lib. They have belief that in doing so, they will be economically prosperous in future.

8. Soon after calving, the colostrum is being fed to the newborn calf and remaining (surplus) colostrum is fed to dam. They believe that, this ration make dam healthy and more productive due to containing of all nutrients and energy source.

9. In case of death of any family member, they donate/gift a healthy animal to the priest of village with belief that departed soul will rest in peace in heaven.

10. At the time of Holi, tribals collect ash from temple of the village goddess “Ghotiaambamata”. The ash is wrapped in the red cloth and hangs on tree at village entrance. Tribals believe that, this ash will prevent the occurrence of any outbreak of diseases among livestock in the village.

11. Tribals reported that the dead animals are thrown away in open areas at distance of about 1-2 km away from houses or in jungle. Carcass is neither buried nor sold and the village sweeper skin off. Gujjars of Nainital district also had the practices to throw away their dead buffaloes in the forest (Samajdar, 2000).

12. For prevention of FMD, tribals keep a piece of aak plant in the shed of their animals.

13. There is also a tradition to collect holly water from “Gomukh” at the temple of “Ghotiaambamata”. This water use for washing the ulcers of FMD affected animal with the belief that, this water will cure their animal.

14. In another belief, they wash ulcers of FMD affected animal by collecting water from fish pond. They also have belief that, milk production of their animal will decrease in the presence of snake in animal shed. Likewise, production of ghee also reduced from animal milk.

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References


INFLUENCE OF SOCIO-ECONOMIC FACTORS ON THE KNOWLEDGE LEVEL OF POULTRY ENTREPRENEURS IN JHUNJHUNU DISTRICT OF RAJASTHAN

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ABSTRACT

Influence of socio-economic factors like age, education, experience, organizational participation and mass media exposure on the knowledge level of poultry entrepreneur in Jhunjhunu district of Rajasthan state was studied. Most of the poultry farmers belonged to 31-50 years age group (60%), had education level at least up to matriculation (60%) and were having poultry farming experience of 5.1 to 10 years (58.66%). Majority of the poultry entrepreneurs had medium level of organizational participation (64%) and mass media exposure (86.66%). Knowledge level of poultry entrepreneurs was found to be medium (65.33%). Correlation between the factors i.e. education, experience and mass media exposure under study and the knowledge level of the poultry entrepreneurs was found moderate to high, positive and significant. However, negative correlation was observed between age of the poultry entrepreneurs and their knowledge level. Correlation between the organizational participation of the poultry entrepreneurs and their knowledge level of found non-significant.

Key words: Adoption, family income, poultry entrepreneurs, socio-economic factor

Introduction

Jhunjhunu district is situated in the North-eastern part of Rajasthan and accounts for 4, 49,248 poultry birds among which 2,95,150 are available at commercial farms (Census 2007). With rapid growth in population, providing food security has taken the government as well as planners in quandary. Increasing production in agriculture front has become a matter of concern for every government due to limited land availability. It cannot be gainsaid that, “agriculture production alone can meet the demand of human population”. In this context, production through poultry sector has become centre stage as means of alternative source protein.

Entrepreneurship is regarded as one of the most crucial factors in the economic development of every region of the country. It widens the horizons of economic development even in the socially and industrially backward regions. Dynamic entrepreneurs are considered to be the agent of change in a society. Entrepreneurs play a very important role in generating new employment and setting up new business. The entrepreneurs are key persons of any country for promoting economic growth and technological change. The appearance of their activities, i.e. the development of entrepreneurship is directly related to the socio-economic development of the society.

In last decade, poultry enterprises in Jhunjhunu district had fluctuated business compared to other enterprises. However, the fact is that most of them are not sustainable in order to utilize the potential and keep their growth continuum. It is necessary to formulate strategies suitable for stimulating, supporting and sustaining the development of poultry entrepreneurship. Such a strategies need to be in congruence with realities and especially take cognizance of that poultry entrepreneurs face within current economic system of liberalization and globalization (GATT/WTO policies).

Management practices are a composite factor involving several components. Among them knowledge regarding poultry management practices, attitude towards poultry management practices, ability in planning, ability to make rational decisions, ability to mobilize resources, ability to coordinate activities, timely adoption, efficient use of resources, competence in evaluation and ability in rational marketing are important. For healthy adoption of improved management practices of poultry enterprise, one should have knowledge about it. The present study is an attempt in this direction.

Materials and Methods

The study was conducted in Jhunjhunu district of Rajasthan state in the month of June to November, 2014. A sample size (n=75) was equally divided (25 each) among the poultry entrepreneurs of Khethri, Chirawa and Nawalgarh tehsils of the district.

Ex-post-facto research design (Kerlinger, 1976) was used in present investigation. The tool used for the study was interview schedule and was pre-tested for its validity. The interview schedule was prepared by keeping in view the objectives of the study and was common for all the respondents. By personal interview method from all the 75 respondents, responses were recorded in the schedule. Frequency and percentage were marked for the various items.

The age of respondents is categorized into three groups i.e., young age group (up to 30 years), middle age group (between 31 to 50 years) and old age group (above 50 years). Education level was measured with the help of socio-economic status (SES) scale developed by Pareek and Trivedi (1963) with due modifications. One score was given to each formal education status of the respondent i.e., illiterate (0), can read and write (1), up to 8th standard (8), 9th to 10th standard (10), up to 12th standard (12), and college level and above education.
According to their experience in poultry farming, the respondents were classified into four categories as 1 to 5 years, 5.1 to 10 years, 10.1 to 15 years and above 15 years of experience. Organizational participation in the present study was operationalized as the degree to which an individual is associated with different formal organization and was measured by using SES scale developed by Pareek and Trivedi (1963). The score was given as no membership in any organization (0), Membership in one organization (1), membership in more than one organization (2) and holding position in organization (3). Mass media exposure refers to the frequency of reading newspaper, magazine and other literature relating to the poultry farming as well as use of radio and television, also attending the agricultural exhibition, demonstration and personal visit by the respondents. This variable was quantified by assigning scoring pattern for each medium as regular use by media by respondents (2), occasionally using media by respondents (1) and never using media by respondents (0).

Knowledge about recommended poultry production technology was measured with the help of teacher made knowledge test based on the scale developed by Jha and Singh (1970) and was administrated with slight modifications to suit the present study. The knowledge index was calculated with the help of following formula,

$\text{Knowledge index (K}_i\text{)} = \left(\frac{X_i + X_2 + \ldots \ldots \ldots + X_n}{N}\right) \times 100$

Where,

$K_i =$ knowledge index

$X_1 + X_2 + \ldots \ldots + X_n =$ Total number of correct answers. i.e., total score

$N=$ Total number of items in the test

The respondents were grouped into three categories with the help of mean and standard deviation i.e., low knowledge (<Mean - S.D), medium knowledge (Mean ± S.D) and high knowledge (> Mean + S.D).

Relationship between socio-economic characteristics of poultry farmers and their knowledge level regarding recommended poultry farming practices was ascertained by calculating correlation co-efficient ($r$) by SPSS version 14. The data were tabulated and statistically analyzed.

Results and Discussion

Distribution of the respondents according to their age, education level, experience, organizational participation, mass media exposure in Jhunjhunu district of Rajasthan are shown in Table 1.

Result showed that majority (60 per cent) of the poultry entrepreneurs belonged to middle age group followed by old age group (21.33 per cent) and young age group (18.66 per cent) in Jhunjhunu district of Rajasthan. The possible reason for this might be that the middle aged respondents had better experience and enough maturity for taking decision for better profitable occupation. Secondly, this occupation might be taken up by them due to unemployment. Old ones were unable to do poultry farming and young ones are in need of white collar job. Present findings were similar to those reported by Thorat (2005) and Esiobu et al. (2014).

There were only 9.33 per cent of respondents who had education level below 9th standard. Majority (60.00 per cent) of poultry owners were having their education level up to 10th standard. Out of these 18.66 per cent and 12.00 per cent of respondents had their education level up to college or above and up to 12th standard, respectively. From the above fact, it can be concluded that great majority of the respondents had education from primary to secondary level of education. Their awareness regarding importance of education to improve their economic condition might be the possible explanation for these types of findings. Similar line of findings was reported by Razzaq (2011), Babalola (2014) and Esiobu (2014).

In respect to experience level of the poultry entrepreneurs, it was found that majority (58.66 per cent) were having 5.1 to 10 years of experience followed by 1 to 5 years and 10.1 to 15 years of experience by 25.33 per cent and 16.00 per cent of respondent, respectively. In Jhunjhunu district none of the poultry entrepreneurs have more than 15 years of experience, which may be possible due to the reason that poultry entrepreneurs in Jhunjhunu district have adopted this profession on large scale in the last decade only. Olagunju and Babatunde (2011), Babalola (2014), and Esiobu et al. (2014) also reported similar findings.

Study on organizational participation of poultry entrepreneur in Jhunjhunu district showed that majority (64.00 per cent) of poultry farm owners had medium level of organizational participation followed by low and high level of organizational participation by 25.33 per cent and 10.66 per cent of poultry entrepreneurs, respectively. Jatto (2012), Babalola (2014) and Esiobu et al. (2014) were in conformity that majority of respondents had middle level of organizational participation.

In Jhunjhunu district, 86.66 per cent of the poultry entrepreneurs had medium level of mass media exposure followed by 13.33 percent who had high level of mass media exposure. None of the poultry entrepreneur in Jhunjhunu district falls under the category of low level mass media exposure, which might be due to high literacy rate in Jhunjhunu. Moreover, it might also be due to availability of mass media and awareness of the respondents regarding programmes broadcasted and telecasted on radio and television and digital media penetration, respectively as well as availability of farm literature published by various agencies. Similar results are reported by Siddhartha (2001), Thorat (2005) and Bothokho and Oladete (2013).

The level of knowledge of poultry entrepreneurs about poultry management practices was also studied and results revealed that 65.33 per cent of the poultry entrepreneurs had medium level of knowledge followed by 18.66 and 16 per cent of the respondents in the category of low and high knowledge level, respectively. Similar results were obtained by Amudha and Veerabhadraiah (2000) and Thorat (2005).

Relationship between various socio-economic factors under study and knowledge level of poultry entrepreneurs about recommended poultry practices is shown in Table 2. Results indicate that all the factors except age had positive relationship with the knowledge level of the poultry entrepreneurs. Except organization participation of the poultry entrepreneurs, all the factors were significantly related to the dependent variable. Range of correlation coefficient was found to be moderate to high. Thus, young poultry entrepreneurs were observed superior in planning, organizing, directing, communicating, leading, supervising, controlling and decision making than
Table 1: Distribution of the respondents according to their age, education level, experience, organizational participation, mass media exposure and knowledge level of poultry entrepreneurs in Jhunjhunu district of Rajasthan

<table>
<thead>
<tr>
<th>Age of the Respondents</th>
<th>Number</th>
<th>Per cent</th>
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</thead>
<tbody>
<tr>
<td>S. No.</td>
<td>Category</td>
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</tr>
<tr>
<td>1</td>
<td>Young age (up to 30 years)</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Middle age (31 to 50 years)</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>Old age (above 51 years)</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>75</td>
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Table 1 continues:

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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>Total</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Experience of Poultry Entrepreneurs</th>
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<tbody>
<tr>
<td>S. No.</td>
</tr>
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</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<tr>
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<table>
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</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
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<table>
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<th>Exposure to Mass Media</th>
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</tr>
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</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
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<td>Total</td>
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<table>
<thead>
<tr>
<th>Knowledge Level of Poultry Entrepreneurs</th>
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<tbody>
<tr>
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<tr>
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</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Table 2: Relationship between characteristic of poultry farmers and their knowledge level of recommended poultry practices (n= 75)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Independent Variable</th>
<th>Correlation coefficient</th>
<th>Significance level</th>
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<td>1</td>
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<td>2</td>
<td>Education</td>
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<td>**</td>
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<tr>
<td>3</td>
<td>Experience</td>
<td>0.295</td>
<td>**</td>
</tr>
<tr>
<td>4</td>
<td>Organizational participation</td>
<td>0.130</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>Mass media exposure</td>
<td>0.328</td>
<td>**</td>
</tr>
</tbody>
</table>

*Significant at 0.05 level of probability. **Significant at 0.01 level of probability. NS-Non Significant