Amelioration of chicken infectious anaemia virus induced immunosuppression by protein and immunoglobulin supplementation in chicks

Article *in* Veterinary Practitioner · June 2011

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TIME COURSE OF APOPTOSIS INDUCED BY INFECTIOUS BURSAL DISEASE VIRUS IN CHICKEN EMBRYO FIBROBLAST CELLS

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ABSTRACT

A therapeutic approach, which uses viruses for the treatment of cancer termed as oncolytic virotherapy has recently emerged. Since cell death can be caused by either necrosis or apoptosis, an investigation was carried out for the types of cell death that occur in Chicken Embryo Fibroblast cells (CEF), infected with infectious bursal disease virus (IBDV). We exposed CEF cells to IBDV and examined the response of these cells to this virus. Light microscopy and Acridine Orange fluroscent microscopy was performed 0, 1, 5, 8, 16, 48 and 72 h pi showed that, within infected CEF cell monolayers, cells underwent programmed cell death after application of IBDV, whereas uninfected cells showed sporadic apoptosis. Incidentally, the apoptotic efficacies in infected cells were increased in a time dependent manner. In conclusion, host cell apoptosis can be induced by IBDV in CEF cells. Ability of IBDV to induce apoptosis may fulfill the urgent need for innovative therapeutic strategies to treat aggressive metastatic cancers clinically that are incurable with standard therapeutic approaches.

Key words: Apoptosis, Infectious Bursal disease, Virus, Chicken Embryo Fibroblast Cells

Complex visible and measurable contortions of the cell membrane and organelles of a cell in response to external and internal stimuli to activate an intrinsic suicide programme and systematic destroying itself is called as apoptosis. Apoptosis plays an essential part in homeostasis, development and disease by facilitating the removal of unwanted, damaged or infected cells. Apoptotic cells show characteristic morphological changes, including shrinkage, blebbing of the plasma membrane, chromatin condensation and DNA fragmentation (Kerr *et al*. 1972). These changes are distinct from those seen in cell death caused by necrosis, but the pathways that lead to necrosis and apoptosis are not entirely separate. Several viruses have been found to induce apoptosis (Roulston *et al*., 1999; Hardwick, 1997; O'Brien, 1998; Radvi and Welsh, 1995), and during virus infection, a variety of signal transduction pathways leading to apoptosis of infected cells can be activated (Mortola *et al.,* 2004) for e.g., human acquired immunodeficiency virus type 1 (HIV-1) (Kumar *et al*., 1998; Meyaard *et al.,* 1992), human cytomegalovirus (hCMV) (Rodems and Spector, 1998), adenovirus (Suomalainen *et al*., 2001), certain DNA tumor viruses like chicken anemia virus (Jeurissen *et al.,* 1992; Noteborn *et al*., 2004), feline leukaemia virus (Rojko *et al.,* 1992), Newcastle disease virus (Lam *et al*., 1995; Lam and Vasconcelos, 1994; Ravindra *et al*., 2008), avian reoviruses (Labrada *et al*., 2002), bluetongue virus (Mortola *et al*., 2010) and many more induces apoptosis in target tissues and cells.

The use of viruses for the treatment of cancers began before the 1950s (Ackermann and Kurtz, 1952; Alemany, 2007). Several viruses have been tested in various animal models for their anti-cancer potency, tumour specificity, efficacy and safety (Ring, 2002). Distinct advantages of oncolytic viruses in comparison to routine chemotherapy or radiotherapy are firstly drugs can inhibit an active target but cannot

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detect or replace the missing function of a protein. During cancer, tumor suppressors are missing targets which leads to the progression of the ontogenesis and secondly, a drug does not amplify itself. Thus a very high concentration is needed to reach every tumor cell. Due to the abovestated shortcoming of conventional drugs oncologists seek for innovative of virotherapy. Antitumor effect is elicited by a number of viruses for example, adenovirus (Bischoff *et al*., 1996; Cervantes-Garcia *et al*., 2008; Kelly and Russell, 2007; Rein *et al*., 2006; Liu *et al*., 2009; Fechner *et al*., 2007; Wei *et al*., 2009), Newcastle disease virus (Ravindra *et al*., 2008), herpes simplex virus (Liu *et al*., 2003), measles virus (Taqi *et al*., 1981), reovirus (Coffey *et al*., 1998; Harrington *et al*., 2010), autonomous parvovirus (Rommelaere and Tattershall, 1990), vesicular stomatitis virus (Stojdl *et al*., 2000), mumps virus (Asada, 1974) etc.

Infectious bursal disease virus (IBDV) is an important acute highly infectious lymphocidal immunosuppressive virus of chickens (Lasher and Davis, 1997; Kumar, 2009). IBDV causes immunosuppression in young chickens by infecting lymphocytes in the bursa of Fabricius (Hudson *et al*., 1975; Rosenberger and Gelb, 1976). This disease is characterized by gross and microscopic lesions in the bursa of Fabricius (van Veen, 2001), which can be attributed to lysis of lymphoid elements (Kaufer and Weiss, 1976). Despite the use of vaccines to control IBDV, it continues, directly and indirectly via immune suppression, to cause economic losses in the poultry industry.

The genome of IBDV consist of two segments of double stranded RNA, larger segment A, encodes five viral proteins (VP1-5) (Sanchez and Rodriguez, 1999). The smaller segment B encodes VP1 protein, an RNA-dependent RNA polymerase (RdRp) for IBDV genome replication (von Einem *et al*., 2004; Zheng *et al*., 2006). The large segment A contains two partly overlapping open reading frames, encoding a precursor polyprotein VP243 and a non-structural protein VP5 respectively (Mundt *et al*., 1995). VP243 polyprotein was cleaved autoproteolytically to give rise to the viral structural proteins VP2, VP3, and a viral protease, VP3 is a groupspecial and major immunogenic protein of IBDV (Birghan *et al*., 2000; Jagadish *et al*., 1988; Kibenge *et al*., 1997), VP5 is a nonstructural protein, no evidence shows that VP1 and VP5 proteins could elicit antibodies in exposed chickens (Mundt *et al*., 1995); VP4 protein is described as a protease produced by the polyprotein VP243 during cleavage (Birghan *et al*., 2000; Feldman *et al*., 2006; Kibenge *et al*., 1997; Lejal *et al*., 2000; Sanchez and Rodriguez, 1999) and found it combining with type II tubule (Granzow *et al*., 1997).

Earlier reports suggests that IBDV induces apoptosis in chicken peripheral blood lymphocytes (PBLs) (Vasconcelos and Lam, 1994), chicken embryos (Vasconcelos and Lam, 1995), chicken embryonic fibroblast (Tham and Moon, 2006) chicken B-lymphocyte cell line (Rodriguez-Lecompte *et al*., 2005). Reports also suggests morphological evidence of apoptosis in chickens infected with infectious bursal disease virus (Lam, 1997), in-situ apoptosis has also been shown in the lymphoid tissues of chickens infected with virulent strains or an attenuated vaccine strain of IBDV (Tanimura and Sharma, 1998). Chicken embryo fibroblasts (CEFs) and Vero cells infected with infectious bursal disease virus (IBDV) exhibited the biochemical feature of apoptosis, viz. the characteristic laddering pattern of DNA fragmentation (Tham and Moon, 1996). Keeping in view the above literature IBDV may act a good candidate for cancer virotherapy. Thus the current study was therefore conducted to investigate the structural and nuclear apoptotic changes in IBDV infected CEF cells.

Materials and Methods

Virus culture

IBDV vaccine strain IM+ was provided by Molecular Biology laboratory IVRI Izatnagar, in secondary CEF culture. The inoculum, containing virus propagated and titrated in chicken embryo fibroblasts, had a $TCID_{50}$ of $10^{5.5}$/0.1 ml. The virus was stored at -20°C for future use. The titre of the purified virus was determined by using method described by Reid and Munch *et al.* (1938).

Culturing the chicken embryo fibroblasts

Fertile eggs were obtained from healthy flock. Eggs were cleaned and pale shelled eggs were selected to simplify candling. After laying they were incubated for 10 days at 37° C, 40-70% humidity and good aeration and turned twice daily. After 6 days they were candled, infertile and dead eggs were discarded. On the day of culture, those with satisfactory development of chorioallantioic blood vessels and showing embryonic movement were marked with pencil to indicate the limits of the air sac. The eggs were placed in an egg cup, air sac upwards and wiped clean with spirit. The shell was broken with the sharp end of a sterile forceps, and the membrane was lift.

The chicken embryo fibroblast (CEF) primary culture was set up from 9-11 day old embryonated chicken eggs under sterile conditions. With a bent forcep the embryo was picked and placed in a petridish containing PBS. The embryo was dissected in the dish, the head, limbs and viscera were removed and discarded. The fibroblastic tissue was picked and transferred into a wide neck bottle

containing PBS. The tissue was minced finely with scissors, and washed several times in PBS to remove blood cells and debris. These minced tissues were suspended in 0.25% trypsin in a conical flask and stirred for 7 minute using magnetic stirrer. Supernatant was poured into another conical flask and medium was added (GMEM with 10% FBS) and stored at 4°C to neutralize trypsin activity. Trypsinization was repeated for 15 minute for the remaining sediment in conical flask. Supernatant was added to previous supernatant in conical flask and mixed properly with growth medium to neutralize trypsin activity. This cells suspension was passed through muslin cloth, centrifuged at 250 g for 10 minute. The supernatant was discarded leaving behind cell pellet. The cell pellet was washed, resuspended in growth medium and centrifuged at 250 g for 10 minute. Incubation was performed at 37° C until monolayer is formed (2-3 days). Cells finally diluted in DMEM media with 10% FBS to the final concentration of $2x10^6$ cells/ml, were dispensed in six well plate and incubated at 37°C till monolayer is formed (Wong *et al*., 2007).

Infection of CEF cells with virus

When cells have formed a monolayer, growth medium was removed, virus was inoculated in maintenance medium (MEM, Eagles base with 1-2% bovine serum) and incubated for one hour. Virus was allowed to adsorb by tilting the tissue culture flask after every 15 minutes. After one hour, the medium was removed from the tissue culture flask and fresh maintenance medium with 10 % of fetal bovine serum was added. Secondary CEF cultures were used in all of the following experiments and maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, USA) in 5% CO2 at 37°C. All secondary CEF cultures were seeded at 1×10^5 cells in 6 well plates. At 24 h after seeding, the culture medium in each plate was removed and the cells were infected with virus at an MOI of 1 in 1.5 ml DMEM, whereas the mock-infected group was treated with an equal volume of virus free medium. At 2 h post-infection (pi), each plate was replenished with 8 ml DMEM supplemented with 1% FBS. The progression of apoptosis and cell viability was monitored 0, 1, 5, 8, 16, 24, 48 and 72 h pi. One 6 well plate was sampled for each of the two assays at each of the time points indicated and the assays were carried out immediately after sampling.

Harvesting of CEF cells

For harvesting of cells for further experimentation the cells are detached from the surface with the help of Trypsin Versine Glucose Solution. Briefly, TVG was poured on to the monolayer in 25 cm2 flask, trypsin was allowed to act for one minute. Cells were separated with the flick of hand. The cells were collected in 1.5 ml centrifuge tube and centrifuged at 250, for 10 minutes using a Hermle BHG centrifuge. Cells were washed with 1 ml of cold PBS thrice. Pellet was used for further analysis.

Acridine Orange Staining after DNA Denaturation

For adherent CEF cells, supernatant (medium and floating CEF cells) were transferred to 15 ml tubes. The rest of the adherent cells were detached with PBS-EDTA, Dulbecco's phosphate buffered saline (Invitrogen, USA) containing 1 mM EDTA. The supernatant and the detached cells from the same sample were pooled together in the 15 ml tubes. Cells were pelleted by centrifugation at 250 g for 5 minutes using a Hermle BHG centrifuge, and washed with 1 ml of cold PBS once. Cells were washed ($1x10^6$) in PBS and centrifuged at 200 g for 5 minute. Cell pellet was re-suspended in 1 ml PBS. Cells were fixed by transferring the cell suspension in 9 ml 1% paraformaldehyde in PBS and incubated for 15 minute on ice. Cells were centrifuged at 200 g for 5 minute and re-suspended in 5 ml PBS. Cells were again washed and centrifuged. Cell pellet was suspended in 1 ml PBS and transferred in 9 ml 70% (vol/vol) ethanol, on ice.

Cells were incubated for 4 h (The cells can be stored in ethanol for weeks). Cells were centrifuged at 200 g for 5 minute and re-suspended in 1 ml PBS. 0.2 ml of RNAse A solution was added and cells were incubated at 37°C for 30 minute. Cell suspension was centrifuged at 200 g for 5 minute and re-suspended in 0.2 ml PBS. 0.5 ml of 0.1 M HCl was added at room temperature. Cell pellets were then re-suspended in 25 ml cold PBS and 2 ml EB/AO dye mix was added. Stained cell suspensions were placed on a clean microscope slide and covered with a coverslip. Cells were viewed and counted using a Nikon eclipse inverted microscope at 400 × magnifications with excitation filter 480/30 nm; dichromatic mirror cut-on 505 nm LP; and barrier filter 535/40 nm (Nikon). Pictures were taken with a Nikon COLPIX digital camera. Tests were done in Quadruplet, counting a minimum of 100 total cells each.

Haematoxylin and Eosin (H&E) staining

Haematoxylin and Eosin staining shows basophilic structures blue, black, purple or grey and acidophilic structures in shades of pink and red. The stain haematoxylin is not a dye, but develops colouring properties on oxidation to haematin. Briefly, equal volume of 8% PFA was added to cell monolayer in culture media to give final concentration 4% for 20 minutes at 22 °C. The cell monolayer was washed twice in PBS. The cell monolayer was incubated in Mayer's Haematoxilin solution at 22°C for 10 minutes. Wash the monolayer

Thrice in PBS to remove the stain and differentiated in the running tap water for 1 minute. Monolayer was rinsed twice with PBS. Afterwards, the monolayer was stained in 1% eosin yellow for 5 to 30 minutes. Again monolayer was rinsed for few seconds in PBS. If cells were over stained with eosin the excess can be washed with more PBS wash. Cell monolayer was allowed to air dry and mounted with circular cover glass using non aqueous mordant and visualized in light microscope (cell nucleus blue and cytoplasm pink).

Results and Discussion

Morphological analysis of apoptosis in CEF cells using acridine orange ethdium bromide apoptotic assay

Typical indicators of apoptosis, including chromatin condensation and plasma-membrane asymmetry, were monitored during the course of infection. To detect chromatin condensation, mock- and IBDV-infected CEFs were stained with a mixture of acridie orange and ethidium bromide to identify viable, apoptotic and non viable cells.

This assay identifies for phases i) viable cell with normal nuclei (VN); ii) Viable cell with apoptotic nuclei (VA); iii) non viable cell with normal nuclei (NVN); iv) non viable cell with apoptotic nuclei (NVA).

The percentage of apoptotic index

= (VA + NVA) / (VN + VA + NVN + NVA) x 100

The percentage of necrotic cells

= (NVN) / (VN + VA + NVN + NVA) x 100

Percentage of dead cells

= (NVN + NVA) / (VN + VA + NVN + NVA) x 100

Apoptotic cell index was 2.25±0.92 at 0 h pi; 3±0.3 at 1 h pi, 3.75±0.54 at 5 h pi, 4.25±1.04 at 8 h pi, 5.25±0.90 at 16 h pi, 17±2.14 at 24 h pi, 32.75±1.66 at 48 h pi and 53.75±3.07 at 72 h pi. The above data is from 4 independent experiments (Fig. 1, 2 and Table 1).

Hematoxylin eosin staining

Cover glass grown CEF cell sheet was observed microscopically and the apoptotic cells were counted (unpublished data). IBDV-infected CEF cells displayed multifocal death of cells, shrunken, hyperchromatic and sometimes fragmented nuclei indicated multifocal apoptotic cell death. In contrast mock infected CEF cells were normal except for some sporadic apoptosis (Fig. 3).

In many experimental animal models of apoptosis there is an association between the severity of apoptosis and the amount of virus or virus antigen, suggesting that apoptosis is a consequence of viral infection. Apoptosis plays an important role in pathogenesis of other poultry viruses also like avian reovirus viral infections. A correlation between virus replication and apoptosis in chicken tissues has been demonstrated.

Oncolysis is a characteristic feature of many avian viruses. The oncolytic nature of reovirus was first recognised when wild-type reovirus was shown to replicate in transformed, but not normal, cells (Hashiro *et al*., 1997). Cells expressing high levels of EGFR were shown to be susceptible to reovirus infection and cytotoxicity (Strong and Lee 1996; Coffey *et al*., 1988; Strong *et al*., 1988). Detailed analysis demonstrated that an activated Ras pathway, a signalling pathway downstream of EGFR, was a prerequisite of sensitivity to reovirus (Bos, 1989). Avian reoviral FAST protein-induced syncytial cells died in a manner characteristic of apoptosis. Chromatin condensation and marginalization, apparent only in large syncytia at later stage of infection. A hallmark indicator of apoptosis, oligonucleosomal DNA laddering was observed in FAST-transfected cells at late transfection stage, laddering is preceded by extensive syncytium formation (Labrada *et al*., 2002; Corcoran and Duncan, 2004).

A chicken anaemia viral protein apoptin or VP3 induces apoptosis in a large variety of transformed cells but not in primary cells (Poon *et al*., 2005). VP3 (apoptin) induces apoptosis in specific lymphoid cells, the chicken thymocytes and chicken lymphoblastoid cell lines (MSB1) (Jeurissen *et al.,* 1992) which is also an important phenomenon during the pathogenesis of CIAV (Noteborn, 2004). It can also induce p53-independent apoptosis in human osteosarcoma cells. Reports are there that the only requirement for the accumulation within the nucleus and the selectivity of cancer cells by apoptin is the protein expression level. Tumor cells are more easily transfected compared with normal cells, which leads to an accumulation of the protein within the cytosol, indispensable for its translocation to the nucleus (Guelen *et al*., 2004). Apototin acts independently of the p53 status (Zhuang *et al*.,1995) and it binds to the anaphase promoter complex (APC/C) with resulting cell cycle block in G2M and p53-independent cell death. This promising viral protein might be useful for the treatment of those tumors which have lost their p53 and are therefore resistant to many forms of anticancer therapy (Teodoro *et al*., 2004). In this study Whether the IBDV infection-mediated cell death was due to apoptosis was examined by monitoring the nuclear morphology of the infected CEF. Chromatin condensation, nuclear fragmentation which is a characteristic of cells undergoing apoptosis, were observed in CEF that were infected with IBDV only but not mock one. The IBDV infection-mediated apoptosis was also confirmed by detecting for the presence of internucleosomal DNA cleavage by fluorescent and light microscopy.

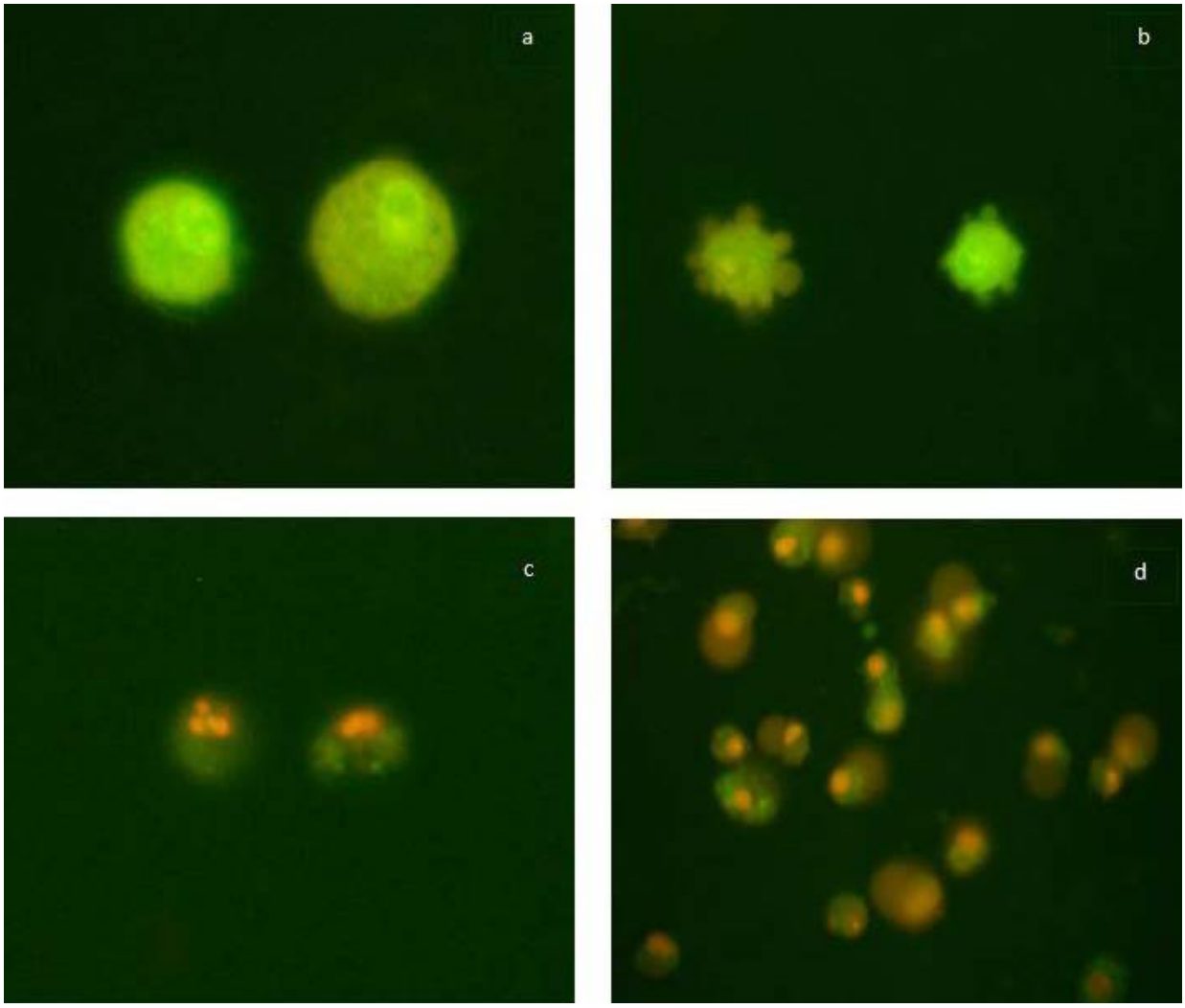

Fig.1: Acridine Orange/Ethidium Bromide apoptotic assay of CEF cells (X 400)
a) Control (VN) Viable Normal Chicken Embryo Fibroblast (CEF) cell;
b) Membrane blebbing (VA) in CEF cell;
c) Non Viable Apoptotic (NVA) CEF cell and d) Non Viable Normal (NVN) or Necrotic CEF cell.

Table 1: Morphological analysis of apoptosis in CEF cells

Hours PI	Apoptotic index	Dead cell	Nectrotic cell
0	2.25±0.92	1.5±0.70	1.0±0.41
1	3.0±0.93	1.75±0.54	1.25±0.25
5	3.75±0.54	1.75±0.54	1.5±0.29
8	4.25±1.04	2.75±0.92	2.25±0.63
16	5.25±0.17	3.0±0.88	2.25±0.63
24	17.0±2.14	5.25±1.04	2.75±0.75
48	34.0±0.11	15.5±0.9	5.0±0.41
72	53.75±3.07	30.75±2.2	5.75±0.63

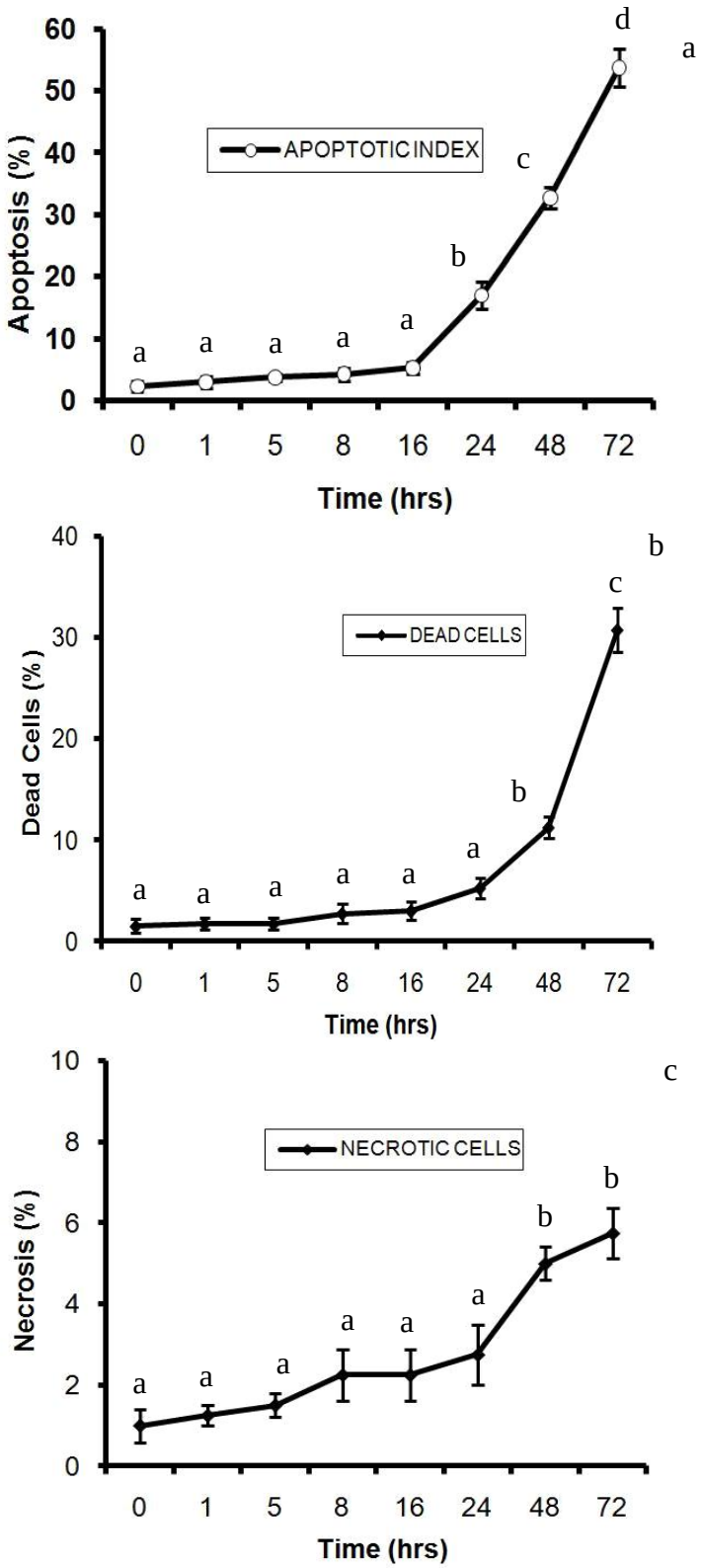

Fig. 2: Line diagram of acridene orange ethidium bromide apoptotic assay of CEF cells. a) Apoptotic Index (VA + NVA) cells; b) % dead cells (NVA + NVN); c) % necrotic cells (NVN). Data shown in the graph is mean±SEM of 4 independent experiments mean within each interval (h pi) that are not denoted by a common letter are different $p<0.05$.

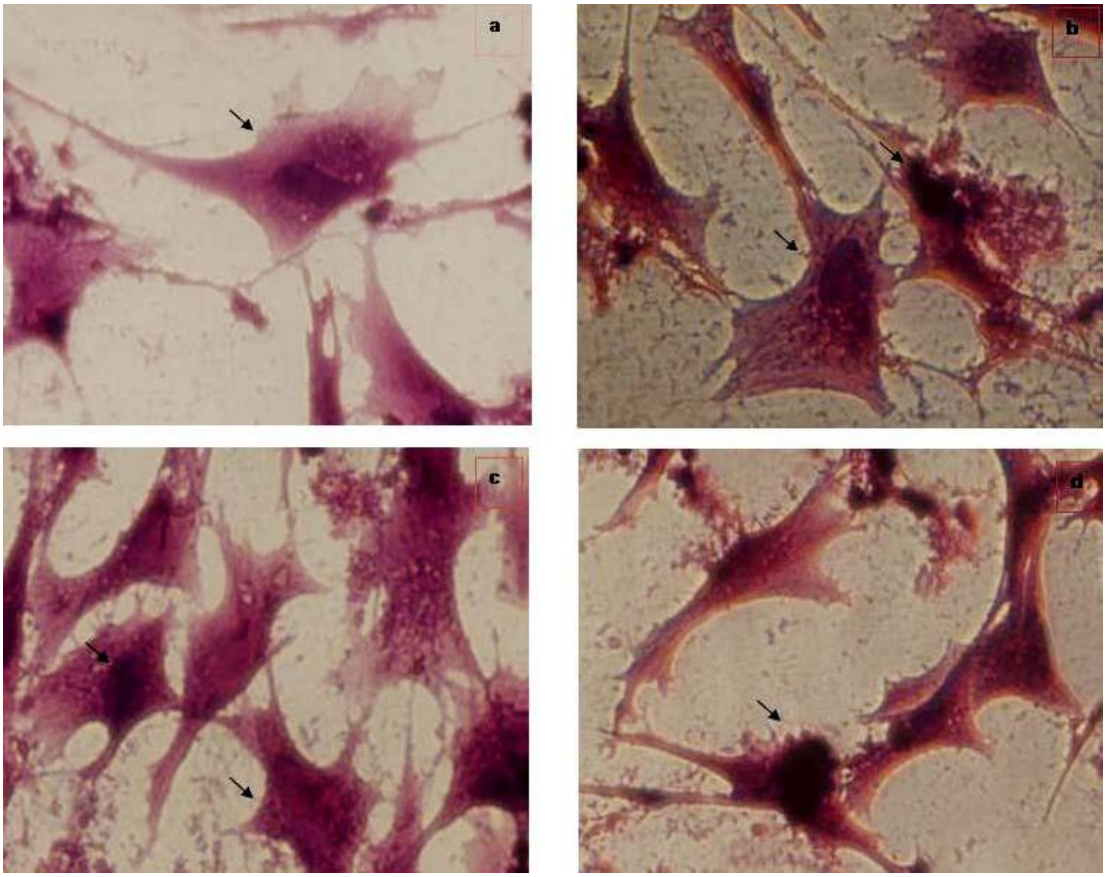

Fig.3: Representative micrograph of CEF cells stained with HE Stain (X 400)
a) Control CEF cells;
b) Control CEF cells with intact nucleus and one apoptotic cell;
c) CEF cells showing Pyknotic nuclei 24 h pi with IBDV IM + strain;
d) CEF cells showing Pyknotic nuclei 48 h pi.

Currently virotherapy using several viruses are in the stage of clinical trials. A clinical trial (phase I and II) using ONYX-015, an oncolytic virus, replicating selectively in p53-defective tumor cells was completed in patients with pancreatic cancer. The phase II trial yielded beneficial results (tumour reduction or stabilization) in about 50% of the patients (Kasuya *et al*., 2005; Bischoff *et al*., 1996). In the present study apoptosis in chicken embryonic fibroblast (CEF) was studied by AO/EB assay after IBDV infection. Acridine Orange (AO) is a metachromatic dye which differentially stains double-stranded (ds) and single-stranded (ss) nucleic acids. When AO intercalates into dsDNA it emits green fluorescence upon excitation at 480-490 nm. On the contrary, it emits red when interacts with ssDNA or RNA. Chromatin condensation is an early event of apoptosis and the condensed chromatin is much more sensitive to DNA denaturation than normal chromatin. Therefore, if RNA is removed by pre-incubation with RNase A and DNA is denaturated *in situ* by exposure to HCl shortly before AO staining, apoptotic cells (which have a larger fraction of DNA in the denaturated form) display an intense red fluorescence and a reduced green emission when compared to non-apoptotic interphase cells. Shrinkage, rounding, and aggregation were the cytopathic effect observed in CEF that were infected with IBDV and became stronger afterwards but not the mock infected one.

With the advent of this new technology we are now in a position to chart the course of the next wave of trials that will go beyond the phase I studies of safety and feasibility. Ultimately, at least in the medium term, the future of oncolytic virotherapy lies in combination regimens with standard anti-cancer agents such as radiation and chemotherapy (Harrington *et al*., 2010; Russo *et al*., 2006). Most of these forms of therapy are still in preclinical development because of their low specifity and susceptibility to drug resistance, but several of them have shown promising results. In particular, this

study aims at providing an oncolytic virus candidate IBDV in order to target cancer using apoptosis and might have the necessary potential for becoming part of the anticancer drug programs

Conclusion

Currently there are a significant number of proposed virotherapy of cancer candidates, with varying levels of efficacy in both pre-clinical models and clinical studies. Thus, the question emerges- are there more oncolytic viruses out there? Are all viruses inherently oncolytic? The study conducted so far has led to a better knowledge of the mechanisms of resistance to standard chemo and radio-therapy, as well as possible strategies aimed at restoring apoptotic sensitivity. The selective replication and induction of cell death in cancer cells induced by virus is essential to evaluate its capacity as antitumor agent. Studies are in progress to detect the oncolytic potential of IBDV. Based on the present knowledge, the use of these 'biological drugs' in synergistic association with the traditional cytotoxic drugs, might represent an important goal in the treatment of malignant cells.

Acknowledgments

The authors wish to thank the Director, Indian Veterinary Research Institute, Izatnagar, for providing necessary facilities to carry out the work. The first author Deepak Kumar also acknowledges Counsel for Scientific and Industrial Research, India, for providing financial support in the form of a Junior Research Fellowship.

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PREVENTION OF EXPERIMENTALLY INDUCED INTRA-ABDOMINAL ADHESIONS WITH COMBINED USE OF SODIUM CHROMOGLYCATE, HYALURONIC ACID AND POLYVINYL PYRROLIDONE IN COW CALVES[#]

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ABSTRACT

An experimental study was conducted on 12 healthy cow calves to assess the effectiveness of sodium chromoglycate (SCG) @ 2 mg/kg body weight, hyaluronic acid (@ 20 ml of 0.1% solution and polyvinylpyrrolidone (PVP) @ 100 ml 30% solution used as pre peri and postoperatively in prevention of intra-abdominal adhesions in an evolved model of ideal adhesions of transmural etiology. Animals were allotted to 2 groups (Group-I = control; Group- II = treatment) of six each. Intra-abdominal adhesions were induced by subjecting a 60 cm loop of ileum to 70 minutes of ischaemia by ligating the mesenteric vessels and lumen at either end if the ideal loop. Venous blood samples were analyzed for plasma fibrinogen and serum total proteins, calcium, inorganic phosphorus, creatinine, creatinine phophokinase and urea nitrogen on 0,1,2,3,5,10 and 12 days postoperatively. Significant differences were observed in plasma fibrinogen levels in both groups. On 12th day, gross observation revealed wide spread and dense adhesions (+4) in 5 out of 6 animals in group- I , while in the treatment group, there was clumping of ischaemic loop of bowel with thin band of adhesions (+1) in 3 animals, in one animal, localized flimsy adhesions were present. Histopathologically ileum showed +3 thickness of serosa with extensive proliferation of young fibroblast in control group, while in group-II, +2 serosal thickening was seen in two animals. Degranulated mast cell count was less in treatment group as compared to treatment. Over and above in the treatment group there was some attenuation of intra-abdominal adhesions as compared to the control.

Key words: Adhesions, polyvinylpyrrolidone, sodium chromoglycate

Introduction

Post-surgical abdominal adhesions developed following trauma to the mesothelium, which is damaged often by surgical handling, instrument contact and foreign materials. A realistic current goal for surgeons is to reduce and prevent the formation of adhesions especial,ly in clinically important areas such as small bowel. The two main strategy for adhesion prevention or reduction are adjusting surgical practice and applying adjuvants. Preventive therapy that has been investigated in human surgery include heparin, surgical barriers, cortico-steroids, antihistaminics, NSAID, fibrinolytic agents, sodium carboxymethylcellulose, hyaluronic acid, polyvinylpyrrolidone and dextran (Holmdahl *et al*., 1997). No single treatment has been shown to reduce adhesion formation consistently: Variation in efficacy of different treatment protocols is most likely a result of the different experimental models and treatment regimens; variation in species used; adhesion model and adhesion scoring system. Further, products developed for human surgery may not be as rewarding in the animals as first envisaged. In view of the transmural etiology of adhesions associated with bowel ischaemia and distention in cattle and buffaloes and also the scanty work done in these species, the effects of different antiadhesion agents in minimizing induced adhesions in male cow calves was studied.

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Materials and Methods

Twelve male calves in the age group of 4-10 months and weighing between 56- 102 kg were allotted to 2 groups of 6 each. In group- I (control) a model of small intestinal adhesion of transmural etiology to study certain aspects of induced intra-abdominal adhesions was evolved. In group-II the efficacy of combined use of sodium chromoglycate (SCG), hyaluronic acid (HA) and polyvinylpyrrolidone (PVP)was evaluated in minimizing intra-abdominal adhesions.

In over night fasted animals, under local infiltration anaesthesia 60 cm ileal loop was exteriorized through right flank laparotomy and immediately 20 ml of SCG (@ 2 mg/kg body weight) was instilled over the bowel loop Identified for inducing adhesions. All mesenteric blood vessels supplying to this ileal segment were ligated. The transmural occlusion at either end of this ileal segment was done by applying file tags (Lundin *et al*., 1989). Before placing the loop in the abdomen 20 ml of HA (0.1%) solution was instilled on the affected loop, and laparotomy wound was closed temporarily by applying a layer of continuous sutures to the skin using cotton thread. After 70 minutes of the ischaemic period the strangulated segment was reexteriorized. All the sutures and file tags were removed and 100 ml solution of 30 % PVP was poured over the ischaemic ileal segment used. After placing a single suture of chromic catgut No. 210 as a marker in least vascular area of mesentery, the loop was finally returned to the abdominal cavity. Laparotomy wound was closed routinely. All animals were given parentally Amoxycillin and Cloxacillin combination (Moxel) @ 6 mg/kg body weight by i/m route twice daily for 5 consecutive postoperative days.

Venous blood samples were collected by jugular vein puncture on day 0 to form base values and post-operative days 1, 3, 5,10 and 12. about 2 ml blood was collected in vials containing EDTA for estimation of fibrinogen (Refractometer method) and 8 ml of blood was collected in sterile test tube to separate the serum for estimation of total proteins (Biuret method), calcium (OCPC method, Autospan Diagnostic kits), inorganic phosphorus (UV molybate method), creatinine (Jaff's reaction), Creatinine phosphokinase (CK Nac method) and serum urea nitrogen (Ned dye method).

The animals were euthanized on 12th post-operative day. The abdomen was opened through a large incision parallel and 5 cm posterior to the previous incision, to evaluate the adhesion. Grading of adhesion was done according to the criteria followed by Fredricks *et al*. (1986). After grading the adhesions grossly, mesentery was used for mast cell count ancl ileal tissue was processed for histopathological study.

Results and Discussion

The method employed for inducing adhesions and the ischaemic period of 70 minutes was based on studies by Lundin *et al*. (1989) in foals and Singh (1999) in buffalo calves. It yielded consistent results in the study. Increase in the rectal temperature after 24 hours of surgery was noticed in both the groups, which returned to near normal by 3rd day post-operatively. The concentration of plasma fibrinogen increased significantly ($P< 0.05$) on day 1 in control group and on day 3 in the treatment group, thereafter the values declined gradually till day 12 in both the groups. The significant rise on day 1 and 3 might be due to acute inflammatory condition (Kaneko, 1989) and thereafter decrease in intensity of inflammation might have resulted in decline of plasma fibrinogen concentration as also observed by Singh (1999), Rathore (2000) in buffalo calves and cow calves, respectively. Moll *et al*. (1991) also noted rise in fibrinogen level on 3rd post-operative day in ponies after surgery. Serum total protein concentration in both the groups was not affected significantly at any stage of observation. Serum calcium concentration declined significantly on day 1 in both the groups, thereafter the concentration returned near normal in group-I, while remained decreased in group-II. Serum inorganic phosphorus concentration increased significantly on day 3 in both the groups, subsequently values declined. The creatinine concentration after initial increase declined towards the end of observation period in both the groups. The serum CPK value increased dramatically on day 1 in both the groups. However, it started declining from day 2 onwards and reached normal level on day 5 in group-II and on day 10 in group-I. The increase in CPK activity 24 hours after surgery might be because of muscle damage due to tissue ischaemia (Aktas *et al.*, 1994). The increase in CPK value on day 1 in group-II was much lower than that of group-I, which might be due to less muscle damage caused by the use of antiadhesion agents in group-II. Serum urea nitrogen increased in group-I ($P >05$) and group- II ($P>0.05$) and thereafter values declined and reached normal level.

Gross evaluation of adhesions was done on 12th postoperative day. The abdominal cavity was approached by giving the incision posterior to the previous laparotomy site. In 5 animals of group-I, widespread dense adhesions (+4) (Fig.1) were noticed and localized dense adhesions (+2) in 1 animal at the site of ischaemia. Adhesions of the ischaemic loop to the adjacent intestinal segment was observed in one animal out of 6 in 4 animals, localized flimsy to widespread flimsy (+ 1, + 2, +3 and + 3) adhesion developed between omentum and sutured peritoneal incision, whereas 2 animals were free from adhesions to the incisional sites. (Table 1 and 2).

Table 1: Grades of intra peritoneal adhesions at different sites in control Group-I.

Animal No.	Incisional site	Within the ischaemic loop	Ischaemic loop to adjacent bowel	Other visceral organs
1	+	++++	0	0
2	+	++++	0	0
3	+++	++++	0	0
4	+++	++++	++	0
5	0	++++	0	0
6	0	++	0	0

Table 2: Grades of intra peritoneal adhesions at different sites in mixed Group-II.

Animal No.	Incisional site	Within the ischaemic loop	Ischaemic loop to adjacent bowel	Other visceral organs
1	0	0	0	0
2	0	0	0	0
3	0	+	0	0
4	0	+	++	0
5	0	+	++	0
6	0	+	++	0

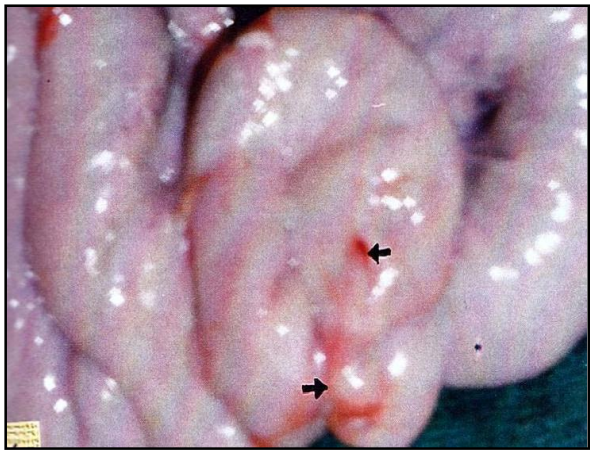

Fig. 1: Clumping if ischaemic loop and dense widespread adhesions (++++) with lieal loop haemorrhagic area at places

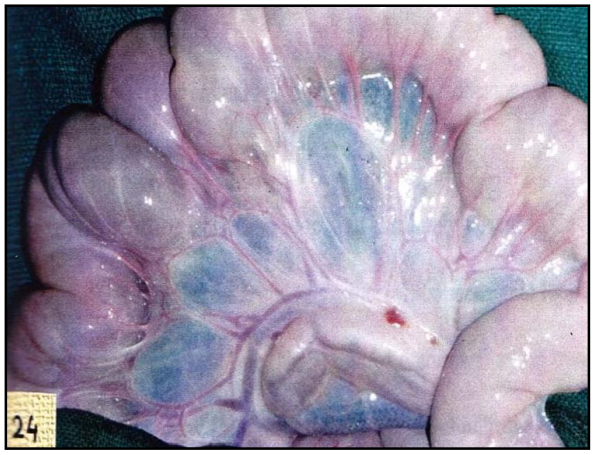

Fig. 2: Zero adhesions in an ischaemic lieal loop

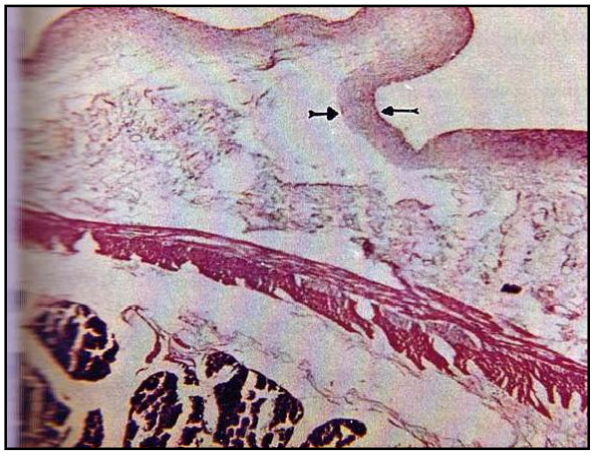

Fig. 3: Sections of ileum with +++ thickening of serosa (H & E x 240)

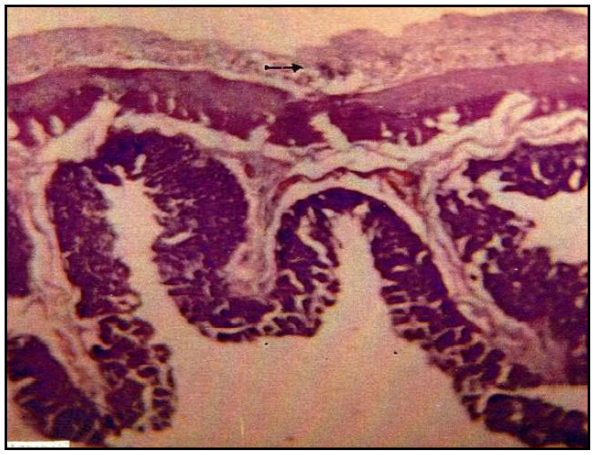

Fig. 4: Section of ileum showing ± thickening of serosa (H & E x 240)

The clumping of ischaemic segment was noted in the animals having +4 grade adhesions (Fig 1). Swanswick *et al*. (1973), after studying the healing of peritoneal defects and peritoneal midline incision in horses concluded that the suturing of peritoneum resulted in a higher incidence of adhesion after surgery than non-suturing of it. Baxter *et al*. (1993) and Singh (1999) reported locatized dense adhesions (+2) at the incisional site with omentum in their studies on horses and buffalo calves, respectively. In group-II, mixed results have been noted as compared to group-I. In 2 animals there was 0 adhesions (Fig. 2), in 1 animal localized flimsy adhesions were seen. In 3 animals, there was clumping of ischaemic loop of bowel with thin band of adhesions (+1) between affected loops and thick dense band of adhesions between affected and adjacent loops of bowel.

Histopathological assessment of section of ileum showed +3 thickening (Fig. 3) of serosa with extensive prollferation of young fibroblasts leading to adhesions between two serosal surfaces in all the animals of control group (Fig. 3). Excessive proliferation of blood vessels was found in the area of fibroblastic proliferation. Heavy infiltration of macrophages with few neutrophils was also noticed. Mucosa showed mild congestion and infiltration of eosinophils. The section of ileum in group II showed variable histopathological lesions. +2 thickening (Fig. 4) of serosa was observed in 3 animals, while 1 animal showed thickening. In 2 cases, there was absolutely no thickening of serosa. Mild infiltration of mononuclear cells with proliferating fibroblasts and blood vessels were noticed in cases with mild to moderate serosal thickening. Rupture of mast cells initiates the cycle of adhesion formation and they increase in number and degranulate after any peritoneal trauma. Degranulated mast cells count were maximum in control (12.63 per 5 high power field) when compared to group II (7 .43 per 5 high power field).

In comparison to group-I, group-II yielded better results. In this group agents were used in combination pre, peri and postoperatively. SCG was used after selecting the loop of ileum. HA was used after creating the ischaemia in the ileal loop and finally PVP was used just before closure of laparotomy wound. SCG was effective only when used 30 minutes prior to laparotomy (Canturk *et al*., 1999). Hence, it can be assumed that SCG did not have any effect in attenuation of adhesions. Similarly, HA works effectively as antiadhesive only when it is ionically. cross-linked with trivalent ions. In the present study, NaHA was used as a formulation of HA, hence had minimal effect in reducing the adhesions. PVP was used just before closure of laparotomy wound. Yaacobi *et al.* (1993) after using PVP in a rat caecal abrasion model concluded that tissue coating with PVP after inflicting the injury failed to inhibit adhesion forrnation, however, tissue coating with PVP prior to ceacal abrasion significantly reduced the formation of postoperative adhesions. Hence, the combination of 3 antiadhesion drugs helped to some extend in attenuating adhesions but not significantly, when compared with the control animals.

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SDS-PAGE CHARACTERISATION OF GRANULOSA CELL PROTEINS OF BUFFALO AT DIFFERENT STAGES OF OESTRUS CYCLE#

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ABSTRACT

The present study was conducted to ascertain the comparative protein profile of granulosa cells at different stages of oestrus cycle of buffaloes. The ovaries of buffaloes were obtained from local abattoir and ovarian stages were determined on the basis of corpus luteum morphology. The follicular fluid was aspirated from the follicles by syringe and the cells were separated by centrifugation at low temperature (4°C) in PBS (0.1M pH 7.4). Supernatant discarded and the pellets of granulosa cells were suspended in 1 ml of PBS and washed by repeated centrifugation. The granulosa cells were lysed first with repeated freezing and thawing and then by sonication. The proteins of isolated granulosa cells of stage III and IV of ovary were analyzed by SDS-PAGE. The proteins of granulosa cells of ovarian stage III revealed eleven bands of molecular weight (202.31, 153.63, 125.86, 94.49, 68.55, 48.25, 41.44, 24.36, 17.15, 14.45 and 6.45 kDa) and ovarian stage IV revealed protein bands of molecular weight (183.99, 145.12, 112.31, 96.41, 58.90, 50.68, 50.60, 41.45, 33.01 and 17.16 kDa) on the silver stained gels. The band of molecular weight 24.36kDa, 4.45 kDa and 6.45 kDa were detected only in stage III and were absent in stage IV.

Key words: SDS-Page, characterisation, granulosa cell proteins, buffalo, oestrus cycle

Introduction

Folliculogenesis involves a series of complex autocrine, paracrine and endocrine regulations which mediate follicular recruitment, selection and dominance leading to formation of a fertilizable dominant follicle. In the course of development of a follicle, granulose cells play a typical central role which secrete different growth factors such as IGF-I, FGF and TGF that bring about signal transduction pathways among theca cells, granulosa cells and the developing oocyte. The interplay of these factors leads to the maturation of follicle and follicular growth by avoiding follicular atresia. That is why it becomes essential to delineate the role of granulosacells and their secretions in the process of folliculogenesis and different phases of oestrus cycle (Fortune *et al.,* 2001).

The buffalo plays a significant role in dairy industry of India but lesser understanding of delayed puberty, poor follicular turnover, poor super-ovulatory response, distinct seasonal reproductive pattern and long post partum anoestrus in buffalo have focused the necessity to understand the mechanism of the molecular cross talks between oocyte, granulosa cells and theca cells. Though, several studies reported the cross talk between the theca and granulosa cells which modulated the follicular growth, morphology and protein of the follicles in different species of animals (Yada *et al.,* 1999; Kotsuji *et al.,* 1990; Sangha and Marwah, 2001 and Spicer and Aad, 2007) yet few reports are available on the distinct functions of granulosa cells proteins in buffalo reproduction (Hynes *et al.,* 1996). Therefore, the present investigation was designed to characterize the protein profile of granulosa cells protein at different stages estrus cycle in buffaloes.

Materials and Methods

SDS-PAGE of buffalo granulosa cells proteins was carried out on 10% slab gels using discontinuous buffer system (Lammeli, 1970). Ovaries as well as serum samples of buffaloes were collected from local abattoir in ice packed thermos and were transferred to the laboratory within one

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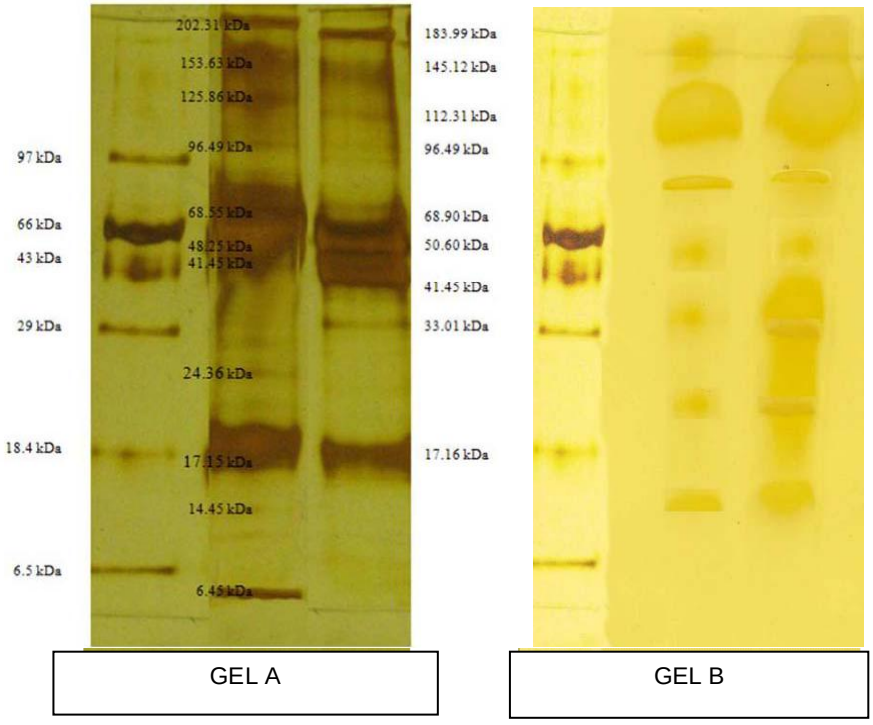
hour of collection. The ovaries were kept in the chilled PBS during transportation to the laboratory. The stage of the ovary was determined on the basis of morphology of corpus luteum as explained by Ireland *et al*. (1979). The stage III and stage IV ovaries were detremined by regressing luteal stage (III) that is 11-17 days of the cycle showing bisected orange to yellowish white corpus luteum, with prominent vasculature. The stage IV was the follicular stage that is 18-20 days of the cycle showing absence of vasculature from the surface and presence of a large follicle. The ovaries were washed with the PBS and the follicles made visible on the surface of the ovaries. Foliicuar fluid from these follicles was aspirated by inserting 2 ml syringe with 20 gauze needle. The cells were separated from follicular fluid by centrifugation at 4000 rpm for 15 minute at 4°C. 1 ml PBS (0.1M pH 7.4) was added to the sediment and re-centrifuged at 4000 rpm for 15 minute at 4°C to remove the traces of follicular fluid and debris. Supernatant discarded and the pellets of granulosa cells were suspended in 1 ml of PBS and cell counting was done by using haemocytometer. The granulosa cells were lysed first with repeated freezing and thawing and then by sonication.

The total protein content of granulosa cells was estimated by spectrophotometric method (Nanodrop, ND-1000, USA) at Protein A^{280} (protein's absorbance at 280 nm). After the estimation of total protein, equal volumes of cold ethanol (-20°C) were added and left with constant stirring for 90 min at 4^0C to precipitate the proteins. Proteins were then recovered by centrifugation at 10,000 rpm for 10 min at 4^0C, re-suspended in phosphate buffered saline (PBS) and stored at "20 ^{0}C until further analysis of granulosa cell proteins.

Serum samples from the same buffaloes were also collected and stored at -20°C for further analysis. The protein and samples were diluted to 10 mg per ml and then mixed with equal volume of sample buffer. The samples were heated at 100°C in a boiling water bath for 5 minutes and centrifuged at 3000 rpm for 10 minutes. The supernatant were stored frozen at -20°C till use. 10ìl of samples as well as molecular marker were loaded in the wells of the gel. The electrophoresis was carried out at room temperature for 6 hours. The voltage was adjusted to 70 V for first 30 minutes and later 120 V till completion. After electrophoresis, the gel was stained by using silver staining kit. The relative molecular weights were determined by using the (broad range molecular weight markers of Merck, Germany) Gel documentation and analysis system (Gel-doc. Model- Alpha imager TM1220, Alpha Innotech Corporation, USA).



GEL A GEL B

Lane- I= Standard Protein Molecular Marker
Lane-2= Granulosa cell stage III
Lane-3= Granulosa cell stage IV

Lane- I= Standard Protein Molecular Marker
Lane-2= Serum stage III
Lane-3= Serum stage IV

Results and Discussion

The granulosa cell proteins isolated from stage III and IV of ovary were analyzed by carrying out SDS–PAGE. The gels obtained were stained by silver staining kit. The granulose cells of ovarian stage III revealed eleven protein bands of molecular weight 202.31, 153.63, 125.86, 94.49, 68.55, 48.25, 41.44, 24.36, 17.15, 14.45 and 6.45 KDa were on the silver stained gels. For granulose cells of ovarian stage IV, ten protein bands of molecular weight 183.99, 145.12, 112.31, 96.41, 58.90, 50.68, 50.60, 41.45, 33.01 and 17.16 KDa were detected on the gel. The comparative electophoretogram of ovarian stage III and stage IV granulosa cell proteins revealed that band number 8 of molecular weight 24.36KDa, band number 10 of 14.45 KDa and band number 11 of 6.45 KDa were only detected in stage III and were absent in stage IV.

The blood serums obtained from the buffaloes in stage III and IV and were analyzed by SDS-PAGE. The SDS-PAGE gels showed similar protein bands of molecular weight 190.51, 134.50, 84.08, 52.55, 35.40, 19.77, 10.43kDa for stage III and 190.57, 133.51, 84.08, 55.07, 31.63, 19.04, 10.53kDa for stage IV.

High molecular weight proteins were more distinct in stage III and IV of ovarian stages. The total protein concentration was found to be more in stage III and IV as compared to stage I and II. Gerard *et al.* (1998) reported a 200KDa protein in granulose cell lysate of equines. This band was not found in the lysate of buffalo granulosa cells in the present study. Rabahi *et al.* (1991) observed three characteristic bands of molecular weight 76, 48 and 30 kDa in pre-ovulatory gralunosa cells and cumulus cells. These proteins bands were not observed in granulasa cells in present study. These differences in protein bands in different studies of granulose cells may be due to difference in species, difference in ovarian stages or difference in experimental conditions (different gel composition).

The proteins bands of molecular weight 24.36kDa, 14.45kDa and 6.45kDa were appeared in stage III and not detected in stage IV. The results of the current study cannot compare due to paucity of availability of literature. Further studies are required to characterize these proteins, the mechanism of synthesis, their nature and their putative role in follicular maturation, growth and ovulatory follicle formation.

The major function of granulosa cells includes the production of steroids as well as growth factors mainly peptides which are supposed to interact with oocyte during development (Gerard *et al.,* 1998). Tonetta *et al.* (1998) reported a steroidogenic protein which is secreted by granulosa cells in porcine. The findings of the present study appeared to be incomplete. Many further studies are required to find out the putative roles of these proteins secreted by granulosa cells and their roles to bring out follicular maturation in buffalo reproduction.

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INFLUENCE OF ZINC DEPRIVATION AND *STAPHYLOCOCCUS AUREUS* INFECTION ON LIVER AND PANCREAS IN ALBINO RATS- A PATHOLOGICAL STUDY#

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ABSTRACT

An experiment was conducted on male albino rats to delineate the effect of zinc on liver and pancreas. The albino rats were offered zinc deficient purified diet and various clinical manifestations occurred due to zinc deficiency were periodically recorded. On 21st day of experiment, when clinical signs of zinc deficiency appeared, animals in group D1-III and D1-IV having six animals in each, were infected with *S. aureus* infection. On 28th day of experiment, the animals showing clinical signs of zinc deficiency were sacrificed and tissues from liver and pancreas were collected for histopathological examination. In zinc deficient infected animals, the liver section revealed congestion and leucocytic infiltration including neutrophils and mononuclear cells in the parenchyma and at places, there were haemorrhages in the liver. In some cases, hepatocytes revealed fatty changes alongwith congestion and neutrophilic infiltration. In zinc adequate infected animals, there were congestion and infiltration of neutrophils and mononuclear cells alongwith Kupffer cell hyperplasia. The sections of pancreas revealed acinar necrotic changes both in exocrine gland as well as in islets of Langerhans in all groups of zinc deficient animals. Most of the pancreatic islets were atrophied and there was hydropic degeneration of the islet cells.

Key words: Albino rats, zinc deficiency, liver and pancreas, *Staphylococcus aureus*

Introduction

Amongst the various dietary essential micronutrients whose significance lies in the deficiency or excess in the diet, the zinc is one of the major trace mineral essentially required for the normal growth of various body tissues including liver and pancreas in animals. In fact, one-third of the world population is at risk of zinc deficiency, ranging from 4 to 73% depending on the country. Zinc deficiency is the fifth leading risk factor for disease in the developing world. In clinical observations, the zinc deficiency has also been found to result in an increased susceptibility to a variety of infectious disorders as well as decreased cell mediated immunity causing significant impairment of cellular and humoral immune response (Beisel, 1976). The various factors causing clinical or sub- clinical deficiency of this mineral in various animal species hinders performance by affecting a number of body functions including retardation of growth, inappetance, alopecia, impaired development and functions of different organs.

The present study had been carried out to highlight the influence of experimental zinc deficiency on liver and pancreas in albino rats.

Materials and Methods

This experimental study was conducted and executed to study the effects of dietary zinc deficiency by feeding of zinc adequate and zinc deficient purified diets to 48 male weanling albino rats divided into two groups. Each group having 24 animals were fed on zinc deficient diet (D1) containing 1.01 ppm zinc and divided further into four sub- groups D1-I, D1-II, D1-III and D1-IV of six animals each having 24 in each dietary group. The basic ingredients of zinc deficient diet were same as that of zinc adequate control diet. On 21st day of experiment, when clinical signs of zinc deficiency appeared, animals in group D1-III and D1-IV having six animals in each, were infected with *S. aureus* @ $1x10^6$ viable cells per rat in 1 ml. NSS intraperitoneally. For their respective controls, animals of sub- groups D2-III and D2-IV were also given infection at the same dose rate.

After 4 weeks of experiment, the animals from both dietary regimens were sacrificed and tissues from liver and pancreas were collected for gross and histopathological examinations. These organs were collected in 10 per cent formal saline and 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 as a routine with haematoxylin and eosin staining method.

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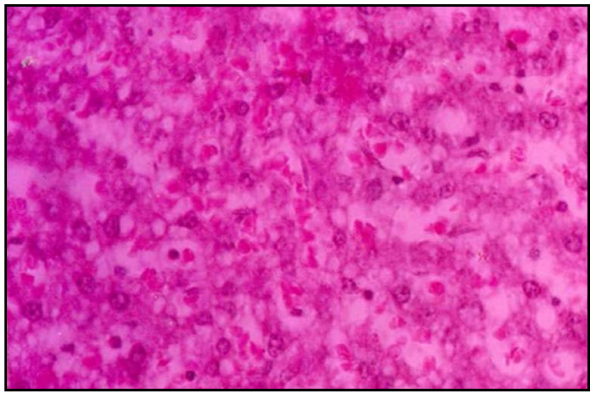

Fig. 1: Microphotograph of liver of zinc deficient infected animal showing fatty changes alongwith congestion and leucocytic infiltration (H&E 200X).

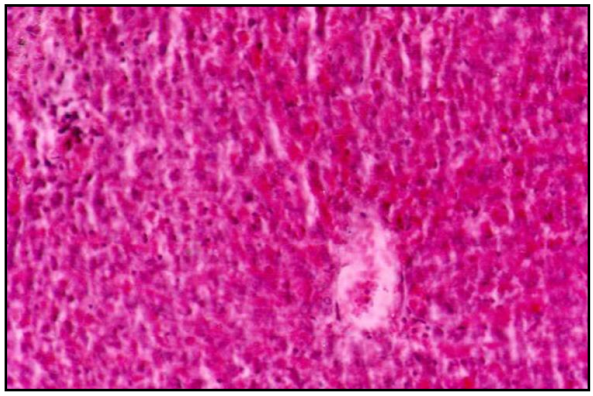

Fig. 2: Microphotograph of liver of zinc deficient infected animal showing Kupffer cell hyperplasia alongwith congestion and inflammatory infiltration (H&E 200X).

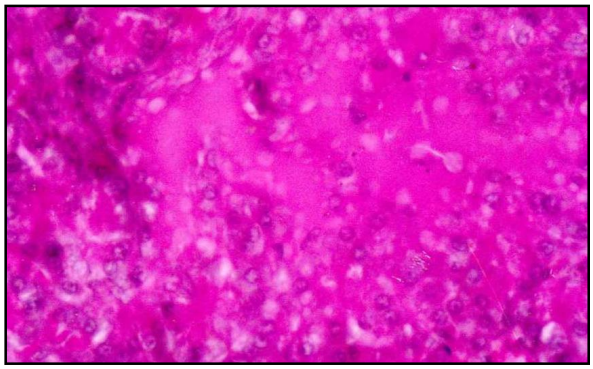

Fig. 3: Microphotograph of pancreas of zinc deficient animal showing areas of acinar necrosis (H&E 200X).

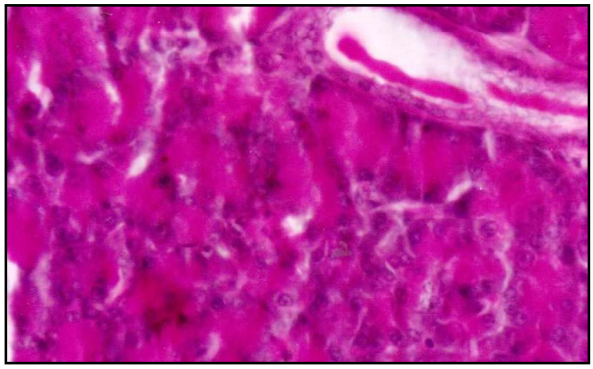

Fig. 4: Microphotograph of pancreas of zinc deficient animal showing intralobular pancreatic ducts with hyperplasia of lining cells and presence of homogeneous eosinophilic mass (H&E 200X).

Results and Discussion

There were no histopathological changes observed in liver of zinc deficient animals due to deficiency of zinc but in zinc deficient infected animals, the liver section revealed congestion and leucocytic infiltration including neutrophils and mononuclear cells in the parenchyma and at places, there were haemorrhages in the liver. In some cases, hepatocytes revealed fatty changes alongwith congestion and neutrophilic infiltration (Fig. 1). While in others, the nuclei of hepatocytes showed pyknosis and focal coagulative necrosis alongwith congestion, infiltration and accumulation of polymorphonuclear leucocytes. Some sections of liver showed Kupffer cell hyperplasia in the sinusoids (Fig. 2).

In zinc adequate infected animals, there were congestion and infiltration of neutrophils and mononuclear cells alongwith Kupffer cell hyperplasia. In portal triad areas, there were proliferation and hyperplasia of bile ducts. Gupta (1995) also observed the similar findings in guinea pigs.

The sections of pancreas revealed acinar necrotic changes (Fig. 3) both in exocrine gland as well as in islets of Langerhans in all groups of zinc deficient animals. Most of the pancreatic islets were atrophied and there was hydropic degeneration of the islet cells. There were proliferation of intralobular pancreatic ducts and epithelium of these ducts becomes hypertrophied showing cytoplasmic vacuolar degeneration. Their lumina contained excessive homogeneous eosinophilic proteinaceous mass (Fig. 4). In the zinc adequate animals, no histopathological alterations were observed. These observations are similar to those described by Fell *et al*. (1973) and Gupta *et al*. (1988).

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EFFECT OF EXPERIMENTAL HYPOTHYROIDISM ON SOME MINERALS IN MARWARI RAMS[#]

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ABSTRACT

Hypothyroidism was induced in Marwari adult rams by oral thiourea administration (@ 50 mg/kg b. wt.). The blood samples were collected and analyzed on 0 (control), 3rd, 5th and 7th day of thiourea feeding. The investigation was carried out to study the effect of experimental hypothyroidism on minerals (calcium, inorganic phosphorus and magnesium) concentration. A significant ($P<0.05$) effect of experimental hypothyroidism was observed on calcium concentration. The calcium was gradually increased in experimental hypothyroidism. The effect of hypothyroidism on phosphorus and magnesium was non- significant ($P>0.05$). The present work evaluates the changes in body mineral status resulting from experimentally induced hypothyroidism.

Key words: Hypothyroidism, thiourea, rams, minerals

Introduction

Thyroid gland and minerals play a central role in maintaining the homeostasis of vertebrate animals. Thyroid gland is responsible for regulation of basal metabolic rate, lipid, and carbohydrate and nitrogen metabolism as well as regulation of energy and growth development. Hypothyroidism adversely affects growth and reproduction. Hypothyroidism can be induced by factors like deficiency of iodine and goitrogens (Raghuprasad *et al*., 1984). There are number of factors causing hypothyroidism, which ultimately alter metabolic status of the animal and therefore, also affect the production efficiency of the animals. Thiourea is the thiouracil type goitrogen, which interfere with the organic iodine binding in the thyroid gland. In agriculture farming thiourea is widely used as fertilizer. Sheep consume thiourea and this chemical causes hypothyroidism by affecting the biosynthesis of thyroid hormone, which may cause heavy losses in production and reproduction. Present investigation will pave the way for soil-water-plant-animal relationship. The other important aspect is that the problem of iodine deficiency co-exists in animals along with human beings in our country, which makes this study important.

Materials and Methods

Nine adult apparently healthy males (rams) of Marwari breed of sheep were included in this study. These animals were provided with standard ration and water *ad libitum* during the course of study. All experimental rams were kept isolated from the rest of the sheep. They were housed in clean and well ventilated sheds. The experimental plan was divided into following phases.

Control (Phase-I)

Initially blood samples were collected from the normal animals, referred as control group or control animals. The samples of these animals were estimated for minerals (calcium, inorganic phosphorus and magnesium). These parameters were treated as normal or control parameters.

Induction of experimental hypothyroidism

Each control animal was further subjected to thiourea feeding to observe the effect of experimental hypothyroidism during the course of study on the mineral status.

To carry out the objectives of present study, thiourea was used as a drug of choice for experimental hypothyroidism @ 50 mg/kg body weight. This stage was further sub-divided into three phases.

3rd day of thiourea feeding (Phase-II)
5th day of thiourea feeding (Phase-III)
7th day of thiourea feeding (Phase-IV)

Blood samples were obtained with anticoagulant (heparin @ 30 IU/ml blood) by puncturing the jugular vein with least stress to animal under aseptic conditions directly into the hot air oven sterile tubes for control and 3rd, 5th and 7th day of thiourea feeding, respectively. Plasma for each sample was separated out on the same day in the laboratory. For separation of plasma the test tubes were centrifuged at 3000 rpm for 20 minutes and plasma was pipette out in clear dry plasma tubes. After collection, plasma samples were stored at -20°C in a deep freeze. The effect of thiourea feeding was observed on the same animals, which have

[#]Part of M. V. Sc Thesis submitted to RAU, Bikaner

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been referred as control animals. On 3rd, 5th and 7th day of sampling during thiourea treatment, thiourea was given just after the sampling. Calcium was estimated by Clark-Collip method, Inorganic phosphorus was estimated by Fiske and Subbarao method and Magnesium was estimated by Titan Yellow method as cited by Oser (1986). The results were presented as mean±SE. The mean values were determined according to the effect of experimental hypothyroidism. The data was subjected to analysis of variance (ANOVA) (Snedecor and Cochran, 1967). The critical difference among various means was worked out by 'Duncun's New Multiple Range Test (DNMRT) method.

Results and Discussion

The mean ± SE concentration (Table 1) of plasma calcium, phosphorus and magnesium (mmol/l) was in accordance with the Pernthaner *et al*. (1993), Baumgartner and Pernthaner (1994) and Purohit *et al*. (2000) in different breeds of sheep.

The analysis of variance (ANOVA) revealed a significant ($p<0.05$) effect of experimental hypothyroidism on plasma calcium concentration. During the course of study, plasma calcium concentration increased gradually by 4.545%, 7.080% and 10.693% in Phase-II, Phase-III and Phase-IV, respectively in comparison to Phase-I (control). Large amounts of calcium, phosphate and magnesium continuously enter and leave plasma via intestine, kidney and bone. Each of the organs contributes to regulation of plasma concentrations of these minerals (Wilson and Foster, 1992). Thyroid hormone has a direct effect on the parathyroid glands, regulating parathyroid hormone secretion, and on the kidney's ability to excrete calcium. Thus, affect calcium homeostasis by decreasing bone turnover and serum calcium level, and by increasing parathyroid hormone and 1, 25-dihydroxyvitamin D concentrations. Hypercalcaemia appeared to result from a combination of reduced renal calcium excretion and a change in the "set point" for calcium feedback inhibition of the parathyroid glands. (Zaloga *et al*., 1984). Kramer (1980) reported that hypothyrodism is one of the conditions that increase serum alkaline phosphates activity and Benjamin (1998) positively correlates the blood calcium concentration with the blood alkaline phosphatase activity. The hypothyroidism results in the increment of blood calcium concentration. Halik and Zavodsky (1978) reported hypercalcaemia in hypothyroid ram, Rajgude *et al*. (2005) observed that plasma calcium levels was significantly and progressively increased as the level of thiourea increased in birds and Belonje (1968) reported long term effect of thyro-parathyrodectomy in Merino weather and observed transient rise in plasma calcium concentration, thus supporting present study. As per the ANOVA, non-significant (P>0.05) effect was observed regarding the plasma inorganic phosphorus and magnesium concentration during various phases of experimental hypothyroidism. The present study evaluates the changes in body mineral status and the animal's ability to maintain body homeostasis after the experimentally induced hypothyroidism.

Table 1: Mean ± SE concentrations of plasma calcium, phosphorus and magnesium according to the effect of experimental hypothyroidism in Marwari rams

Phases	No. of Observations	Calcium (mmol/L)		Inorganic phosphorus (mmol/L)		Magnesium (mmol/L)	
		Mean ± SE	% Incr.	Mean ± SE	% Incr.	Mean ± SE	% Incr.
Phase- I	9	2.518±0.034[a] (2.474-2.697)	-	1.912±0.060[a] (1.743-2.199)	-	0.884±0.016[a] (0.826-0.953)	-
Phase- II	9	2.631±0.050[ab] (2.423-2.872)	4.545	1.921±0.064[a] (1.740-2.231)	0.483	0.903±0.011[a] (0.868-0.954)	2.051
Phase- III	9	2.696±0.053[b] (2.442-2.936)	7.080	1.925±0.066[a] (1.740-2.232)	0.720	0.904±0.016[a] (0.828-0.992)	2.165
Phase- IV	9	2.787±0.071[b] (2.447-2.993)	10.693	1.918±0.056[a] (1.750-2.173)	0.319	0.912±0.009[a] (0.872-0.953)	3.163

Mean comparison have been made with different phases
Mean superscripted with different letters differ significantly (P<0.05) from each other
Data shown in parenthesis are representing respective range

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MICROSCOPICAL STUDY OF THE ADRENAL MEDULLA OF THE GOAT (*CAPRA HIRCUS*)[#]

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ABSTRACT

The microscopic studies on adrenal medulla of 36 adult male goat showed that the follicular cells of medulla grouped in to outer and inner zones. The reticular fibres along with collagen fibres formed a network between medullary cells. The outer zone was made up of large columnar cells in which nuclei were situated in apical portion while the cells of inner zone were polyhedral in shape with centrally placed nuclei. A large central vein was found in almost all specimens. Muscle fibres were not present in the wall of central vein.

Key words: Adrenal gland, adrenal medulla, goat

The adrenal gland regulates the stressful condition, especially 'fight and flight' response of animals. The hormones of adrenal medulla, mainly epinephrine and norepinephrine are the environmental stress factors that lead to permanent strain and overload of the body resulting in structural alteration of the adrenals that in turn are followed by hormonal imbalances. This leads to an increased susceptibility to bacterial and viral diseases.

Materials and Methods

The adrenal glands were collected from 36 apparently healthy male goats (*Capra hircus*) from local abattoir. All animals were above one year of age. For histological observations, pieces of 4 to 6 mm thickness were cut from each gland and the tissues were prepared by routine histological technique. Following staining procedures were adopted for cellular and connective tissue details.

Haematoxylin and Eosin stain (Singh and Sulochana, 1996), the conventional method of H & E staining was used. Ehrlich's haematoxylin and 1% aqueous solution of eosin were used. Van Gieson stain (Luna, 1968) to show the connective tissue details. Verhoeff's elastin stain (Drury and Wallington, 1967) to demonstrate elastic fibres. Gridley's reticulum stain (Luna, 1968) to show reticular fibres. Masson's Trichome stain (Luna, 1968) to demonstrate collagen fibres.

Results and Discussion

Adrenal medulla was the central (inner most), distinct and compact zone of the gland. It comprised of two cellular zones, outer and inner. In the outer zone, the vesicular nuclei were present in the apical portion of the large columnar cells, which were arranged in follicular manner. The inner zone was made up to polyhedral cells having nuclei in the middle portion. This zone was located around the central vein (Fig.2). These observations of present study are almost in accordance with the findings of Smollich (1966) in different species of ruminants, Prasad and Yadava (1973) in buffalo, Baishya *et al.* (1998) in Mithun and Yak and Panchal *et al.* (1998) in sheep.

Medullary tissues were arranged in follicular manner as observed in present study, which was also reported by Smollich (1967) in different species of ruminants and Prasad and Yadava (1973) in buffalo.

In present investigation there was a distinct but irregular demarcation between the cortex and medulla; both were intermingled to each other at some places but were easily distinguishable by pattern of cells (Fig.2). This observation is in line with the findings of Prasad and Yadava (1973 and 1974) in buffalo, Ganguli and Ahsan (1978) in goat and Nagpal *et al.* (1991) camel.

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Extensions of medullary tissue in cortex were also recorded in some specimens, which are similar to the observation made by the Abdalla and Ali (1989) in camel.

Presence of two distrinct catecholomine cells, viz., adrenaline and noradrenaline generator cells were described by Trautmann and Fiebiger (1957) in horse, Narasimhan and Kamat (1970) in goat, Prasad and Yadava (1972, 1973 and 1974) in buffalo, Dellmann and Brown (1981) in ruminants, Prasad and Sinha (1981a) in domestic animals and Yilmaz and Girgin (2005) in porcupine. However, the epinephrine and norepinephrine secreting cells could not be differentiated with haematoxylin and eosin stain in present study, which also supported the findings of Panchal *et al.* (1998) in sheep.

Patches of cortical cells were also observed in the medullary tissue around the central vein (Fig.2). This is in line with the findings of Prasad and Sinha (1981a) in horse and goat, Jamdar and Ema (1982) in donkey and Nagpal *et al.* (1991) in camel. It is quite possible that during development some patches of cortical cells might have found place in to the interior of medulla.

Adrenal medulla was highly vascular in nature. Blood sinusoids were present in between group of cells (Fig.1). A large central vein was present in all specimens in the central portion of medulla (Fig.2). This was lined with endothelial cells along with few fine bundles of reticular and elastic fibres. This view is also supported by the Prasad and Yadava (1973 and 1974) in buffalo, Esther (1978) in porcine, Prasad and Sinha (1981a) in domestic animals, Abdalla and Ali (1989) in camel and Baishya et al. (1998) in mithun and yak.

Prasad and Sinha (1981a) in buffalo and Abdalla and Ali (1989) in camel reported the presence of smooth muscle fibres in the central vein, which is contrary to the present findings.

Fibro-architectural component of medulla consisted of reticular and collagen fibres and both surrounded each ovoid or spherical group of medullary cells. These findings are in consonance with the observations made by Trautmann and Fiebiger (1957) and Prasad and Sinha (1981a) in domestic animal and Prasad and Yadava (1972) in buffalo.

Prasad and Yadava (1974) measured the thickness of adrenal medulla as 1235 µm in buffalo, while in present study it was recorded 1334.26+17.800 µm, which also tallied with the observations of Prasad and Sinha (1981b) in male goat that was 1332.86 µm. But Panchal *et al.* (1998) recorded the thickness of medulla as 295.75 µm in sheep and Yilmaz and Girgin (2005) noted 525 µm in Atlantic bottlenose dolphin. Medulla occupied 41.05% part of total adrenal as noted in present investigation which is almost similar to the findings of Prasad and Sinha (1981b) and Clark *et al.* (2005) in Atlantic bottlenose dolphin as both recorded it 41.23% and 41%, respectively.

Average cells size was measured as 33.34+0.934 µm and average nuclear size was 7.92+0.264 µm, which is contrary to the findings of Prasad and Yadava (1974) in buffalo who recorded average cell and nuclear size as 10.76 µm and 6.32 µm, respectively.

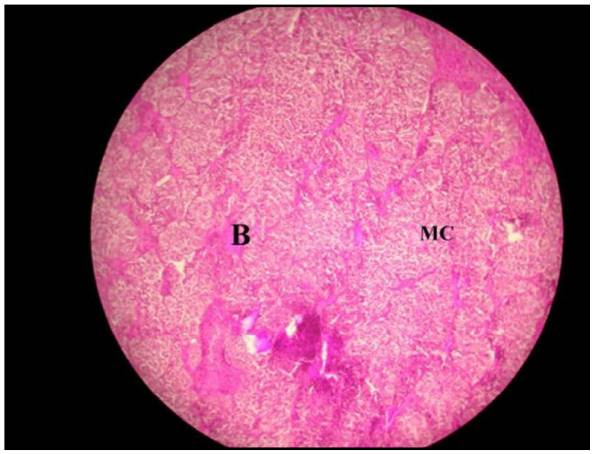

Fig.1: Section of adrenal gland showing vascular medulla (H & E Stain, 100 X) B- Blood vessel, MC- Medullary cells

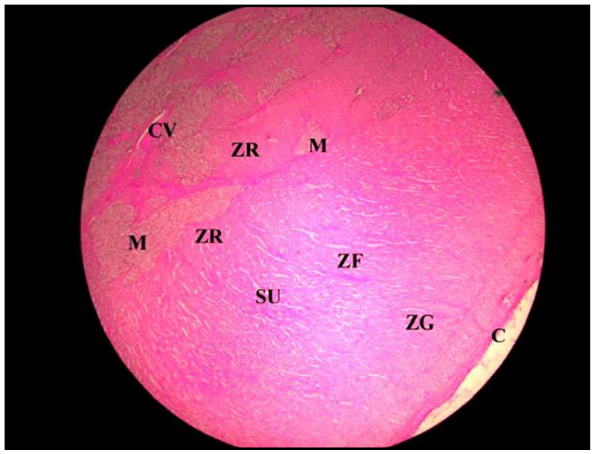

Fig.2 :Section of adrenal gland showing medullary tissue in Adrenal Cortex (H&E Stain, 40 X) M- medullary tissue, CV- central vein, ZR- Zona reticularis, SU- sinusoids, ZF-Zona fasciculate, ZG-Zona glomerulosa, C-capsule

Table 1: Statistical details of different variables used in the present study

S.No.	Name of variable	Mean	SE	SD	CV
1.	Capsular thickness (µm)	77.67	2.683	29.388	37.835
2.	Cortex thickness (µm)	1838.14	28.855	316.091	17.196
3.	Medulla thickness (µm)	1334.26	17.800	194.992	14.614
4.	Z. glomerulosa cell size (µm)	13.54	0.251	2.751	20.318
5.	Z. glomerulosa nuclear size (µm)	6.03	0.195	2.137	35.449
6.	Z. fasciculata cell size (µm)	18.20	0.377	4.127	22.669
7.	Z. fasciculata nuclear size (µm)	6.96	0.202	2.209	31.762
8.	Z. reticularis cell size (µm)	10.32	0.191	2.088	20.229
9.	Z. reticularis nuclear size (µm)	5.59	0.178	1.951	34.931
10.	Adrenal medulla cell size (µm)	33.34	0.934	10.232	30.692
11.	Adrenal medulla nuclear size (µm)	7.92	0.264	2.888	36.480

Table 2 : Average thickness of different zones of adrenal glands

	Capsule(µm)	Adrenal cortex			Adrenal medulla (µm)
		Zona glomerulosa	Zona fasciculata	Zona reticularis	
Overall Mean±SE	77.67 ±2.683	155.06 ±1.824	1509.80 ± 21.356	173.28 ± 2.792	1334.26 ± 17.800

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EFFECT OF INORGANIC ZINC SUPPLEMENTATION ON SEMINAL ATTRIBUTES AND SEXUAL BEHAVIOUR OF CROSSBRED BULLS

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ABSTRACT

Present study was designed to see effect of inorganic zinc supplementation on seminal characteristics and sexual behaviour of crossbred bulls. Twelve healthy, sexually mature and clinically normal crossbred (Holstein Friesian crosses) bulls of almost similar body weight and age group (nearly 2.5 to 5.0 years) were selected. The bulls were randomly divided into two groups (control and treatment). Diets were supplemented with @ 40 ppm inorganic zinc for 150 days including 30 days adaptation period. Zn supplementation had no significant effect on semen volume, pH, individual motility and sexual behaviour indices. However, 6.65 per cent increase of semen volume in the treatment group over control group. Only mass activity was significantly higher in the treatment group over the control group. Finally, it can be concluded that zinc supplementation improves semen volume and individual motility, and no significant change in sexual behaviour of the crossbred bulls.

Key words: Zinc supplementation, sexual behaviour, crossbred bulls

Introduction

The use of AI has greatly increased in India. The increased use of AI has dramatically increased the number of bulls needed for semen collection. The main objective of rearing bulls is to produce a large volume of high-quality semen in an efficient and safe manner. Zinc seems to play an important role in the physiology of spermatozoa; it has been reported to influence the process of spermatogenesis in ram, control motility in goats (Saleh *et al*., 1992), influence in testosterone synthesis (Prasad, 1991) in cattle. The knowledge of reproductive behaviour and semen quality are valuable tools to estimate the reproductive efficiency of a bull (Samo *et al*., 2005). Sexual behaviour is one of the important measures to assess reproductive performance of the bull. Sexual behaviour is differentiated into libido and mating ability (Chenoweth, 1981) and is very critical for breeding bulls to harvest the maximum number of spermatozoa. Libido of the bull depends on testosterone profile and plane of nutrition. Mating ability is the ability to complete service (Hultnas, 1959). The selection of bulls on the basis of sexual behaviour is more important and economical than libido and mating ability alone (Anzar *et al*., 1993). Alexandrov and Zajanckovskii (1969) found that zinc supplementation improved the sexual desire of breeding bulls. However, in tropical countries such studies in diary bulls are scare, in spite of the growing use of diary cattle for milk production in tropics. Keeping in this view, the following study was designed to see effect of zinc supplementation on seminal characteristics and sexual behaviour of crossbred bulls.

Materials and Methods

Twelve healthy, sexually mature and clinically normal crossbred (Holstein Friesian crosses) bulls of almost similar body weight and age group (nearly 2.5 to 5.0 years) were selected. The bulls were randomly divided into two groups (control and treatment). Diets were supplemented with @40 ppm inorganic zinc for 150 days including 30 days adaptation period.

Semen collection was performed by experienced semen collector and the timing was between 8.00 AM and 9.30 AM. Before semen collection, bulls were thoroughly cleaned with water and allowed to dry their skin for 15-30 min. Two successive ejaculates were collected with a 30 min interval, once a week, from each bull into a pre-warmed artificial vagina (42°C). All behavioural

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events expressed by the bulls during the pre-copulatory and copulatory periods were recorded for behavioral study. A water bath was used to maintain the sample at 30°C throughout the evaluation. The two ejaculates were pooled immediately after the second collection, if the mass activities of both samples are similar, otherwise evaluated separately. The maximum interval between two semen collections was 15 days.

Semen Evaluation

Volume: The semen was collected in 15 ml graduated metal free glass tube (0.1 ml accuracy).

Mass Motility: Mass motility was assessed just after the semen collection. Gross swirl rating (GSR) of undiluted semen was performed within 1 min of collection (Matharoo *et al*.,1985).

Individual Motility: Manual progressive motility and percentage motile spermatozoa were determined by placing 100 µl of undiluted semen into pre-warmed tubes containing Tris buffer and mixing.

Colour: Colour was scored creamy to watery for the neat semen.

pH: pH of the fresh semen was determined within 15 minutes of collection with Cyberscan 510 pH meter (Cyberscan 510 pH; Eutech Instrument, Singapore).

Measurement of Sexual Behaviour

Behavioural features were noted at the time of semen collection. Two Murrah (MU) buffalo bulls were used as dummy for semen collection. Different teasers were used on different days to minimize bull sexual satiation from a teaser, to provide uniform stimulus pressure and randomize teaser effects. The semen was collected once a week semen collection schedule (two ejaculates were collected each time) and each bull were observed for behaviours fortnightly. Each bull was assigned to be handled by two experienced handler who were familiar with the bulls. Bulls were led to a restrained teaser and freely permitted to mount and service an artificial vagina. On the day of semen collection, each bull was taken to the collection yard where two MU buffalo bulls were ready to serve as teasers. After first ejaculate was collected from the bulls 30 minutes passed before the same procedure was initiated to obtain second ejaculates of semen form the donor bulls. During this time, observer was stationed near each bull. With the aid of special timing watches and pre-recorded coded work sheets a series of relevant events could be quantified as to the time of occurrence. The sexual behaviour scoring was adopted as described by Anzer et al. (1993) with some modification.

Sexual behaviour score is divided into two parts; libido score and mating ability score. Libido was scored on the basis of reaction time (in seconds), sexual aggressiveness and tactile stimulation. Reaction time (second) classified and scored (in bracket) as up to 10(5), 10-30(4), 31-60(3), 61-120(2), 121-300(1) and above 300 (0). Sexual aggressiveness was classified and scored (in bracket) as aggressive (4), active (3), dull (2) and shy (1). Tactile stimulations (TS) comprises sniffing, flehmen reaction, licking, bleating, pawing, nudging, bunting, chin-resting, licking of penis and urinating.

Libido Score

A libido score (%) of each ejaculate was computed as shown below:

$$\left[\left\{\left(\text{RT score}+\text{SA score}\right)\div 0.2\,\text{per TS}\right\}\div 10\right]\times 100$$

If a bull did not mount on the first attempt, then the teaser was changed. If the bull did not mount the second teaser in the prescribed time (5 min), then refusal to mount designation was noted and a '0' (zero) score was given. Reaction time and sexual aggressiveness of only successful attempt was used for libido scoring.

Mating ability Score

Mating ability was scored on a 10 point scale distributed among different behavioural events displayed by bulls during semen collection i.e., Mounting (MO), penile erection (PE), protrusion score (PS), ejaculatory thrust (ET), grasping of teaser at pelvic level (Gr), penile movement to locate artificial vagina (Pm). After mounting (except false mount) if a bull did not ejaculate, then 1 point was deducted from the total score for each futile attempt. If a bull did not ejaculate in 3 attempts, a refusal to ejaculate designation was noted and a '0' (zero) score was given. The mating ability score was determined in successful attempts only as follows:

$$\left[\left\{\left(\text{MO}+\text{PE}+\text{PS}+\text{ET}+\text{Gr}+\text{Pm}\right)\cdot - \text{Futile attempts}\right\}\div 10\right]\times 100$$

Libido and mating ability scores (%) were averaged for first and second ejaculates separately, and net score (overall mean) was calculated by dividing their sums by 2.

Sexual Behaviour Score

The sexual behaviour score was calculated from the net scores of libido and mating ability as follows:

$$\left(\text{Libido score}+\text{Mating ability score}\right)\div 2$$

Results and Discussion

The frequency distribution of colour of semen is presented in Table 1. Treatment group donated more creamy variety of semen with higher sperm concentration as compared to control (unsupplemented) group. Only a rough estimate of sperm concentration was predicted by observing the colour of the ejaculates. Cattle bulls normally

produce milky white to yellowish semen (Anderson, 1992). There is no literature available correlating zinc and colour and consistency of semen.

The mean of ejaculatory volume has been depicted in Table 1. Zinc supplementation had no significant effect on semen volume. However, a trend of higher volume was observed in the zinc supplemented group than control group. There was 6.65 per cent increase in semen volume over the control group. Zinc supplementation has been shown to increase the ejaculate volume in different mammalian species (El-Masry *et al*., 1994; Tharwat, 1998; Osman *et al*., 2000). However, El-Rahim *et al*. (1995) observed no effect of zinc on seminal volume.

The means of mass activity (4 point scale) of semen are presented in Table 1. The results showed that the difference between groups was significant ($P<0.05$). Overall data indicated that there was higher mass activity in second ejaculate compared to first. The findings indicated that mass activity increased with the zinc supplementation. Mass motility is found to be increased by zinc supplementation in rabbit (El-Rahim *et al*., 1995).

The means of individual motility of semen are presented in Table 1. There was no significant ($P>0.05$) difference between the groups. Over the years, there have been conflicting reports on the effect of seminal zinc on sperm motility. Some researchers reported high zinc concentration to be associated with enhanced sperm cell motility (Caldamone *et al*., 1979; Sorensen *et al*., 1999), while others reported high zinc concentration to be associated with poor sperm motility (Danscher *et al*., 1978). However, Lewis-Jones *et al*. (1996) were unable to find any association between total seminal zinc and sperm motility. In the present study, there was no significant difference of sperm motility between the groups. Selection criterion for the semen sample may be the cause of non-significant difference among the different groups. Because semen sample having more than 50 per cent motility are included in the study. Zinc supplementation has been shown to improve sperm motility in goat (Saleh *et al*., 1992), rabbit (El-Masry *et al*., 1994), Holstein bulls (Wenli and Huiyin, 1998) and buffalo bulls (Osman *et al*., 2000).

The mean values of seminal pH have been depicted in Table 1.The results showed that zinc supplementation did not significantly ($P>0.05$) influence hydrogen ion concentration (pH) of semen. The present findings are contradictory to that of Kumar (2003) who found decreased pH value in zinc supplemented crossbred bulls, but remained within the normal range. In buck, Saleh *et al*. (1992) found increased pH value in zinc supplemented groups.

Pre-copulatory behavioural characteristics of the bulls were recorded while they were presented behind the dummy for semen collection. Zinc supplementation had no significant effect on pre-copulatory behavioural characteristics (Table 2). The most commonly observed pre-copulatory behavioural characteristics (%) were sniffing of the anal region (79.14), followed by Flehman reaction (72.39), licking the teaser, particularly in the preputial area (63.19), butting (63.19) and chin resting (58.28). Other behavioural characteristics were found infrequently. Least observed characteristic was bleating and only 3.68 per cent cases were observed.

The sexual behaviour indices did not show any significant ($P>0.05$) difference between control and treatment groups. Reaction time was recorded as the time lapse between release of bull to the dummy and first attempt to mount. The reaction time (sec) varied from 6.01 to 222.91 minutes. In contrast to the present study, reaction time (RT) was significantly improved following zinc supplementation (ZnO) to Egyptian buffalo (Khalifa, 1997; Osman *et al*., 2000). Total time taken for successful ejaculation was the time lapse between release of bull to the dummy and dismount of the bull after ejaculation. After approaching the teaser, bulls with stronger libido mounted the teaser rapidly without exhibiting very few tactile stimulation. The bulls with poor libido took longer time to mount and displayed various characteristic like sniffing, bunting, licking, chin-resting and flehmen during this period (Anzar *et al*., 1988). Assessment of mating ability of a breeding bull is important because inability to copulate is associated with several factors (Bane and Hansen, 1962; Anzar *et al*., 1993). It appears that the sexual behaviour trends were not influenced by the dietary zinc supplementation. An important indicator that sexual preparation was adequate and that the position of the teaser bull, and semen collector were appropriate for the bull to produce a strong thrust at the time of semen collection, the position of the hind feet was observed before and during ejaculation. Both hind feet moved forward synchronously in 100% of the semen collections, indicative of strong thrust in the present study. Libido of the bull depends on testosterone profile and plane of nutrition. Mating ability is the ability to complete service (Hultnas, 1959). The selection of bulls on the basis of sexual behaviour is more important and economical than libido and mating ability alone (Anzar *et al*., 1993). Alexandrov and Zajanckovskii (1969) found that zinc supplementation improved the sexual desire of breeding bulls, whereas, in the present study we did not find significant change of the same following zinc supplementation. Neathery *et al*. (1973) found lower libido in zinc-deficient goats. Deficient goats attempted mounting, but had muscle spasm when weight was put on rear legs.

Conclusion

From the above study it is concluded that Zn supplementation improves semen volume, individual motility of the crossbred bull semen, while other parameters and sexual behaviour did not show any effect. However, long term study is needed to come to specific conclusion.

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STUDIES ON CLINICO-PHYSIOLOGICAL AND HAEMATO-BIOCHEMICAL CHANGES FOLLOWING EPIDURAL ANALGESIA BY BUPIVACAINE AND ROPIVACAINE IN GOATS[#]

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ABSTRACT

Anaesthetic effect of bupivacaine (1.7 mg/kg) and ropivacaine (0.6 mg/kg) was evaluated by clinicophysiological and haematobiochemical parameters in 6 goats after their administration in lumbosacral epidural space. The onset of analgesia was faster in animals in which ropivacaine was used . Ropivacaine produced complete analgesia of tail, perineum, inguinal and thigh regions in all animals. The physiological parameters (pulse rate, respiratory rate and rectal temperature) did not show any significant change in all the animals in which ropivacaine was used as compared to animals in which bupivacaine was used, which showed significant decrease in all the three physiological parameters. Among the haematobiochemical parameters (Hb, TLC, DLC, PVC, TEC, glucose, total protein, ALP, ALT, total bilirubin, BUN and creatinine) only glucose showed significant increase in animals in which ropivacaine was used as compared to animals in which bupivacaine was used which showed significant increase in glucose, ALT and BUN. Therefore, ropivacaine (0.6 mg/kg) may be used in clinical situations in the patients having impaired kidney and liver functions.

Key words: Ropivacaine, bupivacaine, anaesthesia.

Introduction

Ruminants generally are not considered good subjects for general anaesthesia mainly because of hazards of regurgitation and inhalation of ruminal contents or saliva into the lungs (Hall and Clarke, 1991). Thus, regional anaesthesia produced by the epidural injections of anaesthetic agents are most frequently employed in these species. The epidural analgesics that have been used in small ruminant practice include lignocaine, xylazine (Nelson *et al.*, 1979) and bupivacaine (Mohammed,1997). Bupivacaine hydrochloride is most widely used local analgesic for peri-operative analgesia because of its tendency to block sensory fibres preferentially with relative sparing of motor fibres. (Alvarez *et al.*, 1983). Bupivacaine hydrochloride is a local analgesic agent , which is more stable, more potent than lignocaine hydrochloride and is well tolerated by all tissues. Ropivacaine is the (-) enantiomer of propivacaine, and is long acting amide local anaesthetic agent. (Raffa *et al.*, 1992). Ropivacaine is well tolerated regional anaesthetic agent effective for surgical anaesthesia as well as the relief of post operative pain.

Materials and Methods

The study was conducted on 6 healthy local non-descript breed of goats of same sex (male) weighing between 10-12 kg. All the goats were dewormed prior to experiment by administering the bolus Fenbandazole orally at the dose rate of 5 mg/ kg of body weight. All the goats were maintained under similar standard managemental conditions and feeding schedule. Each experimental animal was subjected to 2 treatments and each treatment lasted for 3 days. An interval of 8 days after the first treatment was given before the onset of the next treatment. Each animal was subjected to two treatments at an interval of 8 days. In treatment I bupivacaine was given alone @ 1.7 mg/kg b.wt. in the lumbosacral space and in treatment II ropivacaine was given alone @ 0.6 mg/kg b.wt in the lumbo- sacral space.

The epidural catheter was placed at the lumbo-sacral space for the delivery of bupivacaine and ropivacaine. The rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute) was recorded at 0, 20, 40, 60, 80,120,180, 240, 300, 360, 480 minutes. The “0” hr values from each animal

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was recorded immediately before the start of the treatment, as the control value. The onset and duration of analgesia and recovery was determined by the pin prick method.

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

Eight blood samples from each animal for each treatment was collected at 0 hr.,15 min., 3 hrs., 6 hrs., 12 hrs., 24 hrs., 36 hrs., and 72 hours for estimation of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count by standard methods. Blood glucose (GOD-POD method), total protein (Biuret method), ALT (IFCC method), ALP (Tris- Carb method), total bilirubin (Jendrassic and Grof method), BUN (Young's method) and serum creatinine (GLDH-Urease method) were also estimated at same intervals. The data obtained during study was analyzed by employing completely randomized design (CRD).

Results and Discussion

The onset of analgesia was faster in treatment II (2.5 minutes)as compared to treatment I (5 minutes) in which bupivacaine was used. Amarpal *et al.* (2002) also recorded faster analgesia with epidural ropivacaine in buffalo calves. Duration of analgesia was longer in treatment II (110 minutes) as compared to treatment I (92.50 minutes). Singh *et al.* (2005) used 0.2% of Ropivacaine in healthy goats and duration of analgesia was 71.5 ± 4.4 min. The animals who received ropivacaine recovered quickly as compared to animals who received bupivacaine.

In treatment I animals showed significant ($P<0.05$) decrease in respiration rate from 20 to 80 minutes , after that it increased and reached to the base value while in II animals showed non significant ($P>0.05$) decrease in respiration rate. Gill *et al.* (1984) and Ameerjan and Rajendran (1985) also reported significant decrease in respiration rate after epidural administration of bupivacaine in cattle and dogs respectively. Singh *et al.* (2005) did not find any significant change in respiration rate after epidural administration of ropivacaine in goats. So in the present study, decrease in respiration rate in treatment I was observed which may due to depression of respiratory centre by bupivacaine (Lumb and Jones, 1984). There was no decrease in respiration in ropivacaine group suggestive of its superiority over bupivacaine.

In treatment I, there was a significant ($P<0.05$) decrease in rectal temperature up to 80 minutes there after the value started increasing and reached to base value while as in treatment II there was non-significant ($P>0.05$) decrease in rectal temperature. Dhage and Pawshe (2010) reported that there was significant decrease in rectal temperature after using 0.5% bupivacaine epidurally in goats. Singh *et al.* (2005) used 0.2% ropivacaine in goats and they also observed non-significant decrease in rectal temperature. Hence it can be concluded that ropivacaine does not have any adverse effect on thermoregulatory centre in hypothalamus. So in present study the decrease in body temperature in treatment I may be due to the peripheral vasodilatation (Mishra *et al*., 1993).

In treatment I, there was a significant ($P<0.05$) gradual decrease in pulse rate after epidural administration of Bupivacaine till 80 minutes. After 80 minute, there was gradual rise in pulse rate and at 480 minute. The value reached nearly to pre-treatment level. There were non significant alterations in pulse rate in treatment II. The present findings on pulse rate are in agreement with Kinjavdekar *et al.* (2000) who reported significant decrease in pulse rate after using bupivacaine subarachnoidally in goats. Singh *et al.* (2005) observed non significant changes in pulse rate after using epidural ropivacaine in goats. So the decrease in pulse rate in present study in treatment I may be due to the cardiotoxicity of bupivacaine (Lumb and Jones, 1984).

The haematological parameters (Hb, TEC, TLC, PCV and DLC) did not show any significant changes in both the treatments. These findings are in accordance with Singh *et al.* (2005) and Raghuvanshi (2008).

Regarding biochemical parameters blood glucose was significantly increased in both the treatments up to 3 hours of post administration of drugs. Dadafarid and Najafpour (2008) used epidural bupivacaine in sheep and observed significant increase in blood glucose level. Singh *et al.* (2002) also observed hyperglycaemia after using epidural ropivacaine in buffalo calves. So in present study the increase in blood glucose level may be due to the release of adrenocorticotrophic hormones due to anaesthetic stress (Mirakhur *et al.,* 1984) which might be responsible for hyperglycemia in both the treatments.

Total protein, ALP, total bilirubin and serum creatinine did not show any significant changes in both the treatments. These results are in agreement with Singh (2005), Singh *et al.* (1999) which suggested that there was non significant changes in these values after epidural use of ropivacaine and bupivacaine in goats, respectively. The mean value of ALT increased significantly (P<0.05) up to 3 hour in treatment I after that the value started decreasing and reached to base value. In treatment II, there was non-significant (P>0.05) increase in ALT values. Tripathi *et al.* (1988) also reported similar increase in level of ALT following epidural use of bupivacaine in dogs. Therefore, in the present study there is possibility that during the process of metabolization of drugs there might have been some disruption in liver parenchymal cells which might have increased the cell membrane permeability leading to elevation in the level of ALT in the blood (Pandey and Rao, 2000). In treatment II, there was no damage to liver as indicated by the values. There was significant (P<0.05) increase in blood urea nitrogen values in treatment I up to 3 hours of observations post administration of drug. In treatment II, there was non significant (P>0.05) increase in blood urea nitrogen values after epidural administration of ropivacaine. Singh *et al.* (2005) reported non significant increase in creatinine and BUN values after using epidural ropivacaine in goats and it matched to the finding of present study. Bisen *et al.* (1994) and Pandey and Rao (2000) reported significant elevation in BUN levels following parenteral administration of opioids like pentazocine in goats. It is therefore, presumed that the increase in BUN in treatment I could be as a result of decrease in glomerular filtration rate due to reduction in renal blood flow, disturbance in urine output leading to urinary retention and other alteration in urinary function (Wright, 1965).In treatment II, there was no increase in creatinine and BUN values suggesting that ropivacaine has no effect on renal functions. Further it is safe as epidural analgesic as far as its effects on clinico-physiological and haemato-biochemical parameters are concerned. The changes in these parameters were minimal and transient. Therefore ropivacaine can be used in animals having impaired some of the clinico-physiological functions.

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IS SERUM GAMMA GLUTAMYL TRANSFERASE A BIOMARKER OF OXIDATIVE STRESS IN CLINICALLY AFFECTED *MARWARI* GOAT?

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ABSTRACT

To find out the serum gamma-glutamyl transferase (GGT) as a biomarker of oxidative stress in clinically affected *Marwari* goat, the present investigation was carried out. The animals were grouped into healthy and affected ones comprising of gastrointestinal parasiticised, pneumonia affected, having enterotoxaemia, and drought affected. In affected group the mean value of GGT increased significantly ($P<0.05$) as compared to respective healthy mean value. The highest value was seen in the animals having pneumonia and least value in the animals which were parasiticised. It was concluded that increased activity of the serum GGT was due to oxidative stress in the affected animals and it can be used effectively as a biomarker of oxidative stress in these animals.

***Key words*:** Gamma-glutamyl transferase, goat, stress

Introduction

Gamma glutamyl-transferase (GGT, EC 2.3.2.2) or Gamma-glutamyl transpeptidase (GGTP), is a carboxypeptidase which cleaves C-terminal glutamyl groups and transfers them to peptides and other suitable acceptors. Several years ago it was proposed that serum GGT should be regarded as a marker of oxidative stress. The concept of serum GGT as primarily either an antioxidant or a pro-oxidant marker presents a challenge in understanding the GGT and disease relationships. As an antioxidant marker, even though the initial increase of GGT may be compensatory to depleted GSH because of oxidative stress, serum GGT would be expected to decrease the risk of clinical outcomes because the parallel increase in cellular GGT would lead to increased intracellular GSH (Lee *et al*., 2008). GGT occurs as a membrane associated aggregate and is also involved in glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water, certain L-amino acids and peptides. Glutathione breakdown results in the formation of cystein, a thiol compound exerting antioxidant effects and helps to preserve intracellular homeostasis of oxidative stress. Increase in environmental oxidative stress may induce GGT via nuclear factor kappa-light-chain-enhancer of activated B cells or NFkB, a protein complex that acts as a transcription factor (Yokoyama, 2007).

To determine the diagnostic value of the serum GGT level, it is necessary to carry out investigations in animals when affected with various diseases (Goranov, 1984) and then compare them with the normal range of values given for those animals, preferrably of the same breed, and in similar environmental conditions. Oxidative stress is a large rise in the cellular reduction potential, increased production of free radicals and reactive oxygen species (ROS), and a decrease in antioxidant defence, contributing to health disorders. Oxidative stress is extremely dangerous as it does not exhibit any symptom and is recognisable with great difficulty by means of laboratory methods only. *Marwari* is a breed of goat in arid tract playing an important role in the economy of marginal farmers and labourers but very little attention has been paid on diagnostic aspects related with oxidative stress. Therefore, the present investigation was planned to determine serum GGT as a biomarker of oxidative stress in clinically affected *Marwari* goat.

Materials and Methods

Serum enzyme levels were determined in adult goat of *Marwari* breed belonging to farmers' stock of arid tract of Rajasthan state, India. The animals were grouped into healthy (200) and affected (200).

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Table 1: Mean ± SEM values of serum ?GT in *Marwari* goats

Serum enzyme	Healthy group (200)	Affected group (175)			
		Gastrointestinal parasiticised (50)	Pneumonia affected (50)	Having enterotoxaemia (25)	Drought affected (75)
?GT, UI^{-1}	24.5±1.3	30.8±1.9 ^{b}	119.8±2.5 ^{b}	100.8±3.5 ^{b}	112.5±4.3 ^{b}

Figures in the parenthesis indicate number of animals.
Superscript 'b' indicates a significant difference (P<0.05) from respective healthy mean value
gGT= Gamma glutamyl transferase

In healthy animals the blood samples were collected as a part of routine health checkup during moderate ambience (maximum temperature varied between 26 and 29 ^{0}C). The affected group comprised of gastrointestinal parasiticised (50), pneumonia affected (50), having enterotoxaemia (25) and drought affected (75) irrespective of sex.

All the samples were collected in sterile tubes without anticoagulants for serum separation. Gamma-glutamayl transferase (GGT, E.C. 2.3.2.2) was determined by the method of Wolf and William (1973). All results of enzyme activities were expressed as per SI units in Units/litre written as U.l. Statistical significance for individual parameter between healthy and affected group was analysed as per Snedecor and Cochran (1994).

Results and Discussion

The mean values of serum GGT are presented in Table 1. In affected group the mean values of serum GGT increased significantly (P<0.05) as compared to respective healthy mean value. The highest value was seen in the animals having pneumonia and least value in the animals which were parasiticised. It showed that the increased levels of serum GGT not only indicated towards the liver involvement but also exhibited the role in oxidative stress as maximum rise was observed in pneumonia cases.

Onat *et al.* (2004) also suggested that moderately elevated serum GGT activity reflected enhanced oxidative stress and hence it can be taken as both markers of hepatic involvement as well as oxidative stress. The role in oxidative stress is due to the fact that GGT is present on the surface of most cells and in serum and mediates cellular glutathione uptake which is an important element of intracellular protective antioxidant mechanisms. It is also regarded as a “pro-oxidant” (Nannipieri *et al*., 2005).

The variations observed in the present study could help in realistic evaluation of the management practice, nutrition and diagnosis of disease conditions as serum GGT belonged to the class whose level in the blood can be used diagnostically to determine the level of damage or dysfunction of liver along with a marker of oxidative stress. Increased activity of serum GGT showed modulation of liver functions. Any disease state of the body can affect the liver functions as a part of stress alleviation response. It is suggested that increased levels of serum GGT should also be looked in terms of oxidative stress along with liver problems. It was concluded that increased activity of the serum GGT was due to oxidative stress in the affected animals and it can be used effectively as a biomarker of oxidative stress in these animals.

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SURGICAL CORRECTION OF CORNEAL DERMOID IN A CROSS BRED CALF

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Corneal dermoids are islands of skin that are histologically normal but displaced to an abnormal location, usually the lateral canthus or limbus, third eye lid, medial canthus and eye lid and described as a heritable autosomal recessive and polygenic trait in Hereford Cattle (Ismail, 1994; Rezaeu *et al*., 2007).

Corneal dermoid is a congenital choristoma, characterized by the presence of heterotopic cutaneous tissue in an inappropriate place (Horikiri, 1994; Slatter, 2001).They may affect the eyelids, conjunctiva (Palpebral and bulbar). Nictitating membrane and cornea (Gelatt, 1973).

Dermoids contain many elements of normal skin such as epidermis, dermis, fat, sebaceous glands, hair follicles, and frequently there is hair. The tissues are usually irritating the eye and associated structures (Gelatt, 1973). Thus, the patients suffer from chronic epiphora and keratitis (Gelat, 1991).

Dermoids are a consequence of abnormal differentiation of tissues of the occular surface (Roberts and Lipton, 1975; Moore *et al.,* 1999).

The present paper reports, a case of corneal dermoid in cross breed calf.

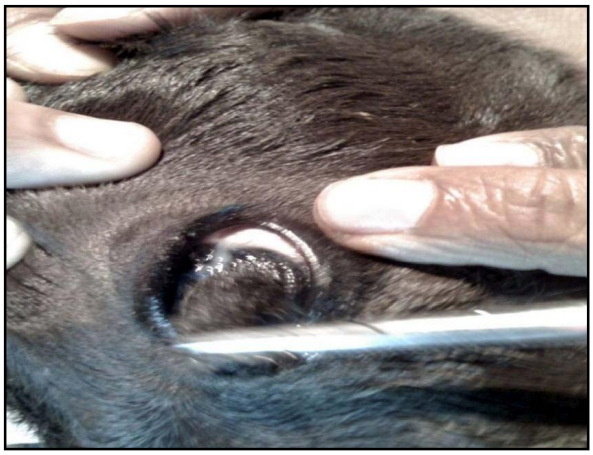

Fig. 1: Corneal dermoid on the left eye of a 10 days old calf, Note the long soft hair (A)

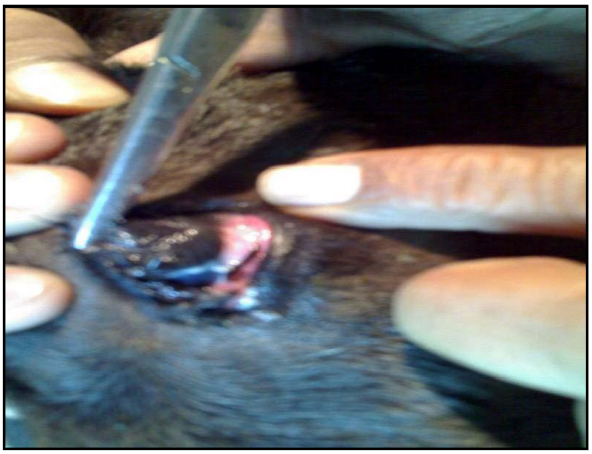

Fig. 2: Left eye of 10 days old calf following superficial keratectomy.

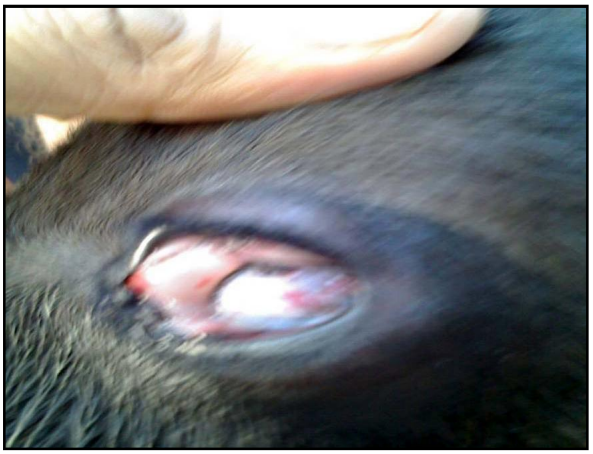

Fig. 3: Almost proper opening between eyelids after superficial keratectomy.

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Case

A 10 days old cross bred calf, was presented with the history of corneal dermoid in left eye at the time of birth. Removal of dermoid by superficial keratectomy was essential to relieve the related clinical signs. If the dermoid had not been totally removed some degree of recurrence could be expected (Gelatt, 1971). Thus, the dermoid excised completely, if possible, without scarring of the cornea.

The animal was restrained and lignocaine hydrochloride was used as a topical anaesthetic.

Superficial keratectomy was performed to remove the mass. The lesion was carefully dissected from the underlying cornea to avoid penetrating the anterior chamber of the eye. The edge of the bulber conjunctival wound was sutured to the limbus with 6-0 cat gut. Gentamicin sulphate ointment was applied TID for 5 days. Recovery was uneventful.

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POST-PARTUM UTERINE PROLAPSE IN BUFFALO - A REPORT OF TWO CASES

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Uterine prolapse is the complication of third stage of parturition involving the eversion of generally both the uterine horns in ruminants (Noakes *et al.,* 2001). Among reproductive disorders, prolapse of reproductive organs either vagina, cervix or both and or uterus, occurs as a common gestational accident, which requires early attention, prompt and efficient management (Roberts, 1971). Prolapsed uterus is highly prone to mechanical injury, trauma and environmental contamination that may lead to increased maternal morbidity and even may lead to death of the animal owing to trauma, laceration, subsequent haemorrhage, tissue necrosis, bacterial contamination, sometimes urinary incontinence, stress and shock (Jana and Ghosh 2004). The report presents two cases of post partum uterine prolapse in buffalo.

Case history and observations

Pluriparous Murrah crossbred buffaloes (n=2) both about ten years of age were brought with the history of complete eversion of uterus immediately after calving live foetuses. Clinical examination revealed hanging down of oedematous, completely everted uterus with severe traumatic injury, necrosis and heavy contamination. The cases brought to notice were delayed by more than 4 hours. The animal appeared restless and showed abdominal straining. Animals were off-fed from last 2-3 days, weak, debilitated, recumbent and with pale conjunctiva.

Treatment and Discussion

The buffaloes were treated as emergency cases under epidural anaesthesia (lignocaine hydrochloride 2% @ 5 ml). The prolapsed mass was washed thoroughly with potassium permanganate solution and hyper-osmotic salt solution but the oedematous prolapsed mass did not get reduced to manageable size and later oxytocin- 30 IU was administered intravenously. Truss of straw was placed beneath the posterior abdomen to rise the hind quarter. Prior to replacement, the heavy weight of prolapsed mass was supported by keeping it over the thighs of veterinarian and xylocaine jelly was smeared over it. Having well soaked hands the uterus was replaced little by little starting from the portions nearest to the vulvur lips. Due care was taken to prevent the injury to exposed maternal caruncles and vuvlar lips do not become turned inwards. Finally with the full extended arm the uterus was pressed forward beyond the cervical ring. But the exhaustive efforts could not manage to save the lives.

Similar to our study, Pandit *et al*. (1982) reported that pluriparous and stall fed animals are more prone to genital prolapse than heifers and freely grazing animals. In present cases animals were old age, off-fed, anaemic as indicated by pale conjunctiva which may develop hypocalcaemia leading to uterine inertia. Uterine inertia along with abdominal straining is the major factor causing the uterine prolapse (Noakes *et al.,* 2001; Morrow 1980). Trauma, injury, necrosis of the prolapsed uterus might result in exaggerated release of inflammatory mediators and toxins which may direct animal towards shock. Further, due to delay in handling the prolapsed mass out weighs and may resulted into rupture of mesovarium and ovarian artery leading to internal haemorrhages and finally death due to shock (Noakes *et al.,* 2001).

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COST ECONOMICS OF RAISING FEEDER PIGLETS ON KITCHEN WASTE AND CONCENTRATE RATION

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ABSTRACT

A kitchen waste feeding trial to adequately replace grains in the feeder piglet diet was undertaken during May-July 2008. The study revealed that kitchen waste contained higher DE in terms of 3300 Kcal/kg then grain ration 2900 Kcal/kg. Piglets maintained on 1:1 ratio of kitchen waste and concentrate in diet had feed conversion ratio of 3.75.These piglets had live weight gain 200.66 gm/day. Considering the cost economics of raising feeder piglets, it was inferred that sale of feeder piglets fetch higher returns than finishers ₹ 964-1464 as feed required per kilogram gain is more during finishing up to 6-7months.

Key words: Economics, feeder piglets, kitchen waste, concentrate, ADG, FCR

In the Indian subcontinent, there has been increasing interest and acceptability of pork to meet the ever increasing demand of animal origin protein and critical growth promoting vitamins in the human diet. Under traditional Indian system of maintaining pig, the production cost is much lower than other animals. They outshine, being omnivores efficient in digesting and assimilating the plant as well as animal materials in their diet and finally converting into edible high caloric value biomass for human consumption. Nevertheless, this has been unethical and injurious to human health. Pig occupies a preferential choice over other livestock farming; on account of the highest feed conversion efficiency, shorter generation interval, faster growth and development, early sexual maturity and above all much less susceptible to infectious diseases (Sharada, 2005). However, the requirement of 50-60% food grains in their normal diet put hogs in a direct competition with human being. Therefore, in order to substitute food grains with alternate system feeding, the present study was planned on waste food materials in the form of kitchen/catering/hostel/ restaurants/dhaba refuge etc., and to work out the underlined techno economics of maintaining new born piglets in captivity.

Materials and Methods

The present study was conducted on 4 male and 4 female suckling white Yorkshire piglets, aged 15 days, with a mean body weight 4.38 ±1.058 kg. They were maintained in captivity under piggery unit comprising of one male and six females aged 2.5 yrs as breeding stock, at the Apollo College of Veterinary Medicine, Jaipur. They were housed in pucca flooring pig shelters of 0.74 covered floor area (m^2) for each piglet constructed recently. The study was of 75 days duration and was undertaken during summer (May-July 2008) in semiarid geoclimatic prevailing in Jaipur.

The piglets were fed *ad libitum,* dividing the feeding time into two halves, *viz.* in the morning at 10 am and in the evening at 3 pm.The piglets were weaned at the age of 6 weeks The daily data on total feed intake were generated and fortnightly gain in body weight was generated to access feed conversion efficiency. Fresh water was made available round the clock at all times during the experimental period.

Piglets were maintained on kitchen waste and pig concentrate in 1:1 ratio on DM basis in conformity with BIS requirement for starter pigs. The calculated digestible energy and estimated crude protein values of kitchen waste and pig concentrate were 3300 and 2900 Kcal/kg, 14.11% and 25.45%, respectively. Ingredient composition of concentrate mixture is given on Table1 a, b. Cost of concentrate was calculated @ ₹13 per kg based on prevailing local market price during the period of study. Adequate kitchen waste was obtained free of cost from the ACVM hostel and hotels located in nearby areas. The main components in kitchen wastes were residues of rice, wheat chapattis/bread, dal, rarely egg, bones, butter raw and cooked vegetables etc. The extraneous material like plastics, foil, broken glass pieces were carefully separated. Cost of transportation of the said material was @ ₹ 1.00/kg.

All the animals were intramuscularly injected with iron dextran injection on 4th and 14th day of age. The piglets on attaining 2 months of age were dewormed with Albendazole and vaccinated against swine fever. Chemical composition of the samples of feeds was determined as per proximate analytical method (AOAC, 1980). The data were statistically analyzed (Snedecor and Cochran 1994).

Results and Discussion

A feeding trial of 75 days duration was undertaken to evaluate growth rate, feed conversion efficiency and cost of live weight gain vis-à-vis sale

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Table 1(a): Chemical composition of piglet diet (% DM basis)

Feed stuffs	DM	CP	CF	EE	NFE	TA
Kitchen waste	23.12	14.11	4.2	7.1	60.57	5.15
Pig concentrate ration	89.12	25.45	8.1	3.2	50.45	8.8

Table 1(b): Ingredient composition of the concentrate ration

Soya DOC	20%
Guar *korma*	20%
Mustard oil cake	15%
Malt sprout	10%
DORB	15%
Rice bran	10%
Molasses	4%
Min mix without salt	3%
DCP	2%
Salt	1%
Vitamin ADEK supplement	++
Vitamin B complex supplement	++
Methionine and lysine	++

Table 2(a): Mean body weight and growth performance of experimental piglets

PARTICULARS	FINDINGS
Birth weight(kg)	0.957 kg±0.16
Average initial body weight(kg)	4.38±1.05
Average final body weight(kg)	19.43±0.48
ADG(gm)	200.66±0.01
Average total weight gain(kg)	15.05±1.30
Average total DM intake (kg)/day	0.750
Feed conversion ratio	3.75:1
Feed conversion efficiency	26.66%
Cost of feeding per kg live weight gain (Rs/kg)	3.850

Table 2 (b): Fortnightly body weight gain, total feed intake, energy consumed in (Kcal/animal) etc

Fortnight	Body weight * (mean +SD)	Mean feed Intake** (mean +SD)
1.	4.38±1.05	3.75±0.07
2.	7.19±1.05	8.50±0.42
3.	9.906±1.09	9.80±0.19
4.	12.577±1.11	12.37±0.24
5.	15.377±1.30	14.25±0.19
6.	19.43±0.48	18.75±0.16

***** in kg ****** feed offered +mothers milk until weaning

Table 3: Production Cost Analysis

Ration component	DM consumption/pig/d (Kg)	Feed consumption (Kg ASB)pig/d	Feed cost (/kg) ASB	Cost in
Pig starter ration	1.875	2.1	13	27.3
Kitchen waste	1.875	8.1	1	8.1
Total	3.75	1.902	-	35.4

price of feeder piglets in order to minimize cost of production and dispense with cereal grains in pig ration. The average CP content in the diet was 19.75% approximately analogues to values 19.34% in boiled kitchen waste and raw kitchen waste as earlier reported by Kumar *et al.* (2009).

Mean body weight and growth performance of the piglets total feed intake, energy consumed in terms of Kcal/animal under the trial are summarized in Table 2 a, b.

Successive fortnightly average body weights of piglets from zero to 6th fortnights were 0.957±0.16, 4.38±1.05, 7.19±1.05, 9.906±1.091, 12.57±1.11, 15.37±1.30, 19.43 ± 0.48 kgs. The final body weight of piglets during 3rd month ranged from 18.892 kg to 19.962 kg. The calculated mean body weight gain by each piglet during the period of study work out to 200.66±0.017gm. On similar lines, Shiv kumar *et al*. (2009) reported that body weight gain in pigs from weaning to 210 days on feeding 50% garbage and 50% concentrate ration higher growth rate of 316 gm/day, as compared to those exclusively fed on kitchen waste/residue gain 287gm/day and those exclusively consuming concentrate ration manifesting with 209 gm/day. The reason for higher daily body weight gain in trial conducted Shiv kumar *et al*. (2009) seems to be on account of piglets being in active phase of growth for a longer period of trial (2-7 months) as against 0-3 months in our study.

On the contrary in another trial documented by Kumar *et al.* (2009). Relatively lower ADG of 176.11 gm in piglets reared from weaning to 255 days when fed on ration having 75% kitchen waste and 25% concentrate. The lower weight gain of 176.11 gm in his trial as compared to 200.66 gm in the present trial attributed to kitchen waste and pig concentrate in 50:50 ratios. Thus, ultimate gross composition of mixed ration seems important factor regulating the energy protein requirement as per laid down nutritional standard. Probably the higher energy level in kitchen waste in combination with protein and vitamin rich concentrate mixture seems to have contributed better growth rate in the present feeding trial.

It is, therefore, concluded that higher growth rate in piglets maintained on kitchen waste may be due to the fact that kitchen waste played important role in meeting rich nutrient requirement of growing animals. During the early phase of growth the ratio of feed component could be 50:50, which may be increased to 66.5:33.5 during the later phase of life i.e. up to finishing phases. During starter, grower and finisher phase the two components of ration may be at the rate of tentatively 1:1, 2:1, 3:1 as exclusive feeding of kitchen waste though meets energy requirement of the animal, lacks good protein quality including deficiency of mineral and vitamin etc. The imbalance in energy, protein, mineral vitamin etc., requires further planned trial before making a concrete recommendation on the subject.

The trends of feed conversion efficiency of piglets under present study are similar to earlier observation of Shiv kumar *et al.* (2009) with FCR of 3.61 to 3.90 concentrate and garbage based ration as compared to 4.30 to 4.38 under exclusively concentrate fed group and 4.14 to 4.25 under exclusively garbage fed group. Under exclusively concentrate fed growing piglets FCR of 4.02 was reported by Medhi *et al.* (2009) and Kumar *et al*. (2009) also observed feed conversion ratio of 4.10.The results of present study indicate superior feed conversion efficiency in pigs fed kitchen waste and concentrate ration than conventional ration. This might be due to better quality of energy, protein and other nutrients in total mixed diet comprising of kitchen waste and concentrate resulting in better utilization of nutrients in by piglets.

Cost economics

Feed cost per kg live weight gain recorded herein as ₹ 35.4/- (Table 3) besides, overhead charges in the form of the initial cost, wages of labour and vet aids etc @ ₹ 18/kg. The net cost of production works out to ₹ 53.4/kg live weight. Taking the production cost @ ₹ 53.4/kg live weight in this trial, the total production cost of feeder piglets weighing 19.43 kg will work out to 1035.96. However, the market sale price of feeder pigs aged between 2-3 months, fetch nearly ₹ 2000 to ₹ 2500 against the production cost of ₹ 1035.96 and a net profit margin of ₹ 964 to 1464 for each feeder piglet. In conclusion raising feeder piglets in the present trial has been a better farming enterprise, to fetch higher returns besides, saving on recurring expenditures incidental to cost of feeding, labour, space etc., when raised up to finishing stage. Replacement of pig concentrate feed with 50% kitchen waste also helps in reducing the cost of grains while rearing piglets.

Acknowledgements

The authors express their deep sense of gratitude and sincere thanks to Dean, Apollo College of Veterinary Medicine, Jaipur, India for facilities provided.

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EFFECT OF TREADMILL EXERCISE ON SOME PHYSIOLOGICAL AND HEMATOLOGICAL PARAMETERS IN GERMAN SHEPHERD DOGS

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ABSTRACT

A study was conducted on twelve healthy adult German Shepherd dogs to observe the effects of treadmill exercise at 20, 40 and 60 minutes of exercise. As the exercise progressed, there was significant increase in rectal temperature and marked increase in respiratory rate leading to panting. The heart rate increased gradually but significantly at each stage of exercise. There was simultaneous significant increase in haemoglobin and packed cell volume but plasma total proteins concentration was not affected. The results showed that several responses are invoked in these dogs for thermoregulation and to meet increased oxygen demand during exercise.

Key words: Treadmill exercise, German shepherd, physiological and haematological parameters

Introduction

The capacity to exercise depends upon the availability of oxygen and metabolic substrates to working skeletal muscles. Therefore to meet increased demands of oxygen and metabolic substrates, it is expected that a number of modulatory changes would takes place in the body. The exercise physiology of the human athlete, the racehorse the greyhound dogs and the camel has been extensively investigated (Erickson and Pool, 2004). The objective of the present study was to investigate the effect of treadmill exercise on some physiological and haematological parameters in German Shepherd dogs.

Materials and Methods

This study was conducted on 12 apparently healthy German Shepherd dogs of either sex, 24-42 months of age and weighing 38-44 kg. All animals were maintained under similar managemental and feeding schedule. Water was provided *ad-libitum*.

The treadmill used was designed at fitness world, U.S.A. for laboratory studies and for Gyms of persons. All treadmill exercises were done in a chamber with a temperature of 24-26^{0}C and relative humidity of 60-65%.

The exercise in each case was done four hour after morning feeding and one hour rest in the chamber. The animals were familiarized to the machine with pilot trials before actual experiment. Pre-exercise venous blood samples were collected to form the base values. Once the dogs started to walk on treadmill the speed was slowly increased to 9 km/hour. Subsequently blood samples were collected from the cephalic vain at 20, 40 and 60 minutes of exercise at 9 km/hour.

Parameters investigated were rectal temperature, respiratory rate, heart rate. Haemoglobin, packed cell volume and plasma concentration of total proteins, albumin and globulin. Standard laboratory methods were used. The statistical analysis of the data was done by least significant difference methods (Snedecor and Cochran, 1990).

Results and Discussion

The changes in various parameters at different stages of exercise are show in Table 1. As the exercise progressed the rectal temperature and respiratory rate increased gradually and significantly. At 60 minutes the rectal temperature was 106.66 ± 0.5 ºF as compared with the base value of 101.94 ± 0.1ºF. The respiratory rate increased tremendously by 20 minutes itself and at 60 minutes was 194 ± 1.0 when compared with the base value of 20 ± 1.0. It means that dogs were panting. Increased metabolism during exercise increased the body temperature. In hot climate and during summer racing season, racing greyhound dogs often show symptoms of heat stroke (Erickson and Poole, 2004). During strenuous exercise, a considerable portion of energy librated by contracting muscles is also converted into heat (Ilkiew *et al.,* 1989).

In canines respiratory centre also responds to body core temperature. During panting there is dead space ventilation which is the volume of air that does not take part in gas exchange over a certain period of time. In dogs panting is an important thermoregulatory mechanism as unlike other domestic animals sweating plays no significant role in this regard. (Erickson and Poole, 2004; Reece, 2004).

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Table1: The effects of treadmill exercise on certain parameters in German Shepherd dogs. Mean ± SE (n = 12)

Parameters	Rectal temp (^{o}F)	Respiratory rate (breath/min)	Heart rate (beats/min)	Haemoglobin (gm/litre)	PCV (%)	Total Protein (gm/litre)
Exercise base value	101.94^{a}±0.10	20.00^{a}±1.00	83.00^{a}±1.00	151.25^{a}±3.41	42.75^{a}±6.77	66.49±0.15
At 20 min of exercise	103.45^{b}±0.40	123.00^{b}±1.00	123.00^{b}±1.00	169.17^{b}±3.82	52.83^{b}±6.82	66.71±0.14
At 40 min of exercise	101.74^{b}±0.30	160.00^{b}±1.00	145.00^{b}±1.00	185.83^{b}±2.76	58.33^{b}±6.79	67.07±0.15
At 60 min of exercise	106.66^{b}±0.50	194.00^{b}±1.00	178.00^{b}±1.00	198.75^{b}±2.89	63.83^{b}±6.90	67.63±0.15

During dead space ventilation respiratory rate is increased but tidal volume is decreased and so alveolar ventilation remains constant and reparatory alkalosis does not occur. During inspiration cooling of body is provided by evaporation of water from tissues involved especially the nasal passages (Reece, 2004). Similar changes in rectal temperature and respiratory rate during exercise have been reported in Greyhound dogs (Ilkiew *et al.,* 1989).

The heart rate increased gradually but significantly at each stage of exercise. At 60 minutes of exercise the mean heart rate was 178 beats/ minutes when compared with the base value of 83 beats/minutes. The PCV and haemoglobin values also increased markedly but plasma total proteins remained unaffected.

During exercise the demand of the body tissues for oxygen increases tremendously to sustain increased metabolic rate. The body can respond three ways to meet this increased oxygen demand i.e. by increasing cardiac output; by increasing oxygen carrying capacity of the blood and by extracting more oxygen from the blood at tissue level (Reece, 2004). Increase in heart rate under this situation is achieved by autonomic nervous system through increased stimulation of sympathetic system and release of catecholamines along with simultaneous withdrawal of parasympathetic inhibition of heart. Increase in heart rate shall increase the cardiac output. Moreover, release of catecholamines shall also have inotropic effect on myocardium to increase the stroke volume which will also add to increase in cardiac.

The increased PCV in the absence of any change in plasma total proteins indicates that the increase was not due to any body fluid shift. The increased sympathetic activity contracts the spleen and the discharge of erythrocytes from the spleen under such a situation would markedly increase the PCV and haemoglobin. This enhances the capacity of the blood to carry more oxygen. Moreover, observed increase in body core temperature in the present dogs would also shift the oxygen dissociation curve to the right so that each reduction in PO_2 at tissue level would yield more oxygen. It is so because shift to the right shall decrease the affinity of haemoglobin at tissue level. Similar changes in dogs during exercise have been reported by Hinchchilff *et al.* (1993) and Saleh and Ibrahim (2003). However Rose and Bloomberg (1989), during exercise of Greyhounds, observed increase in serum protein along with increased Hb, PCV and TEC and opined some fluid movement from the vascular compartment.

The results of the present study show that in German Shepherd dogs respiratory system plays on important role in thermoregulation during exercise. In addition several responses are involved to meet increased oxygen demand during this period.

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DIETARY MANAGEMENT OF CHRONIC RENAL FAILURE CASES IN DOGS

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Introduction

Chronic renal failure (CRF) is a clinical syndrome occurring under the circumstances when the compensatory mechanisms in the kidneys suffering from long-term disease are unable to maintain their regular function and when this inability becomes irreversible (Kuaera, 1999). It is primarily a disease of the older animal and most of the patients died due to uraemic complications as the condition is progressed. Cells of the kidney cannot replace or regenerate themselves as they do in the liver, lungs, bone and skin. Once a glomerulus ages and is lost, it is lost forever. This is probably the most common cause of kidney failure in dogs and cats. Clinical signs frequently observed in chronic renal failure are anorexia, polyuria, polydipsia, vomiting, lethargy, weight loss and pallor or ulceration of mucous membranes while laboratory findings are metabolic acidosis, azotaemia, hyperphosphataemia, hypercalcaemia or hypocalcemia, hypermagnesemia and hypokalemia (Polzin *et al.,* 2005). Although there is no cure for CRF and since existing renal damage is irreversible, measures to correct biochemical abnormalities, alleviate clinical signs, and slow the progression of renal damage are of considerable clinical importance (Markwell,1998).

Indication for dietary therapy

Dietary manipulation is a cornerstone in the conservative medical management of CRF, but this represents only one aspect of the therapeutic strategy. An often-cited guideline has been to initiate dietary therapy when serum creatinine exceeds 2.5 mg/dl and serum urea nitrogen exceeds 60-80 mg/dl. More recently, one investigator recommended the restriction of dietary phosphorus and omega-3 dietary PUFA dietary supplementation in dogs with serum creatinine values exceeding 4.0 mg/dl (Polzin *et al.,* 2005). Where an underlying primary disease is identified, or if prerenal components are involved, specific therapy to correct these may be possible. Additional measures may include: unlimited access to drinking water or fluid replacement, avoidance of stress, sodium bicarbonate administration, anabolic agents, intestinal phosphorus binders, calcium and calcitriol supplementation, H_2 antagonists, erythropoietin, anticonvulsant, avoidance of nephrotoxic drugs and antihypertensive therapy.

The aims of dietary management of CRF

- to promote optimal growth
- to assist conservation of renal function
- to normalise body fluid composition
- to minimise symptoms of nausea, fatigue and anorexia
- to minimise hyperphosphataemia and renal osteodystrophy

Dietary changes are needed when renal function is less than 25% of normal (i.e. when Glomerulus Filtration Rate (GFR) is <25 ml/min/m^2). To achieve these aims, the diets recommended for dogs and cats with renal failure are modified from typical maintenance diet in several ways, including reduced protein, phosphorus and sodium content, increased vitamin content, and caloric density and a neutral effect on acid base balance (Polzin *et al.,* 1994; Finco *et al.,* 1998)). Feline renal failure diets are typically supplemented with additional potassium. Canine renal failure diets may have an increased omega-3 or omega-6 polyunsaturated fatty acids (PUFA) ratio (Polzin *et al*., 2005). Diets may also have added fibre designed to enhance GI excretion of nitrogenous wastes

Phosphorus

Renal diets are limited in phosphorus content to limit phosphorus retention, hyperphosphataemia, renal secondary hyperthyroidism, and progression of renal disease. Hyperphosphataemia is a common finding in patients with CRF. It occurs when the GFR falls to approximately 20% of normal, resulting in impaired renal phosphate excretion. Phosphorus retention can cause renal mineralization, secondary hyperparathyroidism and potentially, an increase in renal damage. Decreased calcitriol concentrations with hypocalcaemia result in stimulation of parathyroid hormone (PTH) synthesis and release, leading to secondary hyperparathyroidism (Nagode

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et al., 1993). Parathyroid hormone (PTH) may be an important uremic toxin, which could contribute to anaemia, neurotoxicity, dyslipoproteinaemias, insulin resistance, promotion of soft tissue calcification, renal osteodystrophy and, most importantly, progression of renal damage (Massry, 1980). Phosphorus restriction, therefore, should be initiated early in the course of CRF and should be considered for any dog or cat in which azotaemia is shown to result from primary renal failure. Dietary therapy aims to normalize serum phosphorus concentration and control secondary hyperparathyroidism. If dietary phosphorus restriction does not correct hyperphosphataemia or hyperparathyroidism, the next stage is to commence therapy with oral phosphorus binding agents. These should be used only in conjunction with phosphorus-restricted diets and after the patient has become accustomed to the diet. They should always be administered with food. If phosphorus-binding agents do not achieve the goal of normalizing PTH concentrations, supplementation with calcitriol may be considered. A once-daily oral regimen at an average dose of 2.5 ng/kg has been recommended. If calcitriol therapy is initiated, it is essential that serum calcium and phosphorus concentrations are monitored regularly. Dietary phosphorus restriction has been shown to enhance survival and a slow decline in renal function in dogs with induce renal failure (Polzin *et al.*, 2005). Experimentally induced chronic kidney disease (CKD) in 24 dogs with the restriction of dietary phosphate and protein has been shown to slow progression of CKD and improve survival (Brown *et al.,* 1991). In dogs with more severe renal dysfunction, dietary phosphate restriction alone may not prevent hyperphosphataemia (Jacobs *et al.,* 2003).

Omega-3 polyunsaturated fatty acid

A variety of effects attributed to Omega-3 PUFA supplementation in dogs with CRF may have contributed to the favourable renal effects observed, including their tendency to reduce hypercholesterolaemia, suppress inflammation and coagulation, lower blood pressure, favourably influence renal haemodynamic, provide antioxidant effects or limit intra renal calcification. A subsequent study supported possible roles of altered lipid metabolism, glomerular hypertension and hypertrophy, and urinary eicosanoid metabolism in the beneficial renal effects of omega-3 PUFA **(**Polzin *et al.,* 2005**)**.The optimum quantity of omega-3 PUFA supplementation and ratio of omega-3/omega-6 PUFA have not been conclusively established.

Antioxidants

Oxidative damage, via generation of reactive oxygen species, may be cause of renal injury that contributes to the progression of chronic kidney disease (Brown, 2008). Effects of dietary supplementation of a dry therapeutic renal food with antioxidants (vitamin E, C and carotenoids) evaluated in a marked study of 10 dogs with spontaneous stages 2 to 3 living with their owners (Yu *et al.*, 2006). Dogs were fed a renal food for 6 weeks and then the same food was supplemented with antioxidants and fed for an additional 4 weeks compared with baseline, antioxidant supplementation significantly reduced oxidative stress and serum creatinine concentration compared to dog receiving the renal food without antioxidants (Yu *et al.*, 2006).

Protein

Studies have shown that reducing dietary protein intake can bring about clinical benefits in uraemic patients. In addition to reducing the level of protein catabolites, dietary protein restriction may also help by reducing the intake of dietary phosphorus, decreasing the protein-related solute load (lessening the severity of polydipsia and polyuria), decreasing the acid load. (alleviate metabolic acidosis). Uraemia is a catabolic state, which may adversely affect several aspects of protein metabolism. Renal failure may also lead to increased urinary losses of protein or specific amino acids. The protein requirements of dogs and cats in CRF have not been established, but it is likely that they may be different, and probably higher, than those of the healthy animal. It is important, therefore, that high quality protein sources are used in the formulation of restricted protein diets to minimize the risks of essential amino acid deficiency. Very low protein diets may be poorly accepted and have been associated with protein malnutrition (as indicated by weight loss and decreased serum albumin) in dogs with CRF (Polzin, 1991; Barsanti and Finco, 1985). Other reported side-effects in the dog include hypertension and increased serum ionized calcium and cholesterol. These observations have led to the recommendation that protein intake should not be restricted to less than 1.9 g/kg body weight/day (approximately 11 g protein/ 400 kcal metabolizable energy [ME] in the diet for a 10 kg dog), unless further restriction was required to control clinical signs of uremia (Polzin,1991). Dietary protein restriction is justified in dogs and cats showing clinical signs of uraemia. Restriction of dietary protein at an earlier stage, before the onset of clinical signs, would be appropriate; it played a significant role in delaying the progression of renal damage. High protein intake in the presence of renal injury contributed to the increased perfusion, but it was found that a reduction of dietary protein from 24% to 6% reduced the haemodynamic changes and slowed the progression of tissue destruction (Brenner, 1985). Dietary protein (unlike dietary phosphorus) was not

found to influence the progression of renal failure in a number of studies (Bovee *et al.*, 1979; Robertson *et al.*, 1986).

Potassium

Hyperkalaemia is usually not a problem for most patients until in end stage renal failure when urine production is reduced. The mechanism of controlling of potassium secretion operates at near maximal level in end stage renal failure, so a sudden addition of more potassium through dietary indiscretion or an acute catabolic event can easily overwhelm the ability to excrete excess potassium, this can result in high serum potassium levels (Harte *et al.*, 1994).

Calcium

Calcium concentrations in the blood of patients with CRF may be low, normal, or high (Polzin, *et al.*, 1984). Decreased ionized calcium, where present, may contribute to increased PTH release (Nagode, *et al.*, 1993). A number of factors may contribute to hypocalcaemia, including hyperphosphataemia, poor gastrointestinal absorption associated with decreased levels of calcitriol, and reduced intake following anorexia. It has been recommended that calcium intake should be normal or supplemented in patients with CRF (Bovee, 1983). Conversely, if the product of the concentrations of calcium and phosphate ions in the blood exceeds the solubility product of calcium phosphate, soft tissue calcification may occur, leading to the progression of renal damage (Massry, 1980). Calcium supplementation, which would be desirable in the presence of known hypocalcaemia, would be contraindicated in the presence of hypercalcaemia.

Sodium

When GFR falls in the diseased state, surviving nephrons increase their fractional excretion of sodium to cope with the increased load delivered to each one. In general, this response is adequate to maintain sodium balance until the condition is very advanced. However, the ability of the kidney to adapt to changes in sodium intake becomes progressively limited (Polzin *et al.*, 1989). There is controversy over the prevalence of hypertension among dogs with chronic renal disease. Hypertension, where it occurs, may be important for two reasons: It can result in a variety of pathophysiologic consequences, including left ventricular hypertrophy, neurologic abnormalities, and ocular lesions. It may contribute to the progression of renal damage. Reduction of blood pressure in patients with documented hypertension is thus a desirable goal of therapy (Ross *et al.*, 1989). It has been reported that expansion of extracellular fluid volume, hypertension, and oedema may occur in uraemic dogs receiving normal or high sodium intake (Cowgill, 1983). These findings suggest that the traditional recommendation to supplement with sodium is not appropriate for most cases of CRF. Conversely, severe sodium restriction should probably also be avoided. This could promote volume depletion in some dogs with CRF that are unable to adapt to varying sodium intake. It may also result in a decreased capacity to reabsorb bicarbonate, thus contributing to metabolic acidosis. However, this latter effect was not seen when diets containing 0.25% DM sodium were fed (Polzin *et al.*, 1989). For these reasons, most recommendations are for diets with 'normal' to 'moderately restricted' sodium content for dogs with CRF include 0.25 to 0.8% DM (Polzin *et al.*, 1989) and 0.1 to 0.3% DM (Ross *et al.*, 1989).

B-complex vitamins

Dogs and cats with CRF are also potentially at risk of water-soluble vitamin deficiency through reduced intake from in appetence and increased urinary losses in polyuric cases. Supplementation with B-complex vitamins is therefore likely to be beneficial, and at least twice the maintenance level is recommended.

Energy

The metabolism of protein to provide energy is undesirable in patients with CRF since this increases the amount of nitrogenous waste products that must be excreted through the failing kidneys. An adequate energy supply in the diet is therefore important to prevent further tissue catabolism and, as far as possible, should be derived from non-protein sources. Appetite is often poor in affected animals, so the energy density of the diet should be high to enable the animal to obtain its nutritional requirements from a relatively small volume of food. In this respect, fat offers advantages over carbohydrate as a non protein source of energy: It provides approximately twice the energy per gram and aids palatability in the diet. For this reason, canned diets designed to support dogs and cats with CRF tend to be high in fat. One concern about this design is whether the high fat content could adversely influence lipid metabolism in CRF patients, perhaps contributing to progression of renal damage. Abnormalities in lipid metabolism have been documented in a variety of human renal diseases and have also been reported in dogs with both spontaneous and induced renal disease (Keane *et al.*, 1998; Down and Krawiec,1995). In addition to creating a more atherogenic environment, these lipoproteins may also be responsible for glomerulosclerosis, a process which may have similarities to atherosclerosis (Attaman and Allaupovic, 1991).

Assisted feeding

Malnutrition is among the most important complication of CKF as it contributes to complications to mortality in most dogs that die of or are euthenised for uraemia. Evidence supporting a recommendation for assisted feeding for dogs with CKD that fail to consume adequate calories is limited to the expert opinion. It appears likely that assisted feeding can reverse malnutrition in some patients and improve their quality of life (Roudebush, 2010).

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ELECTROPHORETIC PATTERNS OF SERUM PROTEIN FRACTIONS IN ANOESTRUS CROSSBRED COWS: ROLE OF VITAMIN E AND SELENIUM[#]

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ABSTRACT

High incidence of post-partum anoestrus affects the overall productivity of cross-bred cattle. Among the supplementations, vitamin E and selenium is prominent. The experiment was aimed to detect changes in the serum proteins of anoestrus cows on the basis of their electrophoretic mobilities and their sizes. A total of twenty (20) post partum anoestrus crossbred cows in and around Khanapara area of Guwahati were selected for the experiment. Animals were randomly divided into two groups. Control group was injected with 4 ml distilled water intramuscularly at weekly interval for five occasions, whereas Treatment group was injected with E care Se @ 1 ml/25 kg body weight for equal period. Electrophoretic pattern in serum proteins were evaluated. Six distinct protein bands *viz*. albumin, α_1 globulin, α_2 globulin, β_1 globulin, β_2 globulin and γ globulin were found in serum of both anoestrus control and oestrus responded treated cows. The mean serum total protein concentrations on day 0, 7, 14, 21, 28 and 35 of anoestrus cows of control group were found to be 7.20 ± 0.23, 7.25 ± 0.22, 7.16 ± 0.21, 7.23±0.21, 7.19±0.21 and 7.24±0.23 g/dl, respectively. The values were 7.06 ± 0.18, 7.16±0.18, 7.30±0.18, 7.58±0.27, 7.72±0.28 and 7.93±0.29 g/dl, respectively, on day 0, 7, 14, 21, 28 and on the day of induced oestrus in responded cows of treated group. It can be concluded that Vitamin E and selenium were effective to help in changing the blood biochemical constituents in anoestrus cows.

Key words: Postpartum anoestrus, crossbred cow, electrophoretic patterns, *E Care Se*

Introduction

Dairy cattle population has got enormous contribution towards economic growth of our country, particularly in Assam. But the most discouraging factor which affects the overall productivity of cross-bred cattle is measurably higher incidence of post-partum anoestrus during which the animal shows tranquality in their reproductive activities causing an economic loss to the farmers. To address this problem different supplementation as well as medication has been used out of which vitamin E and selenium is prominent. Literature relating to electrophoretic patterns of serum proteins of cyclic and non cyclic cows is meagre. However some authors (Satija, 1979; Sarmah *et al*., 2001; Devi *et al.,* 2003; Keay and Doxey, 2005; Batavani *et al*., 2006; Bonia *et al.,* 2009) studied the electrophoretic patterns in buffalo, pregnant cows, pregnant goat, normal cattle, sheep and horses, ewes and suboestrus cows, respectively. Therefore, the present investigation was designed to study the changes in electrophoretic patterns of serum protein in order to detect changes in the serum proteins of anoestrus cows on the basis of their electrophoretic mobilities and their sizes.

Materials and Methods

Experimental Animals

A total of twenty (20) post partum anoestrus crossbred cows belonging to different private cattle owners in and around Khanapara area of Guwahati were selected for the experiment. The selected cows failed to show oestrus even after more than 90 days to 180 days of parturition with sub-active ovaries. All the cows were in lactating condition. Animals were kept under uniform managemental condition during the period of study. They were randomly divided into two groups-control and treatment group comprising of ten cows in each group.

Treatment of Animals

Control group was injected with 4 ml distilled water intramuscularly at weekly interval for five

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Table 1: Relative mobility (Rm) and relative proportion (Rp) of different protein fractions in serum of anoestrus control

On days	Relativity of protein fractions	Protein fractions						Total protein (g/dl)
		albumin	α_1 globulin	α_2 globulin	β_1 globulin	β_2 globulin	γ globulin	
0	Rm (Av.)	0.49 ± 0.03	0.40 ± 0.04	0.25 ± 0.03	0.18 ± 0.05	0.12 ± 0.02	0.04 ± 0.01	7.20 ± 0.23
	Rp (%)	53.64 ± 2.21	1.80 ± 0.03	2.45 ± 0.05	6.46 ± 0.03	16.25 ± 0.03	20.38 ± 0.05	
7	Rm (Av.)	0.46 ± 0.03	0.38 ± 0.03	0.27 ± 0.03	0.17 ± 0.03	0.14 ± 0.04	0.07 ± 0.01	7.25 ± 0.22
	Rp (%)	52.02 ± 0.03	2.34 ± 0.03	2.02 ± 0.04	7.11 ± 0.03	16.34 ± 0.02	20.17 ± 0.02	
14	Rm (Av.)	0.51 ± 0.03	0.43 ± 0.02	0.31± 0.03	0.24 ± 0.02	0.19 ± 0.03	0.08 ± 0.01	7.16 ± 0.21
	Rp (%)	51.56 ± 2.34	1.43 ± 0.03	2.34 ± 0.05	6.27 ± 0.03	17.76 ± 0.01	21.08 ± 0.03	
21	Rm (Av.)	0.48 ± 0.02	0.39 ± 0.04	0.28 ± 0.03	0.20 ± 0.04	0.13 ± 0.03	0.06 ± 0.02	7.23 ± 0.21
	Rp (%)	53.87 ± 2.21	1.93 ± 0.03	2.61 ± 0.04	7.89 ± 0.06	15.97 ± 0.04	17.75 ± 0.03	
28	Rm (Av.)	0.48 ± 0.02	0.41± 0.04	0.26 ± 0.04	0.15 ± 0.02	0.10 ± 0.02	0.05 ± 0.02	7.19 ± 0.21
	Rp (%)	50.58 ± 2.31	1.89 ± 0.03	2.39 ± 0.01	6.46 ± 0.03	16.06 ± 0.01	22.47 ± 0.05	
35	Rm (Av.)	0.52 ± 0.02	0.43 ± 0.02	0.28 ± 0.04	0.19 ± 0.05	0.11 ± 0.01	0.07 ± 0.01	7.24 ± 0.23
	Rp (%)	51.38 ± 2.32	1.97 ± 0.02	2.79 ± 0.02	7.34 ± 0.07	16.37 ± 0.02	20.13 ± 0.05	

occasions, whereas Treatment group was injected with E care Se (500 mg α-tocopherol acetate and 15 mg selenium) @ 1 ml/25 kg b. wt. intramuscularly at weekly interval for five occasions. Electrophoretic pattern in serum proteins were evaluated by the method of Davis (1964) at 8.5% poly acrylamide gel concentration.

Relative Proportion

Relative proportion of protein present in serum was calculated from the optical density values of eluted dye from polyacrylamide gel stained with Coomassie Brilliant Blue (CBB). Elution of dye was performed by cutting out stained zone, extracting the dye with 4 ml 25% pyridine for 6 hours at room temperature (Fenner *et al.*; 1975). Eluted dye was collected by passage through a small syringe stuffed with glass wool and the optical densities were recorded at 580 mì using atomic absorption spectrophotometer.

Relative Mobility

The relative mobility of all the serum proteins was recorded by the following formula:

$$\text{Mobility} = \frac{\text{Length of gel before staining}}{\text{Distance of dye migration}} \times \frac{\text{Distance of protein migration}}{\text{Length of gel after destaining}}$$

Table 2: Relative mobility (Rm) and relative proportion (Rp) of different protein fractions in Serum of responded cows

On days	Relativity of protein fractions	Protein fractions						Total protein (g/dl)
		albumin	α_1 globulin	α_2 globulin	β_1 globulin	β_2 globulin	γ globulin	
0	Rm (Av.)	0.52 ± 0.02	0.43± 0.04	0.28 ± 0.04	0.21± 0.04	0.11 ± 0.01	0.07 ± 0.02	7.06 ± 0.18
	Rp (%)	51.35 ± 2.34	1.87 ± 0.03	2.58 ± 0.02	6.47± 0.02	14.68 ± 0.02	23.09 ± 0.02	
7	Rm (Av.)	0.49 ± 0.02	0.38 ± 0.03	0.25 ± 0.04	0.16 ± 0.03	0.12 ± 0.01	0.05 ± 0.03	7.16 ± 0.18
	Rp (%)	52.93 ± 2.12	1.56 ± 0.06	2.87 ± 0.02	6.47 ± 0.02	14.45 0.03	21.38 ± 0.02	
14	Rm (Av.)	0.53 ± 0.02	0.37 ± 0.04	0.27 ± 0.04	0.17± 0.01	0.10 ± 0.02	0.03 ± 0.03	7.30 ± 0.18
	Rp (%)	53.34 ± 2.50	1.44 ± 0.04	2.28 ± 0.02	6.24 ± 0.02	16.87 ± 0.03	20.82 ± 0.01	
21	Rm (Av.)	0.56± 0.02	0.34 ± 0.03	0.24 ± 0.08	0.18 ± 0.01	0.13 ± 0.02	0.04 ± 0.03	7.58 ± 0.27
	Rp (%)	54.97 ± 2.21	1.46 ± 0.02	2.51± 0.05	6.78 ± 0.03	15.08 ± 0.03	20.77 ± 0.02	
28	Rm (Av.)	0.58 ± 0.03	0.44 ± 0.07	0.32 ± 0.07	0.24 ± 0.01	0.19 ± 0.02	0.07 ± 0.02	7.72 ± 0.28
	Rp (%)	56.57 ± 2.46	1.23± 0.06	2.13 ± 0.03	5.78 ± 0.04	15.92 ± 0.03	18.63 ± 0.03	
Induced oestrus	Rm (Av.)	0.59 ± 0.02	0.44± 0.09	0.35 ± 0.09	0.26 ± 0.02	0.18 ± 0.02	0.09 ± 0.02	7.93 ± 0.26
	Rp (%)	58.32 ± 2.32	1.54 ± 0.03	2.46 ± 0.04	6.27 ± 0.04	13.34 ± 0.03	18.03± 0.03	

The data generated were analysed for mean ± SE using standard statistical procedure (Snedecor and Cochran, 1994).

Results and Discussion

In the present study, polyacrylamide gel electrophoresis showed six distinct protein bands (Fig. 1 and 2) *viz.* albumin, α_1 globulin, α_2 globulin, β_1 globulin, β_2 globulin and γ globulin in serum of both anoestrus control and oestrus responded treated cows. The relative mobility and relative proportions of different serum protein fractions in both the groups were shown in the Table 1 and 2. There were minor variations with relative mobilities and relative proportions of different protein fractions. The mean serum total protein concentrations on day 0, 7, 14, 21 , 28 and 35 of anoestrus cows of control group were found to be 7.20 ± 0.23, 7.25 ± 0.22, 7.16 ± 0.21, 7.23 ± 0.21, 7.19 ± 0.21 and 7.24 ± 0.23 g/dl, respectively. The values were 7.06 ± 0.18, 7.16 ± 0.18, 7.30 ± 0.18, 7.58 ± 0.27, 7.72 ± 0.28 and 7.93 ± 0.29 g/dl, respectively, on day 0, 7, 14, 21, 28 and on the day of induced oestrus in responded cows of treated group at different days of observations.

From the findings of the present study, it could be concluded that parental administration of antioxidant-vitamin E and selenium in combination could alter the level of different biochemical constituents in the blood. Vitamin E and selenium were effective to help in induction of oestrus in anoestrus cows.

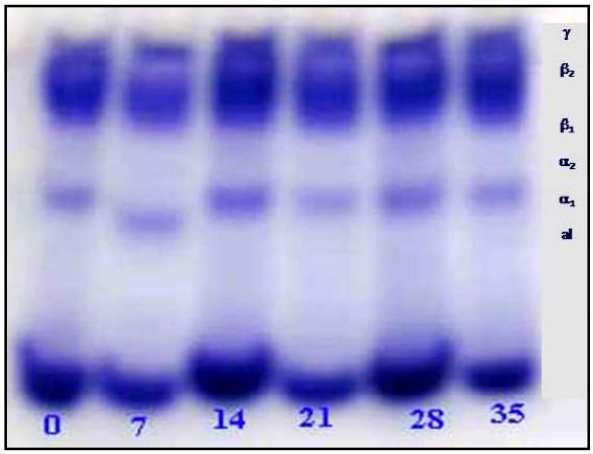

Fig. 1: Electrophoretic pattern in serum of anoestrus control cows

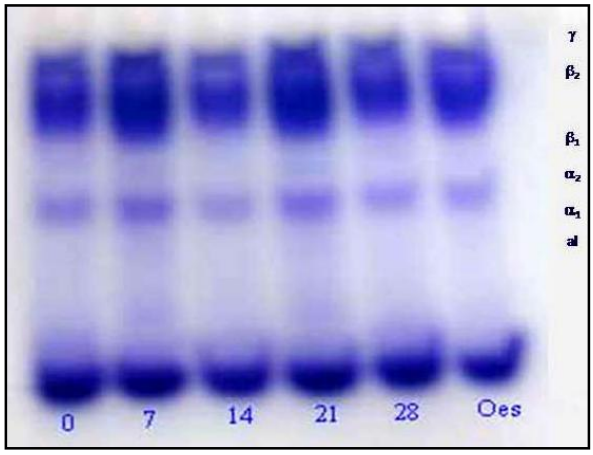

Fig. 2: : Electrophoretic pattern in serum of responded cows

γ = gamma globulin, β_2 = beta 2 globulin, β_1 = beta 1 globulin, α_2 = alpha 2 globulin, α_1 = alpha 1 globulin, al = albumin,
Oes = induced oestrus

Acknowledgements

The authors are thankful to the Dean, Khnanapara Veterinary College, AAU as well as the in-charge of cattle farm for providing necessary facilities for carrying out the research work.

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INTER-RELATIONSHIP BETWEEN HUSBANDRY PRACTICES AND OCCURRENCE OF MANGE IN DOGS[#]

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ABSTRACT

The present study was conducted on inter-relationship between husbandry practices and prevalence of mange in dogs. Higher prevalence was found in the dogs which were purchased from unorganized sectors. The dogs which spent more than 8 hours out of home/ kennel showed more prevalence of sarcoptic mange as compared to demodectic mange. Similarly, dogs kept at shady and damp places were more prone to sarcoptic mange in comparison to demodectic mange. The prevalence of demodectic mange was more in the cases which were bathed too often or too rarely. Both types of mange were more common in dogs which were fed home made food and dewormed occasionally. Dogs which were suffering from hormonal imbalance and other concurrent diseases were more prone to demodectic mange.

Key words: Prevalence, kennel, mange.

Introduction

Among infectious skin diseases of dogs, mange is very old and still continues to pose problems for the dog keepers directly and to the veterinarians indirectly. Mange is one of the contagious diseases of the dogs and has zoonotic importance. It is caused by the arthropod insect carried mites belonging to the class Arachnida and order Acarina. Various mange mites *Demodex canis* causing demodectic mange or 'red mange', *Sarcoptes scabiei var. canis* causing sarcoptic mange or scabies and *Otodectus cynotis* causing ear mange have been isolated from cases of dermatitis in dogs (Mishra and Basistan, 1972); (Chittawar,1981) and (Yathiraj *et al.*,1990). Though, extensive research work has been conducted in India and abroad with regard to symptomatology, diagnosis and treatment of canine mange, yet, there are some research gaps which need proper attention. As evident from the literature, the inter-relationship between husbandry practices and prevalence of mange has not been studied sufficiently, thus the present study was conducted.

Materials and Methods

A total of 401 dogs brought to veterinary clinical complex CSK HPKV were undertaken for the study from March, 2009 to February, 2010. Sixty three dogs were diagnosed to be suffering from mange based on standard procedures viz. skin scarpping examination (Sastry, 1985) and ELISA (Arlian *et al.*, 1995). A questionnaire was prepared and comprehensive history in relation to habit and habitat of the dogs was recorded.

Results and Discussion

The inter-relationship between husbandry practices and occurrence of mange in dogs is illustrated in Table 1. Higher prevalence was found in the dogs which were purchased from unorganized sectors. The reason for this could be that managemental practices might not be good at such places.The dogs which spent more than 8 hours out of home/kennel showed more prevalence (67.85%) of sarcoptic mange as compared to demodectic mange (31.42%). Similarly the dogs kept at shady and damp places were more prone to sarcoptic mange in comparison to demodectic mange. This was probably because of the reason that sarcoptic mite survives for longer period in such type of environment than demodectic mite (Soulsby, 2005). The present study also revealed that sarcoptic mange was more contagious than demodectic mange as also mentioned by Soulsby (2005).The prevalence of demodectic mange was more in the cases which were bathed too often or too rarely. The reason for this could be that excessive bathing alters

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Table1: Inter-relationship between husbandry practices and occurrence of mange in dogs.

Husbandry practices		Demodectic mange[#]	Sarcoptic mange[##]
Place of purchase	Organized sector	11 (31.42)	9 (32.14)
	Unorganized sector	20 (71.42)	19 (67.85)
Time spent outdoor/day	<2hrs.	12 (34.28)	4 (14.28)
	2-8 hrs.	12 (34.28)	5 (17.85)
	>8hrs.	11 (31.42)	19 (67.85)
Location of kennel	Shady/damp	18 (51.42)	18 (64.28)
	Sunny/dry	17 (48.57)	10 (35.71)
Floor of kennel	Cemented	9 (25.71)	5 (17.85)
	Kuccha	26 (74.28)	23 (82.14)
Disinfection of kennel	Regular	9 (25.71)	13 (46.42)
	Occasional	26 (74.28)	15 (53.57)
Effect on in contact animals	Dam	9 (25.71)	10 (35.71)
	Litter	3 (8.57)	3 (10.71)
	Other pets	1 (2.85)	4 (14.28)
	Non-affected	22 (62.85)	11 (39.28)
Frequency of bathing/ month	Once	1(2.85)	2 (7.14)
	Twice	1 (2.85)	2 (7.14)
	Thrice	2 (5.71)	3 (10.71)
	>3	13 (37.14)	8 (28.57)
	Rarely	18 (51.42)	13 (46.42)
Deworming status	Regular	11 (31.42)	11 (39.28)
	Occasional	24 (68.57)	17 (60.71)
Type of food	Non-scientific	27 (77.14)	19 (67.85)
	Scientific	8 (22.85)	11 (39.28)
Hormonal status	Oestrus	5 (14.28)	1 (3.5)
	Pregnancy	13 (37.14)	2 (7.1)
	Castration	6 (17.14)	4 (14.28)
	Normal	11 (31.42)	21 (75)

Figures in parentheses are per cent of cases suffering from mange.
[#] Figure is out of 35, [##]Figure is out of 28

the normal microfauna and pH of skin resulting in increased susceptibility to Demodex mite which is present as part of normal microfauna. Both types of mange were more common in dogs which were fed home made food and were dewormed occasionally. This observation simulated the findings of Gupta (2008) who also reported that poor nutrition was responsible for increased susceptibility of dogs to skin infections. Soulsby (2005) also mentioned about the increased susceptibility of dogs to mange which are not dewormed regularly. Similarly the dogs with hormonal imbalances were more susceptible to demodectic mange. Dogs which were suffering from other concurrent diseases were more prone to demodectic mange perhaps because of suppressed immune system as recorded by Toman *et al*. (1997).

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PREVALENCE OF SOME INFECTIOUS DISEASES IN DROMEDARY CAMEL FROM BIKANER REGION IN RAJASTHAN

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ABSTRACT

A study was undertaken to determine the prevalence of brucellosis, *peste des petits ruminants*, trypanosomosis, mange and gastrointestinal nematodosis in camles in Bikaner region. Out of total 94 camels examined all were seronegative for brucellosis by RBPT and STAT whereas prevalence of PPR was 4.52 % by c-ELISA and that of trypanosomosis was 1.06% by mercuric chloride test. The prevalence of mange and gastrointestinal nematodosis was 8.51% by skin scrapping examination and 44.68% by faecal examination. The eggs of *Strongyle*, *Strongyloide* and *Trichuris* spp. were observed with prevalence of 38.29%, 4.20% and 2.12%, respectively. Mean ± SE value of eggs per gram of faeces was 897.16 ± 62.24. The haematological parameters were altered in camels affected with mange and gastrointestinal nematodosis and serum protein levels decreased in camels affected with intestinal parasites but remained unchanged in mange affected animals.

Key words: Camel, brucellosis, gastrointestinal parasites, mange, prevalence, PPR, trypanosomosis

Introduction

In Rajasthan state some communities depend on the camel for their livelihood. Wernery and Kaaden (2002) stated that infectious diseases caused 50% fatalities in new world camelids and 65% in old world camelids. The camels are frequently infected with brucellosis particularly when they are in contact with affected ruminants (Wernery and Kaaden, 2002). *Peste des petits ruminants* (PPR) is an infectious and highly contagious viral disease responsible for huge economic losses in sheep and goats but reports on PPR in camel are scanty.

Trypanosomosis is endemic in almost all the camels inhabited areas but the true account of its epidemiological picture in Rajasthan is lacking because the infections often do not manifest overt clinical signs and when develop, these clinical signs are so varied and non-specific that they are by themselves not diagnostic (Pathak and Khanna, 1995). Asymptomatic infections are also very common (Raisinghani, 1977).

Sarcoptic mange, an ectoparastic infestation caused by *Sarcoptes scabiei var cameli* poses a major threat to camel health because of its extremely contagious nature and is regarded as one of the most prevalent and serious camel diseases (Higgins, 1983). Gastrointestinal nematodosis is prevalent in camels and when coupled with mineral deficiency cause pica (Bansal *et al.*, 1971; Sharma and Satija, 1974).

Considering the importance of the infectious diseases in the camel, the investigation was undertaken with the objectives of determining the seroprevalence of brucellosis, PPR, trypanosomosis, mange and gastrointestinal nematodosis in camels in Bikaner region.

Materials and Methods

In the present study blood for serology, parasitology and haematobiochemistry, skin for mange mites and faecal samples for GI nematodosis were collected from same camels presented to Clinics, College of Veterinary and Animal Science, Bikaner over a period of eight months including summer, rain and winter season. A total of 94 adult camels of which 84 were males and 10 were females ageing 5-10 years brought from the surrounding areas of Bikaner were screened.

The tests for brucellosis used were Rose Bengal Precipitation Test, RBPT (Morgan *et al.*, 1969) and Standard Tube Agglutination Test, STAT (Alton *et al.*,1975). Competitive Enzyme-linked Immunosorbent Assay (c-ELISA), developed at IVRI, Mukteshwar was used for the diagnosis of PPR. Trypanosomosis was diagnosed by wet blood film examination (Killick-Kendrick and Godfrey, 1963), buffy coat smear examination (Murray *et al.*, 1977) and mercuric chloride test on serum (Rutter, 1967) and by blood smear examination after staining with Giemsa.

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Skin scrappings for diagnosis of mange were collected from the lesions by using the method as described by Soulsby (1982). Qualitative examination of faeces was conducted to record the gastrointestinal nematodosis by Willi's floatation technique (Soulsby, 1982) and quantitative examination of faeces was conducted to record the intensity of parasitic infestation by McMaster's technique (Gordon and Whitlock, 1939).

For determination of some haematobiochemical parameters in camels affected with mange and GI nematodosis, the haematology was carried out as per the methods described by Jain (1986) and serum protein assays were done with Alfa Wasserman clinical chemistry systems (ACEO) using kits supplied by Alfa Wasserman, BV, New Zealand.

The data obtained were analysed as per the Snedecor and Cochran (1968).

Results and Discussion

In the present study none of the samples revealed presence of brucella antibodies by RBPT and STAT. Similar findings were reported by El-Ansary *et al.* (2001) who tested 65 camels by slide agglutination test and did not record any positive case. Megersa *et al.* (2011) showed prevalence of brucella antibodies in 2.2% of camel from Ethiopia, however, association of brucella seropositivity was not associated with abortion, which is a hallmark of brucellosis (Olsen and Tatum, 2010). Radwan *et al.* (1992) reported higher incidence of camel brucellosis in intensive farming than in free-grazing desert camels in S. Arabia. Ghanem *et al.* (2009) and Musa *et al.* (2008) observed higher seroprevalence of brucellosis in camels kept mixed with cattle, sheep, and goat. As all the camels in present study were stall fed mainly being used for transportation purpose, there were less chances for them to come in contact with brucellosis infected animals and materials. Secondly, there was no history of abortion, stillbirth or calf born weak, orchitis or hygroma in the investigated camels.

In the present study the overall seroprevalence of PPR in camels was 4.25% with 3.5% in males and 10.0% in females. Our results are in accordance with those of Ismail *et al.* (1992) where they found 4.2% camels from Egypt positive for PPR antibodies. However, Saeed *et al.* (2010) employing immunocapture competitive ELISA on tissue sample and competitive ELISA on serum observed prevalence of 42.6 and 0.3%, respectively, in the camels of Sudan. Roger *et al.* (2001) observed seroprevalence of 7.8% of PPR virus antibodies in Ethiopian camels. Contrary to the prevalence observed in the study, Albayrak and Gur (2010) found seronegativity to PPR in all the camels tested in Sudanese camels.

The seropositivity of Indian camel for PPR demonstrates that the camel could play a role in the epidemiology of PPR. Considering the necessary programmes of eradication and control of PPR, camel population should be integrated in the epidemiological surveys.

The prevalence of trypanosomosis in the present study was 1.06% on the basis of mercuric chloride test which detects a high level of protein in the blood rather than the parasites themselves and is a simple but not very reliable test (Kohler-Rollefson *et al.*, 2001). However, Abdel-Latif (1958) and Leach (1961) confirmed the diagnostic value of this test and indicated that though this test is not highly specific; there are, presumably, no other infections in the camel which give similar result with mercuric chloride, hence it can be considered very close to a specific test. Chand and Singh (1970) also employed mercuric chloride test in 124 camels and detected 33 of these to be positive. They further concluded that the mercuric chloride test was reliable in camels but not in other species. In our study, Trypanosomes were not detected in any of the blood smears. Wernery and Kaaden (2002) suggested that the parasites in the blood of the vertebrate host are often scarce, particularly in the chronic and sub-clinical stages and difficult or impossible to find even when the camel is in moribund state. Localisation of the parasite in lymph node could be the reason for absence of parasite in the blood.

The low prevalence of trypanosomosis is in accordance with findings of Dia *et al.* (1997) from Mauritania and Ngaira *et al.* (2002) from Kenya who observed prevalence of 1.3 and 2.0% in camels by blood smear and buffy coat examination, respectively. Pathak *et al.* (1993a) observed 7.5% prevalence of *T. evansi* infection in Rajasthan by blood smear examination and 31.66 % with double antibody sandwich ELISA.

Swai *et al.* (2011) screened 193 camels from Northen Tanzania by Giemsa stained blood smear examination and observed 8.2% of prevalence of trypanosomosis. Similarly, Bhutto *et al.* (2010) observed prevalence of 11.25% in camels from Sindh, Pakistan. Ravindran *et al.* (2008) found still a higher prevalence of *T. evansi* in camels through PCR (34.4%) than with blood smear examination (3.3%) in camels from Bikaner. The prevailing drought around the period of study could have been the reason of low prevalence of camel trypanosomosis as during this situation the fly could not breed and were reduced in numbers. Also frequent relief and veterinary aid camps organized during drought years for mass treatment/prophylaxis against endemic trypanosomosis could be responsible for low prevalence (Pathak *et al.,* 1997).

The prevalence of mange in the present study was 8.51% as only eight males showed presence of sarcoptes mites in skin scrappings. Similar results were reported by Alhendi (2000) from Saudi Arabia and Mahran and Saleh (2004) from Egypt in sarcoptic mange affected camel. However, higher prevalence of 21.3, 28-32 and 35% were reported by Chauhan *et al.* (1986); Bett *et al.* (2009) and Muhammad *et al.* (2006), respectively whereas Sena *et al.* (1999a) reported a wider range of prevalence (5.38-34.91%) in camel from Bikaner.

The overall prevalence of gastrointestinal nematodosis in camels was 44.68% (42/94). But higher prevalence of 69% (Bekele, 2002) and 75% (Borji *et al.,* 2010) were reported in camels Ethiopia and Iran, respectively.

In the present study prevalence of *Strongyles* spp. was the highest followed by *Strongyloides spp*. and *Trichuris* spp. with 38.29%, 4.20% and 2.12%, respectively. The prevalence of *Strongyle* spp is much below than that reported by Chauve *et al.* (1990) (95.00%); Partani *et al.* (1996a) (82.37%) and Tekle and Abebe, 2001 (94.60%) in camels but higher than that reported by Troncy and Oumata,1976 (9%) and Abdel-Aal and Sahlab, 1998 (5%), respectively in camels.

The prevalence of *Strongyloides spp.* in the present study was also much below than that reported by Trocy and Oumata, 1976 (92%).

Prevalence of *Trichuris* spp. in the present study was much lower than that reported by Malek, 1959 (70%) in Sudanese camel, Borji *et al.* (2010 (40%) in Iranian camel and Partani *et al.* (1996a) (22.45%) in camels from Bikaner.

Out of positive cases 85.71% showed single and 14.48% mixed infection. The mixed infection of gastrointestinal nematodes in camels has been reported by many workers (Lodha, 1977; Tager-Kagan, 1984; Sharma, 1991; Kayum *et al.*, 1992; Kumar *et al.,* 1993 and Pathak *et al.*, 1993b). *Strongyloides* and *Trichuris spp* eggs were present as mixed infection only.

The faecal eggs count in the present study ranged from 200-2000 eggs per gram of faeces (EPG) with mean value of 897.16±62.24. The concentration of *Strongyle* and *Strongyloides* in positive samples ranged between 200"2000 and 200-600 eggs per gram of faeces. In contrary, Amr *et al.,* (2008), reported faecal egg count range of 900-2700 EPG with mean EPG of 2035.

Maximum number of cases of gastrointestinal nematodosis were recorded in the month of July and minimum number of cases were recorded in the months of January to May. Arzoun *et al.* (1984) also observed that during rainy season camels generally harboured mature parasites. However, the present finding is contrary to Lodha (1977) and Raisinghani (1992) who reported high prevalence in autumn and winter than in summer.

Sex-wise, the prevalence was high in female camels in comparison to males. However, Partani *et al.* (1996a) observed that sex had no effect on the prevalence of gastrointestinal nematodosis.

The mean±SE values of haematological parameters in healthy and camels affected with mange and GI parasites are presented in the Table 1. A significant ($P<0.05$) decrease in Hb, PCV, TEC, lymphocytes and a significant ($P<0.05$) increase in neutrophils and eosinophils were observed in camels affected with mage. These values are in conformity to those of Radwan *et al.* (1987); Raisinghani *et al.* (1989) and Premalatha *et al.* (2010). Almost similar results were reported by Sena *et al.* (1999b) and Mal *et al.* (2000). Al-Saad *et al.* (2000) also observed significant decrease in Hb, PCV and TEC in camels affected with sarcoptic mange.

The decreased value of PCV, Hb and TEC could be due to malnutrition or worm infestation, as these 8 mange affected camels were also harbouring mild *Strongyle* spp. infection.

Leucocytosis in the present study was in accordance with Raisinghani *et al.* (1989) and Sena *et al.* (1999b). However, Mal *et al.* (2000) observed significant leucocytopaenia in camel infected with sarcoptic mange.

Parija *et al.* (1995) also observed neutrophilia in goats suffering from sarcoptic mange. But contrary to the present findings Sena *et al.* (1999b) and Mal *et al.* (2000) reported neutropoenia in camels with mange.

Eosinophilia recorded in mange affected camels in the present study was in accordance with the report of Kataria and Kataria (2004) who recorded higher counts of eosinophils with raised levels of IgE in camels suffering from mange.

Significant lymphocytopoenia was observed in the present study. However, Sena *et al.* (1999b) found no significant change in the lymphocytes and contrarily Mal *et al.* (2000) and Premalatha *et al.* (2010) observed lymphocytosis in camels affected with mange.

In gastrointestinal nematodosis the haemato- logical findings *viz.* significant decrease in Hb, PCV and TEC were in accordance with Arzoun *et al.* (1984); Egbe-Nwiy and Chaudhary (1994); El-Magawary *et al.* (1998); Partani *et al.* (1996b) and Sena *et al.* (2000) in camels. Leucocytosis in the present study is in conformity with the findings of Grabber *et al.* (1967); Arzoun *et al.* (1984) and Sena *et al.* (2000) in camels. This finding is however,

Table 2. : Mean ± SE values of serum proteins in healthy and camels affected with mange and GI nematodosis.

Parameter	Camels affected with		**Healthy camels
	Mange	GI Nematodosis	
Total serum protein (g/dl)	07.11±0.50 (4.82-8.75)	05.85±0.11 (5.01-7.50)	06.13±00.19 (04.69-06.86)
Serum Albumin (g/dl)	03.79±0.35 (2.42-5.02)	03.76*±0.11 (2.22-5.31)	03.32±00.12 (02.81-04.17)
Serum globulin (g/dl)	03.32±0.42 (2.2-5.44)	02.09*±0.08 (1.20-4.44)	02.80±00.21 (01.46-03.91)
Albumin:Globulin	01.27±0.20 (0.60-2.05)	01.93*±0.09 (0.62-3.58)	01.29±00.13 (00.75-02.01)

* Significant ($p < 0.05$), figures in the parentheses indicate range, ** (Partani *et al.*, 1995).

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HAEMATO-BIOCHEMICAL CHANGES IN PYOMETRA AFFECTED BITCHES#

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ABSTRACT

The present investigation was carried out in 30 clinical cases of canine pyometra. Various haematobiochemical parameters like Hb, PCV, TLC, DLC, serum creatinine, BUN and alkaline phosphatase were evaluated and their importance as diagnostic aid for canine pyometra was studied. There was significant decrease ($P<0.05$) in the Hb content (g%), PCV (%) and significant increase in the TLC (thousand/cumm) in pyometra affected bitches as compared to control group animals. DLC showed significant increase ($P<0.05$) in the number of neutrophils, significant decrease in the number of lymphocytes and monocytes and non-significant ($P<0.05$) difference in the number of eosinophils and basophils between pyometra affected bitches and control. The mean serum alkaline phosphatase (IU/l), serum creatinine (mg/dl) and BUN (mg/dl) levels were significantly higher ($P<0.01$) in pyometra affected bitches as compared to control group animals.

Key words **:** Pyometra, haematobiochemical, canine.

Introduction

Pyometra in bitches is an acute or chronic polysystemic metoestral disorder associated with secondary bacterial infection and accumulation of pus in the uterus leading to toxaemia in dogs (Nath *et al*., 2009). Significant alterations in haematobiochemical parameters and kidney damage due to bacterial endotoxins during canine pyometra have been reported (Dabhi and Dhami , 2006). Therefore, the present work was planned to evaluate haematobiochemical parameters as diagnostic aid for pyometra in bitches.

Materials and Methods

The study was carried out in 30 clinical cases of canine pyometra presented for treatment at Teaching Veterinary Clinical Service Complex Mhow. Further 6 normal non pregnant bitches were taken as control. After retrieval of history and sonographic evaluation 5 ml of blood was collected from each affected and control group animals. About 1ml of blood was poured in a sterile vial containing anticoagulant ethylene diamine tetra acetic acid (EDTA) @ 2 mg/ml of blood for haematological studies. Remaining blood was collected in a tube and was allowed for clotting. After clotting serum was separated out, centrifuged @ 2500 rpm for ten minutes and supernatant was then collected in sterile vials and kept at -20° till biochemical estimations. The haematobiochemical parameters evaluated were Hb, PCV, TLC, DLC, serum creatinine, blood urea nitrogen and alkaline phosphatase. Haematological parameters were carried out as per the procedure described by Jain (1986).Serum creatinine was estimated by Alkaline Picrate method using Qualigens diagnostic kit as described by Bonses and Tausskey (1945). BUN was estimated by DAM method using Acesurce urea reagent kit as described by Marsh *et al*. (1965). Alkaline phosphatase was estimated using Qualigens diagnostic kit as described by Searcy (1968). The data so obtained was subjected to statistical analysis using “t” test (Snedecor and Cochran, 1980).

Results and Discussion

Haematobiochemical values of these parameters are illustrated in Table 1. There was significant decrease in the Hb content (g%) and PCV (%) in pyometra affected bitches as compared to control group. This decrease might be due to loss

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Table 1: Haemato-biochemical parameters (Mean ± SE)

Parameters	Control	Pyometra
Hb (g %)	11.56 ± 0.32	10.10 ± 0.30*
PCV (%)	34.85 ± 0.91	30.42 ± 0.89**
TLC (th/cumm)	12.33 ± 0.18	22.22 ± 1.13**
% DLC Neutrophils	64.16 ± 1.01	72.0 ± 0.54**
Eosinophils	5.16 ± 0.79	5.26 ± 0.17^{NS}
Basophils	1.50 ± 0.22	1.23± 0.14^{NS}
Lymphocytes	23.50 ±0.99	18.09 ± 0.34**
Monocytes	5.66 ± 0.33	2.53 ± 0.17**
Serum creatinine (mg/dl)	0.76 ± 0.08	3.37 ± 0.08**
Blood Urea Nitrogen (mg/dl)	14.31 ± 0.78	31.04 ± 0.73**
Alkaline phosphatase (IU/l)	10.66 ± 1.76	32.03 ± 1.29**

* (P< 0.05) ** (P< 0.01)

of erythrocytes into the lumen of uterus coupled with toxic depression of bone marrow (Schalm *et al*., 1991). Significant increase in the TLC (thousand/ cumm) in pyometra affected bitches as compared to control group animals was observed. This leucocytosis might be due to increased stress on body defence mechanism which in turn produces increased leucocytes to combat the infection (Kaymaz *et al*., 1999). Neutrophilia with regenerative shift to left, lymphocytopaenia and monocytopaenia was a common finding in blood picture of pyometra affected bitches as compared to control group. Neutrophilia with regenerative shift to left might be due to retention of purulent exudates in the uterus which exerts a chemotactic effect on neutrophils resulting into accelerated granulopoiesis (Schalm *et al*., 1991). Lymphopaenia and monocytopaenia might be due to the severe stress during the disease (Stone *et al*., 1988). The mean serum creatinine (mg/dl) and BUN (mg/dl) were significantly higher in pyometra affected bitches as compared to control group animals. Elevated serum creatinine and BUN levels might be due to kidney damage due to bacterial endotoxins and dehydration caused by pyometra (Dabhi and Dhami, 2006). Significantly higher serum alkaline phosphatase levels (IU/l) were observed in pyometra affected animals as compared to control group animals. Higher serum alkaline phosphatase levels during pyometra are suggestive of its diagnostic value. Similar findings have been reported by Panday and Pandit (2000) and Bondade (2006).

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PREVALENCE OF NUTRITIONAL ANAEMIA IN GOATS OF ARID ZONE OF RAJASTHAN#

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ABSTRACT

A total of 300 goats were screened for anaemia on the basis of clinical manifestations and laboratory examination. The overall prevalence of anaemia in goats in and around Bikaner district of Rajasthan was found to be 19.66 per cent irrespective of age, sex and breed. Prevalence of anaemia in relation with sex revealed that out of 59 positive cases of anaemia, 48 (81.36%) were female and 11 (18.64%) were male. Age wise the prevalence of anaemia was highest at 4 months to 1 year of age (62.71%) followed by 1 to 2 years of age (20.34%) and above 2 years of age (16.95%). Hb, PCV, TEC, MCH and MCV parameters showed significantly lower values in anaemic goats as compared to control. No significant variations in neutrophils, monocytes, eosinophils and basophils values were observed in healthy and anaemic goats. The biochemical parameters revealed decrease in total serum protein, albumin, A:G ratio and glucose values in goats suffering from anaemia. The level of copper, cobalt and iron decreased significantly ($P<0.01$) in goats of group T_2 as compared to group T_1. It was concluded that overall prevalence of anaemia in goats was 19.66 per cent. Mineral deficiencies or imbalances in soil, feed and fodder have long been held responsible for development of nutritional anaemia in goats of arid zone of Rajasthan.

Key words: Anaemia, goats, prevalence, nutritional, arid zone

Introduction

Anaemia is the common and predominantly seen clinical manifestation in goats. The anaemia is characterized physiologically by insufficient circulating haemoglobin and clinically by reduced exercise tolerance and pale mucous membrane (Armour *et al.,* 1991 and Fraser *et al.,* 1991) increased destruction or loss of red blood cells (Radostits *et al.,* 2000). Thus, in anaemia quantity or quality of circulating red cells is reduced below the normal level. Important causes of anaemia in goats included bacterial, viral, haemoparasitic diseases viz., anaplasmosis, babesiosis and theilariasis, toxicity of chemicals, regional poisonous plants and venom, autoimmune haemolytic diseases, congenital defects, carcinoma, trauma and chronic antigenic stimulation. Apart from these, anaemia caused by decreased production of erythrocytes result mainly due to nutritional deficiency of certain micronutrients as copper, cobalt and iron are important minerals in such conditions (Chaudhary *et al.*, 2008). Since majority of goats are grazing and are kept under imbalanced diet, they often suffer from anaemia due to malnutrition. Further, state of Rajasthan experiences regular famines and because of low rainfall, animals of this region do not get green fodders which provide minerals, micronutrients and other vitamins. The goat husbandry in this area is mostly dependent on grazing land especially in rural areas and under such feeding practices the minerals deficiency in animals is expected due to poor contents of grazing resources and non availability of green fodder in the tropics. Further, most of the goats in urban areas are kept as stall fed animal which may also lead to nutritional problems and deficiency diseases causing great economic loss to goat breeders either due to the loss of meat or milk production. Therefore, a study was conducted to find out the prevalence of nutritional anaemia in goats of arid zone of Rajasthan.

Materials and Methods

A total of 300 goats irrespective of age, sex and breed brought to the Medicine Clinic of Department of Clinical Veterinary Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Science, Bikaner, goats from Gadwala, Pemasar,

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Udasar, Udramsar, Shivbari and Sujandesar village of Bikaner as well as goats belonging to individual holdings of the owner in and around Bikaner district of Rajasthan were screened for anaemia on the basis of clinical manifestations and laboratory examination. Routine faecal examination was carried out for each goat to rule out the possibility of parasitic load. The positive cases having parasitic load were excluded from the study.

The blood samples collected from all the goats separately to estimate haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) as per the standard method described by Jain (1986). Biochemical analysis of serum samples was also carried out to estimate serum total protein, albumin, globulin, albumin-globulin (A:G) ratio and blood glucose by the methods of Tietz, (1990), using standard kit (SPINREACT, ARK, Diagnostics Pvt. Ltd, Bangalore). Serum copper, cobalt and iron levels were estimated by Atomic absorption spectrophotometer as per the method of Pinta (1979).

Diagnosis of anaemia was arrived on the basis of history, general examination and clinical manifestations. The final diagnosis was established on the basis of haemato-biochemical parameters. The goats having haemoglobin values (g%) less than mean -2 standard deviation of healthy goat were considered as anaemic goat (Welchman *et al*., 1988). All the estimated values were compared with the normal values recorded in healthy goats maintained on a well balanced diet in goat farm, Beechwal, Rajasthan University of Veterinary and Animal Sciences, Bikaner. The collected data were analyzed as per the methods of Snedecor and Cochran (1994).

Results and Discussion

Out of total 300 goats (93 males and 207 females) screened and 59 goats were found anaemic. The goats showing haemoglobin values less than 8.07 g% were considered as anaemic. The affected goats reveled partial to complete anorexia, loss of body condition, depression, dry and rough hair coat, pallor mucous membranes, weakness, shuffling gait, tremor, tachycardia, pulse with large amplitude, slight increase in heart beat intensity, loud heart sounds. In few cases exaggerated apex beat, poor exercise tolerance and coughing were also noticed with low productivity in few milking goats. The clinical signs of anaemia as recorded in present study corroborated with the findings of Sarkar and Mishra (1991), Smith and Sherman (1994), Rajkhowa and Hazarika (2002) and Bhikane *et al*. (2006) in goats.

The overall prevalence of anaemia in goats was 19.66 per cent irrespective of age, sex and breed. Calculations of prevalence in relation with sex revealed that out of 59 positive cases of anaemia, 48 (81.36%) were female and 11 (18.64%) were male. Age wise the prevalence of anaemia was highest at 4 months to 1 year of age (62.71%) followed by 1 to 2 years of age (20.34%) and above 2 years of age (16.95%). Similar type of prevalence have also been reported earlier by Sarkar *et al.* (1992a) reported higher prevalence of anaemia in young goats with in age group between 7 months to 1 year (35.9%) followed by 3 to 6 months (34.1%) and 1 to 2 years of age group (19.4%). Ramesh and Suryanarayana (1999) recorded 13.46 per cent of overall prevalence of anaemia in goats. Age wise highest incidence of caprine anaemia (31.11%) was recorded at 3 months of age. Sex wise highest incidence of anaemia was recorded in females (14.28%), followed by males (11.76%). Chandra *et al.* (2000) reported 34.84 per cent nutritional anaemia in Black Bengal goats. Similarly, Shinde and Rajguru (2009) observed 20.68 per cent overall prevalence of anaemia in goats. Age wise highest prevalence of anaemia was recorded in 6-12 months age group (28.27%) followed by 0-6 months (24.00%), 12-24 months (19.51%), 24-36 months (17.24%) and 36 months and above (13.51%).

Comparison of haematological profile of healthy and anaemic goats indicated significantly ($P<0.01$) lower values of haemoglobin, packed cell volume, total erythrocyte count, mean corpuscular volume and mean corpuscular haemoglobin in anaemic goats (Table 1). The decreased value of red cells, PCV, haemoglobin and alteration in MCH and MCV was either due to mechanical damage to erythrocyte or due to some nutritional deficiencies resulting in the development of anaemia (Sarkar *et al*., 1992b). Significant decrease in the level of erythrocytic indices attributed to the production of hypochromic anaemia and microcytic hypochromic anaemia was recorded in nutritional deficiencies. The goats of group T_2 were supposed to be suffering from nutritional anaemia as the positive cases of parasitism were already excluded from the study. Non-significant changes in neutrophils, monocytes, eosinophils and basophils values indicated that the development of caprine anaemic state might be due to nutritional deficiency either in feed, fodder or soil.

The biochemical profile of healthy and anaemic goats revealed significantly ($P<0.01$) lower values of total serum protein, albumin, albumin-globulin (A:G) ratio and blood glucose in anaemic goats (Table 2). However, non-significant difference was observed in globulin values in both the groups. The present observation is in conformity with the findings of Sarkar *et al*. (1995) recorded significant decrease in the value of blood glucose (46.92 mg/dl) and total

serum protein values (5.3 g%) in anaemic grazing goats suffered from micro mineral deficiency. Similarly, Dalpati and Bhowmik (1996) also recorded significant decrease in blood glucose (47.25 ± 0.84 mg/dl) in anaemic goats. High significantly low levels of blood glucose in all cases of caprine anaemia were also recorded by Sarkar *et al*. (1991) and Sarkar *et al*. (1992b). In nutritionally deficient anaemia, hypoglycaemia might be due to prolonged inappetance and defective glucose metabolism.

The level of copper, cobalt and iron decreased significantly ($P<0.01$) in goats of group T_2 as compared to group T_1. Sarkar *et al*. (1990a) reported significantly lower levels of iron and copper in grazing goats irrespective of parasitism. Cobalt deficiency in cattle of Bikaner district of Rajasthan was also reported by Bal and Dwarkanath (1989). Jain *et al*. (2000) inferred that under grazing conditions the trace minerals like copper, cobalt and zinc, which are deficient in many forages should be supplemented in the diet to meet the requirement of these minerals for optimum productive and reproductive efficiency of goats. Natural feed stuffs usually contain enough iron (McDowell *et al*., 1983) which ruled out deficiency in grazing livestock, however, low levels of serum iron in the study might be due to low level of cobalt which may cause decrease in level of iron possibly due to the interference in the uptake of iron by the cells (Samanta *et al.*, 1995). The other cause of iron deficiency is possibly due to inadequate utilization of readily available iron or dietary iron deficiency may be observed in animals grazing on pasture deficient in iron (Coles, 1980). Interference in the uptake of iron and copper by the cell in case of anaemic goats possibly also resulting from deficiency of cobalt in the pasture plants of arid zone (Sarkar *et al*., 1990b).

It was concluded that overall prevalence of anaemia in goats was 19.66 per cent. Mineral deficiencies or imbalances in soil, feed and fodder have long been held responsible for development of anaemia in goats. Nutritional deficiency anaemia may be due to higher atmospheric temperature and heavy erosion of soil due to storms in arid zone of Rajasthan, making the soil deficient in plant minerals. In the present study, nutritional deficiency anaemia in goats might be due to exclusive grazing practices without supplementation of either mineral

Table 1: Haematological profile in healthy and anaemic goats.

Profile	Healthy goats (T_1)	Anaemic goats (T_2)
Haemoglobin (g%)**	10.28±0.39^b	7.31±0.06^a
Packed cell volume (%) **	32.37±1.29^b	23.72±0.27^a
TEC (million/cumm)**	9.97±0.31^b	8.46±0.08^a
TLC (thousand/cumm)**	9.10±0.26^a	11.11±0.15^b
MCV (fl)**	32.63±1.54^b	28.15±0.38^a
MCH (pg)**	10.40±0.55^b	8.68±0.10^a
MCHC (g/dl)^{NS}	31.85±0.66	30.96±0.31
Neutrophil (%)^{NS}	37.75±1.97	40.91±0.64
Lymphocyte (%)*	54.37±1.82^b	49.40±0.63^a
Monocyte (%)^{NS}	3.50±0.42	4.79±0.27
Eosinophil (%)^{NS}	4.00±0.37	3.28±0.26
Basophil (%)^{NS}	0.37±0.18	1.59±0.24

NS- Non significant; *Significant at ($P<0.05$); **Significant at ($P<0.01$)

Table 2: Biochemical profile in healthy and anaemic goats.

Profile	Healthy goats (T_1)	Anaemic goats (T_2)
Total serum protein (g/dl)**	7.15±0.07^b	5.35±0.08^a
Albumin (g/dl)**	4.09±0.12^b	2.24±0.04^a
Globulin (g/dl)^{NS}	3.05±0.15	3.10±0.08
A: G ratio**	1.37±0.11^b	0.75±0.03^a
Blood glucose (mg/dl)**	62.97±2.11^b	50.52±0.75^a
Serum copper (µg/dl)**	93.27±3.89^b	67.43±1.84^a
Serum cobalt (µg/dl)**	27.15±0.79^b	18.70±0.67^a
Serum iron (µg/dl)**	168.06±5.77^b	119.14±3.83^a

NS- Non significant; **Significant at ($P<0.01$)

mixture or good quality concentrates, that might resulted in deficiencies of vitamins and minerals and those are most essential for erythrocyte production.

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CLINICAL HAEMATOLOGY IN DOGS AFFECTED WITH HAEMORRHAGIC GASTROENTERITIS

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ABSTRACT

Haemorrhagic gastroenteritis in dogs is a common disease of multiple etiology seen in all breed and age groups. The present paper discusses the study that was conducted on 30 dogs having history of vomition and diarrhoea with blood of any etiology, and 10 dogs presented at TVCSC, Apollo College of Veterinary Medicine, Jaipur for routine examination deworming or vaccination constituted the apparently healthy dogs. There was significantly decrease in the values of haemoglobin and packed cell volume which might be due to loss of blood in vomition and stool. Twenty five dogs showed leucocytosis probably due to bacterial/secondary bacterial infections where as five cases showed leucopoenia. Neutrophilia with lymphopoenia was seen in 25 cases while five cases showed neutropoenia with lymphocytosis.

Key words: Haemorrhagic gastroenteritis, haematology

Introduction

Haemorrhagic gastroenteritis is a commonly seen disease in all breed and age groups of dogs having multiple etiologies. Sudden onset of bloody vomition and diarrhoea, dehydration, anorexia and depression are the common clinical symptoms. Causative factors responsible for haemorrhagic gastroenteritis are viruses such as parvovirus (Appel *et al.,* 1978; Mohan *et al*., 1993 and Hoskins, 1997), corona virus (Toma and Moraillon, 1980), rotavirus (Barrios *et al*., 1989), bacterial infections like, *Salmonella* spp. (Chaudhary *et al*., 1985), *Escherichia coli* (Prada *et al*., 1991), *Clostridium* spp. (Turk *et al*., 1992 and Prescott *et al*., 1978), endoparasites such as *Dipyllidium caninum, Ancylostoma caninum* (Ndiritu and Sadi, 1977 and Kumar *et al*., 2001), dietary errors (Guilford, 1994), food allergy (Kumar *et al*., 2003) and irritant drugs (Cumming, 1991 and Waters *et al*., 1992).

Materials and Methods

Collection of blood samples

Two ml of blood from 30 haemorrhagic gastroenteritis affected dogs was collected from cephalic or recurrent tarsal vein with the help of sterile disposable syringe fitted with a 22 gauze needle before the start of treatment when the case was presented at the TVCSC, Apollo College of Veterinary Medicine, Jaipur. The blood was poured immediately in glass vials containing EDTA as anticoagulant for haematology.

The blood samples from 10 apparently healthy dogs (control group) were collected as described above when they were presented for routine examination, deworming or vaccination and was subjected to estimation of haematological values.

Haematological examination

Haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC) and differential leucocyte count (DLC) were estimated by standard procedure as described by Schalm *et al*. (1975). Statistical analysis was done as described by Snedecor and Cochran (1968).

Results and Discussion

Haemoglobin

Mean ± SE value of haemoglobin of control group and haemorrhagic gastroenteritis affected dogs were 14.27 ± 0.28 and 9.07 ± 0.12 gm %, respectively. The mean haemoglobin value of haemorrhagic gastroenteritis affected dogs was found significantly low ($P<0.05$) when compare with apparently healthy dogs. (Table 1).

In present study significantly decrease in haemoglobin value might be due to loss of blood in vomition and stool. Additionally low haemoglobin values could be due to decreased erythropoiesis as a result of direct effect of parvovirus on the bone marrow (Boosinger *et al*., 1982) and accumulation of toxic waste products during viraemia and febrile phase (Jones and Hunt, 1983) and preexisting poor health status of the dog.

Packed cell volume

Mean ± SE value of packed cell volume of control group and haemorrhagic gastroenteritis affected dogs were 42.30±1.42 and 28.21±0.38%, respectively. The packed cell volume of haemorrhagic gastroenteritis affected dogs was found significantly low (P<0.05) when compare with apparently healthy dogs (Table 1).

The finding of present study is in conformity with Biswas *et al.* (2005) and Rai *et al.* (1994) who have also reported a decrease PCV values in parvo viral affected dogs but contrast to Burrows (1977) reported that elevated packed cell volume secondary to fluid loss is the most consistent finding in acute haemorrhagic gastroenteritis. In majority of the dogs in the present investigation, the packed cell volume values were low. O'Sullivan *et al*. (1984) have reported an initial increase in packed cell volume with the onset of vomition and diarrhoea which subsequently decreases to below normal values with the progression of the diseases.

Total leucocyte count

Mean ± SE value of total leucocyte count of apparently healthy dogs and haemorrhagic gastroenteritis affected dogs were 8.75 ± 0.50 and 30.18 ± 3.23 thousand/cumm, respectively. The total leucocyte count of haemorrhagic gastroenteritis affected dogs was found significantly higher (Pd"0.05) when compare with apparently healthy dogs. (Table 1).

In the present study five haemorrhagic gastroenteritis affected dogs were showing mild leucopoenia which could be due to viral infection which was reported by several workers (Appel *et al*., 1978; Pletcher *et al*., 1979; Greene, 1984; Macartney *et al*., 1984; and Nayak *et al*., 1984). Majority of haemorrhagic gastroenteritis affected dogs were showing leucocytosis which might be due to primary or secondary bacterial infection, acute intravascular haemolysis with the damage to the tissues of liver and other organs or from secondary resurgence of the bone marrow leading to increased production of cells with a shift of the cells from the marginal pool to the circulating pool. These findings are in conformity with the results of several workers (MaCandish *et al*., 1981; Greene, 1984 and Ghermai and Kraft, 1987) who have reported that with the progression of the disease, a reactive leucocytosis as a response to complicating bacterial infections and a reactive leucocytosis associated with myeloid hyperplasia occurs leading to leucocytosis.

Differential leucocyte count

Mean ± SE value of neutrophils, lymphocytes, monocytes and eosinophils of apparently healthy dogs and haemorrhagic gastroenteritis affected dogs were 68.20±0.78, 25.40±0.89, 4.11±0.48 and 2.50±0.36, and 78.60±2.83, 15.85±4.25, 1.30±0.05 and 4.25± 3.15%, respectively. The neutrophils and eosinophils value of haemorrhagic gastroenteritis affected dogs were found significantly higher and value of lymphocytes and monocytes were significantly low (Pd"0.05) when compare with apparently healthy dogs (Table 1).

In the present study, five haemorrhagic gastroenteritis affected dogs were showing neutropoenia with lymphocytosis might be due to the secondary affect as a result of increased loss of neutrophils through the damaged intestinal wall. Perry (1970) has reported that there is considerable loss of neutrophils into the intestinal lumen even in normal dog and this may be greatly increased in enteric diseases. Macartney *et al*. (1984) have also reported profound neutropoenia in severely affected dog after the appearance of clinical enteric disease. Potgieter *et al*. (1981) have also reported that neutropoenia occurs in dogs with severe enteric disease.

In the present study, majority of haemorrhagic gastroenteritis affected dogs were showing neutrophila and lymphopoenia could be due to severe inflammatory reaction caused by bacterial infections. This is in agreement with the findings of Macartney *et al*. (1984) and Ramprabhu *et al*. (2002).

Table 1: Mean ± SE volume of haemoglobin (Hb), packed cell value (PCV), total leukocytes counts (TLC) and differential leucocyte counts (DLC) of healthy control dogs and haemorrhagic gastroenteritis affected dogs.

Group	Parmeters Hb (g%)	PCV (%)	TLC (Th/cumm)	N (%)	L (%)	M (%)	E (%)
Healthy control	14.27^{b}±0.28 (12.8-15.00)	42.30^{b} ±1.42 (34-48)	8.75^{b}±0.50 (8.25-13.4)	68.2^{b}±0.78 (65-75)	25.40^{b}±0.89 (24-30)	4.11^{b}±0.48 (3-8)	2.50^{b}± 0.36 (2-9)
Affected dogs	9.07^{a}±0.12 (4.8-18.6)	28.21^{a} ±4.18 (15-55)	30.18^{a}±3.23 (2.5-48.00)	78.60^{a}±2.83 (28-94)	15.85^{a}±4.25 (13-18)	1.30^{a}±0.05 (0-5)	4.25^{a} ± 3.15 (0-8)

Mean values with the different superscript are differ significantly (P≤ 0.05)

Acknowledgements

The authors are very thankful to the Dean of the College for providing necessary facilities to carry out this research work.

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USE OF HUMAN RECOMBINANT ERYTHROPOIETIN AND NANDROLONE DECANOATE IN A COMBINATION FOR TREATMENT OF HYPOPLASTIC ANAEMIA IN A GERMAN SHEPHERD DOG

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Hypoplastic anaemia is characterised by pancytopenia i.e. decrease in red blood cells, leucocytes and platelets in peripheral blood due to failure of the bone marrow to produce all haematopoietic cell lines. It is also known as aplastic anaemia and aplastic pancytopenia. It is present in two forms-acute form which occurs within two weeks of bone injury and chronic form which results from injury to haematopoietic stem cells. Anaemia could occur due to infections, drugs, toxins or radiations. It can also be immune mediated or idiopathic which is the most common cause (Cote, 2007).

Erythropoietin (EPO) is a haematopoietic factor produced in the kidney, which stimulates erythropoiesis and promotes red blood cells survival by protecting these cells from apoptosis (Langston *et al*., 2003). It is widely used to treat hypo-proliferative anaemia associated with chronic renal failure (Sukullaya *et al.*, 2008), cancer patients on chemotherapy, haematological disorders and other conditions with blood loss (Cowgill *et al.*, 1998; Langston *et al.,* 2003; Plumb, 2005).

Nandrolone decanoate is used as an adjuvant therapy to treat anaemia in human patients (Sheasha *et al.,* 2005). It is an injectable anabolic steroid and useful to stimulate erythropoiesis in patients with anaemias e.g. secondary to renal failure, aplastic anaemias and to stimulate appetite. Nandrolone may potentially increase the response to erythropoietin (Plumb, 2004).

Case study

A German shepherd, 5 years old, male dog was brought to the clinic with the history of lethargy, inappetance and fever since last 5 days. Physical examination revealed fever, tachypnoea, pale mucus membrane and dyspnoea. Blood sample was collected for haemogram and serum biochemistry profile. Haematological examination revealed decrease in haemoglobin concentration (3.8 g/dl), reduced PCV (10.9%), decreased RBC (1.63 million/ cu mm), decreased white blood cells (6.4 $\times 10^3$/µL) and decreased platelet count (10,000$\times 10^3$/µL). Serum values for liver and kidney function were within the normal range.

The dog was treated with EPO (100 IU/kg SC) twice weekly and nandrolone decanoate (2mg/kg deep IM) once a week. Along with this the dog was put on oral broad spectrum antibiotics, oral vitamin B complex and oral iron supplement for two weeks. After one week the haematological examination as repeated and it revealed the remarkable increase in haematological values i.e. Hb (15.9 g/dl), RBC (6.98 million/cu mm), PCV (46.2%), white blood cells (24.1$\times 10^3$/µL) and platelets (419$\times 10^3$/µL). EPO and nandrolone treatment was not given after two weeks and after one month and three months of treatment haematological values were found within the normal limits. Assessment of other parameters after one week, one month and three month of treatment revealed clinical well being of dog, good appetite, weight gain, strength, energy and playfulness.

Summary

Use of nandrolone decanoate and EPO together is very effective in treatment of anaemia. Oral iron supplementation is essential in conjunction with erythropoietin so that bone marrow will have all necessary supplies to produce RBC. A multivitamin with iron is typically used as this will provide the B vitamins needed in red blood cells production as well (Langston *et al.*, 2003).

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MANAGEMENT OF POLYARTHRITIS IN KIDS

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Polyarthritis in kids is a disease with mulifactorial etiology affecting the joints either unilaterally or often bilaterally. Many therapeutic protocols have been tried with success but appear uneconomical for a goat owner. A case of successful and economical management of polyarthritis in kids is reported.

Polyarthritis is characterized by inflammation of synovial membrane and articular surfaces of joints of all the legs resulting in pain and lameness. Fever, inappetance to anorexia, loss of body weight and discomfort may occur in animals with severely affected joints (Radostits *et al.*, 2000). Etiology of disease is varied with mycoplasma being frequently associated with the disease (Rodriguez *et al*., 1994; Nayak and Bhaumik, 1989; Bajmócy *et al*., 2000). Many drugs like Tylosin, Lincomycin and Tiamutin have been evaluated for its efficacy in polyarthritis (Nayak and Bhaumik, 1990; Chakrabarti *et al.,* 1995; Varshney *et al.,* 2000). Therapeutic management of polyarthritis with these drugs appears to be uneconomical for a goat owner. Enrofloxacin has been reported to successfully treat experimental *Mycoplasma bovis* infection in calves (Stipcovits *et al.,* 2005). In the present study, enrofloxacin, a cheap and easily available drug was used for successful treatment of polyarthritis in kids. Two non descript male goat kids around 1½ months of age were presented to veterinary teaching hospital of the college.

On clinical examination the animals were dull with pyrexia (105°F), laboured breathing and accelerated heart rate. Painful swelling was observed on knee, stifle and hock joints with no free fluid in the affected joints. Haemogram revealed relatively low haemoglobin (9.8 g%), haematocrit (30%), neutropenia (28%) and lymphocytosis (67%). Also, no haemoprotozoan was found on goat kids around 1½ months of age were presented to movement. Painful swelling was observed on knee, stifle and hock joints with no free fluid in the affected joints. Haemogram revealed relatively low hemoglobin (9.8g%), haematocrit (30%), neutropenia (28%) and lymphocytosis (67%). Also no haemoprotozoan was found.

Coprological examination performed was negative for any parasitic ova. The case was diagnosed as polyarthritis and treated accordingly.

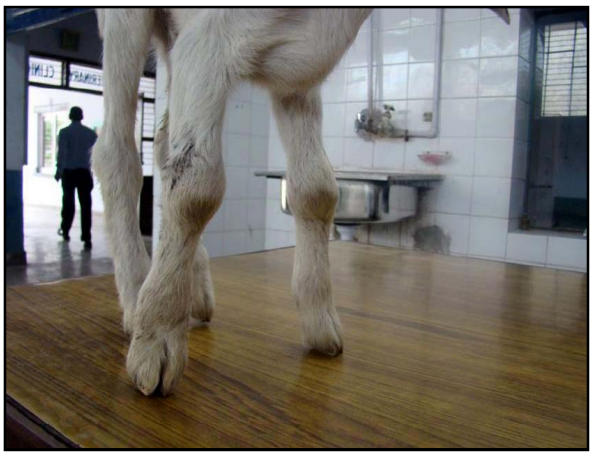

Fig. 1: Swelling of joints in affected kids.

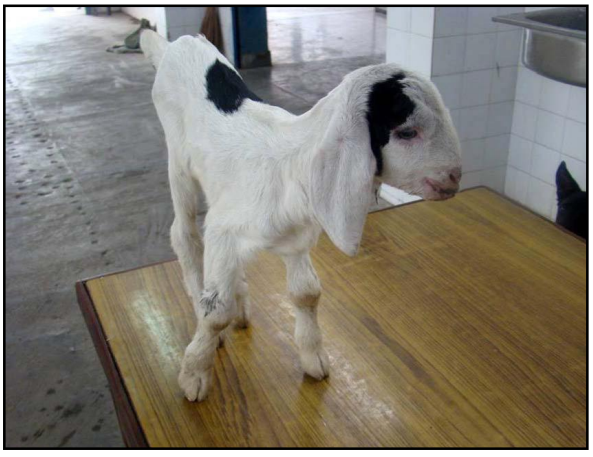

Fig. 2: Swelling of joints in affected kids.

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The kids were treated with enrofloxacin (Enrocin®) given @ 10 mg/kg wt. bid intramuscularly along with anti-inflammatory antipyretic combination (Proxy Vet DS®) @ 1 ml i/m bid for period of 5 days. Supportive medication included liver tonic (Liv. 52®) and haematinic (Cofecu®) in prescribed doses for a week. The animals showed considerable improvement by day 2 and by day 5, there was significant reduction with regard to swelling and movement was observed. Same treatment was advocated for another three day. The owner did not turn up thereafter with animals but reported that animals have recovered completely, No untoward effect was observed.

Therefore, enrofloxacin along with anti inflammatory drugs and supportive medication may be advocated as an economical treatment for managing polyarthritis in kids.

Acknowledgement

Authors are thankful to Dean, College of Veterinary and Animal Sciences and Professor and Incharge, Veterinary Clinics for providing necessary facilities.

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PREVALENCE OF GASTROINTESTINAL HELMINTHS IN HORSES IN MALWA REGION OF MADHYA PRADESH

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ABSTRACT

Faecal samples of 162 horses (104 males and 58 females) of organized and unorganized government as well as private farms of Malwa region of M. P. were examined using standard coprological procedures. Further differentiation of strongyle species was done by Baermann method. Highest prevalence was observed in private unorganized farm (55.12%) followed by that of government organized (16.66%) and private organized unit (36.11%). The mean EPG values also followed the same sequence as above. Regarding the sex wise prevalence, male horses showed lower prevalence (33.65) as compared to that of female horses (50%). Age wise prevalence of helminths was highest in horses of age group 6-9 years (48.94%) followed by above 9 years (37.5%), 3-6 years (34.69%) and least in horses up to 3 years (30%) while highest mean EPG was found in horses above 9 years group (828.19±232.85) followed by that of 6-9 years (798.65±218.75), 3-6 years (690.47±130.99) and 0-3 years (545.33±57.74). Strongyles (24.82%) were the predominant followed by *Parascaris equorum* (6.03%), amphistomes (3.31%), *Oxyuris equi* (2.47%) and Anoplocephala (1.28%). Among the strongyle further differentiation by larval culture revealed highest prevalence of Cyathostomes sp. (62.22%) followed by *Oesophagostomum* sp. (17.77%), *Strongylus vulgaris* (11.11%), *Trichostrongylus axei* (4.44%), *Strongylus edentatus* (2.22%) and *Strongylus equinus* (2.22%).

***Key words*:** Gastrointestinal helminths, parasites, horses, prevalence

Internal parasites of animals are very important in countries like ours when control methods mainly depend on the use of anthelmintics. Extensive use of anthelmintics resulted in resistance problem and a relative increase in the prevalence of some parasite species (Kalpan, 2002; Klei *et al*., 1993; Herd, 1990). In equids, helminths are very important parasites; of which nematodes seem to be more prevalent while trematodes and cestodes are less occurring (Arslan and Umar, 1998; Bakirci *et al*, 2004; Demir *et al*., 1995). In order to design a rational control programme for parasitic diseases of equines, it is very essential to understand the prevalence of parasitic infections in the area and its impact on health status of horses. The parasite epidemiology plays a major role in effective and sustainable control of parasites in a particular animal species (Herd, 1993). Although equine population is very widespread in India, a precise systematic study on their gastrointestinal parasite is lacking however, there are a few reports (Kaur and Kaur, 2008). Therefore, the study was planned with an objective to know the prevalence and some other epidemiological factors related to gastrointestinal parasites of horses in Malwa region of Madhya Pradesh.

Materials and Methods

Freshly collected faecal samples of 162 horses (104 males and 58 femals) belonging to organized and unorganized government and private farms of Malwa region, were kept in nylon bags and examined qualitatively and quantitatively using standard parasitological procedures (Sloss *et al*., 1994). For further differentiation of strongyle species, Baermann method of larval culture (Soulsby, 1995) was performed.

Results and Discussion

The observations of the study are shown in Table 1. The overall prevalence and intensity of gastrointestinal parasites in horses were 35.96% and 720±172.23. This overall prevalence is somewhat lower than those reported earlier (Kaur and Kaur, 2008). The cause behind this may be anthelmintic usage. Comparatively higher prevalence and intensity were observed in unorganized farms as compared to that of organized farms. This may be due to the fact that the horses of unorganized farms are not dewormed properly. Out of 162 horses (104 males and 58 females), male horses showed lower prevalence (35/104) as compared to that of females horses (50%). Higher prevalence of parasites in female horses may probably be due to the common practice of avoiding deworming during pregnancy. Mean intensities of infection were higher in males (775.81±219.84) as compared to that of females (751.19±190.86). Although there were considerable individual variations with in all age groups. Age wise prevalence of these helminths was highest in horses of age group 6-9 years (48.94%) followed by above 9 years (37.5%) group, 3-6 years

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group (34.69%) and least in horses up to 3 years group (30%). Though some workers have found higher mean intensities of parasites (with reference to spiruroids) in both young and aged horses (Bucknell *et al*., 1995) but higher prevalence in middle age group of horses is almost similar to the findings of Dunsmore and Jue Sue, 1985; Miftilodze and Hutchinson (1989) who found higher prevalence of G I helminths in age group upto 5 years. Higher mean intensities of infection was found in horses more than 9 years (828.19±232.85) group followed by that of 6-9 years (798.65±218.75), 3-6 years (690.47±130.99) and 0-3 years (545.33±57.74). In contrast, Ostermanlind *et al*., (1999) found a decline in EPG values of horses with increasing age, though they have also found considerable individual variations within the age groups.

Regarding the species of helminths, strongyles were the predominant parasites (24.82%) followed by *Parascaris equorum* (6.03%), amphistome (3.31%), *Oxyuris equi* (2.47%) and *Anoplocephala sp.* (1.28%). The lower overall prevalence of GI helminths along with variable data of individual prevalence (i.e. nematodes more prevalent and cestodes and trematodes less prevalent) have also been recorded from other parts of the world (Sotiraki *et al*., 1997) and country (Kaur and Kaur, 2008).Occurrence of *Anoplocephala* sp. is indicative of its intermediate host's presence in the region. Prevalence of strongyles was higher in horses of unorganized units as compared to that of organized units. Larval cultures of strongyles revealed the highest prevalence of *Cyathostome* sp. (62.22%) followed by *Oesophagostomum* sp. (17.77%), *Strongylus vulgaris* (11.11%), *Trichostrongylus axei* (4.44%), *Strongylus edentatus* (2.22%) and *Strongylus equinus* (2.22%). This is similar to those reported in other parts of the world (Chander, 1991). An abattoir survey performed in central Sweden during 1992 to 1993 by Hogland and coworkers (1997) and coprological examination performed by Ostermanlind and coworkers (1999) found approximately 80% prevalence of strongyles. The reason behind this might be the lesser efficacy of some drugs against inhibited developemental stages of these parasites (Eysker *et al*., 1992). Further the prevalence of Cyathostomes was higher than the earlier reported (Chaudhari *et al*., 1985, Sengupta and Yadav, 1998). Develpoement of resistance in Cyathostomes against most of the commonly used benzimidazoles might be another reason for higher prevalence of this species.

Conclusion

From the study it can be concluded that many species of gastrointestinal helminth (both with direct and indirect life cycles) are prevalent in the area and special attention should be paid to these infections in horse enterprise. Here the study sample size was small, larger field studies throughout the country are required to know the actual status.

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Table 1: Prevalence of various gastrointestinal helminths in horses.

S. No.	Sources	No. examined	No +ive (%)	Intensity Mean±SD	No positive (%)				
					Strongyles	*Parascaris equorum*	Amphistome	*Oxyuris equi*	Anoplocephala
1.	Government organized unit	48	08 (16.66)	643.13 ±131.63	6 (12.5%)	1 (2.08%)	-	1 (2.08%)	-
2.	Private organized unit	36	13 (36.11)	709.23 ±161.96	8 (22.22%)	3 (8.33%)	1 (2.77%)	1 (2.77%)	-
3.	Private unorganized unit	78	43 (55.12)	808.60 ±223.09	31 (39.74%)	6 (7.69%)	3 (3.85%)	2 (2.56%)	1 (1.28%)
	Total	162	64	720.32 ±172.23	45 (24.82%)	10 (6.03%)	4 (3.31%)	4 (2.47)	1 (1.28%)

PREVENTION OF RECURRENCE OF CERVICO-VAGINAL PROLAPSE BY ROPE TRUSS METHOD IN ANTE-PARTUM BUFFALOES AT FIELD LEVEL

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ABSTRACT

Study was carried out in buffaloes presented to clinic with history of first time cervico-vaginal prolapse during 3rd to 6th parity. The cases selected in present report were recorded with increasing tendency of prolapse due to excessive abdominal pressure at the time of recumbent position in advance pregnant buffaloes and in majority of cases, the prolapse was noticed during night hours. The cases were treated with epidural anaesthesia and the prolapsed masses were reposed only after proper dressing and medication. Out of 23 cases, vulval suture were applied to 08 cases (34.78%), and recurrence of prolapse was observed in 04 cases (17.39%). Prolapsed mass was retained successfully with the help of rope truss in 15 cases (65.21%) and no recurrence was recorded. It can be conclude that rope truss is effective, safe and easy method for retention of prolapse in ante-partum cervico-vaginal prolapse.

***Key words*:** Ante-partum, prolapse, rope truss, vulval suture and buffaloes.

Introduction

Prolapse of genitalia is considered as one of the major reproductive disorder causing great economic loss to dairy industry. Any delay in treatment may lead to oedema, ischaemia, laceration, haemorrhages and shock, resulting in prognosis as poor to hopeless (Pande and Pande, 2002). Many factors play major role for prolapse of genitalia like deficiency of calcium (Pandit *et al*., 1982), deficiency of selenium (Diminov and Dimmintrov, 1988), chronic infection causing continuous irritation of uterine tract (Sharma *et al.,* 1977). Hormonal imbalances during pregnancy and after parturition may also cause prolapse (Roberts *et al*., 1971). Present report deals with management of ante-partum prolapse in field cases with different preventive strategies for retention of prolapse.

Materials and Methods

The present study was carried out in 23 buffaloes presented to Teaching Veterinary Clinical Service Complex (TVCSC), College of Veterinary and Animal Sciences, Parbhani with the history of ante-partum cervico-vaginal prolapse. The cases selected in the present report were recorded with increasing tendency of prolapse (Fig. 1) due to excessive abdominal pressure during recumbency specifically during 3rd to 6th parity and in majority of cases the prolapse of vagina was noticed during night hours only.

The buffaloes were restrained with 10 ml injection of 2% lignocaine hydrochloride injected epidurally. After relieving urine, the vulva and the perineum were scrubbed thoroughly with mild disinfectant solution. The prolapse masses were cleaned thoroughly with lukewarm water with 2 per cent potassium permanganate solution. Reduction of prolapse mass was carried out by saturated solution of sugar and ice cubes. Lacerated wound on prolapsed mass was dressed with antibiotic jelly and repositioned without disturbing cervical portion. The retention of the prolapses was attempted with rope truss (Fig. 2) method (Roberts *et al*., 1971) in 15 cases for 5 days regularly where the cotton rope truss was loosened in standing position of the animal. Vulval tape retention suture (Arthur *et al.*, 1996) was used in 08 animals and the sutures were retained for 5 days. Clinical observations were recorded in all the treated cases till parturition and also during post-partum.

Observations and Discussion

Efficacy of methods to retain genital tract in pregnant buffaloes was assessed and the clinical observations were recorded as detailed in Table 1. On attempting regular gynaeco-clinical treatment in ante-partum prolapse cases (Fig. 1), higher efficacy of rope truss method was recorded as against vulval suture method for retention of prolapse mass. It was noted that the straining were continued for more

Fig. 1:Recto-vaginal prolapse in buffalo

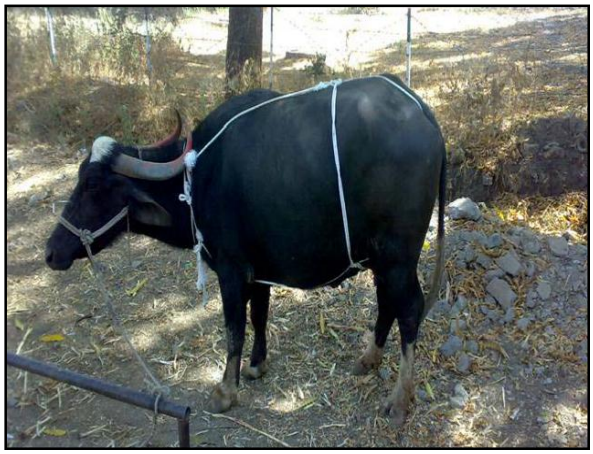

Fig. 2:Rope truss applied for retention of prolapse

Fig. 3: Recovery from prolapse

duration after vulval sutures (2.25 ± 0.75 hrs) as against rope truss method (1.30 ± 0.30 hrs). No recurrences of prolapse and successful completion of term were the added benefit of use of rope truss method as 04 cases (17.39 %) showed recurrence of condition and 02 cases (08.69%) delivered prematurely in vulval suture method. It was further recorded that vulval sutures were ineffective in preventing even post-partum prolapse as 2 cases (08.69%) were recorded with recurrences and injury at the site of suture was turned into maggotted wound in 01 case (04.34%). On the basis of clinical observations, it was recorded that rope truss method is more effective method for retention of prolapse mass against vulval sutures in ante-partum buffaloes (Fig. 3).

Excess pre-partum relaxation of pelvic tissues combined with increased intra-abdominal pressure may be responsible for the vaginal prolapse occurring before parturition. Calcium is required for cell membrane permeability, muscle contraction and nerve impulse transmission, and its deficiency can result in reduced uterine muscle tone and ultimately the resultant uterine prolapse (Herrick, 1977). Excessive relaxed or loose pelvic ligaments near the term and loosing of vaginal muscles may be the possible reason for the higher incidences of prolapse in pluriparous animals.

Lack of exercise and close confinement to the stanchion were reported to be the predisposing factors for genital prolapse in cattle. Generally stall fed animals were more prone (82.54 per cent) to prolapse as reported by Mishra (1997a). However, all the clinical cases of the present study were on grazing practices indicating the possible role of even exercise and increased locomotors activity for prolapse in buffaloes. Buffaloes in present study were having good body score condition and even then there was ante-partum prolapse. Similar finding were recorded by Mishra (1997b) that carrying good body score which are also prone for cervico- vaginal prolapse.

Bladder distention with retention of urine was observed in 95.00 per cent cases during present study.

Rope truss was found to be effective, safe, non invasive and easy method for retention of prolapse in ante-partum cervico vaginal prolapse cases in present study. The rope truss can be applied by para veterinarian or even by animal owner also. As this method is non invasive, post applicative complication were nil and also there found reduced chances of injury to the external genitalia. No disturbance in the process of parturition by application of rope truss method was recorded. No recurrence of prolapse in any case before parturition or even after parturition was the remarkable observation in rope truss method as against vulval

Table 1: Clinical observation in ante-partum prolapse cases

Observations	Retention of prolapse	
	Rope truss	Vulval sutures
Number of cases	15	08
Continuation of straining after retention (hrs)	1 to 2 (1.30±0.30)	2 to 3 (2.25± 0.75)
Recurrence of prolapse	Nil	04
Successful completion of term	All cases	Pre-mature delivery in 2 cases
Injury to external genitelia	Nil	Maggotted wound at suture site in 1 case.
Post-partum complications	Nil	Reappearance of prolapse in 2 cases

suture method. Hence it can be concluded that the tendency of prolapse can be curtailed by rope truss which creates no injury to the external genitalia during retention in advance pregnant animals and also the same can be possibility used as preventive strategy to avoid the prolapse in threatened cases.

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SOCIO-ECONOMIC STATUS OF SPITI HORSE OWNERS *VIS-A-VIS* HORSE MANAGEMENT IN NATIVE TRACT

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ABSTRACT

Spiti horses are widely used for carrying men and material in the hilly terrains of Lahoul and Spiti in Himalayan region. People living in Pin Valley rear these horses mainly for breeding purpose. The study was planned to know socio-economic status of Spiti horse owners and managemental practices followed by them. The primary data was collected from 50 horse owners belonging to Pin Valley of Spiti Sub-Division of Lahoul and Spiti District. The people engaged in horse rearing belong to mainly Buddhist community. Majority of them were poor and illiterate. Women and children contribute significantly in equine husbandry. Selection of breeding stallion, sharing of upkeep of stallion, castration of surplus males etc are the important practices which are performed under the supervision of elderly and experienced horse breeders more or less on scientific lines. The mares freely roam with the stallion from April onwards and get covered through natural mating. Foaling generally occurs in the month of April and May and most of the mares conceive during the foal heat. They sell their equines in International Lavi Fair at Rampur in Shimla District and Ladarcha Fair Kaza in Lahoul and Spiti District. Selling price varies from 10,000 to 30,000 depending upon age, sex and physical condition of the horse. It was concluded that horses were an important component in the life of people of Pin Valley. Potential areas for intervention were identified as training of veterinarian for AI, treatment, husbandry practices of equines and disease investigation as well as including of height as a criterion in stallion selection.

Key words: Spiti, horse, socio-economic status, managemental practices

Introduction

Although Spiti or Chamurthi horses are found in most of the parts of Himachal Pradesh but they are originally habitant of Pin Valley, breeding tract of these horses. People of this Valley are engaged in horse breeding since ages. As per estimates, there were about 400-450 Spiti horses in Pin Valley. As per survey report of CSK H. P. Krishi Vishvavidyalaya, Palampur the total population of Spiti horses in Himachal Pradesh is approximately 4000 (http://ahdhp.nic.in/chamurthi.pdf retrieved on May 26, 2010). True to breed Spiti horses are confined to 15 villages of Pin Valley in Spiti Sub-Division of Lahoul and Spiti District. People residing in this valley have great affinity to Spiti breed of Indian horses. They use these horses for journ from 50 horse breeders belonging to villages namely Sagnam, Kaa, Teling, Teha, Fuckchung and Mudd of Pin Valley in Spiti Sub-Division of Lahoul and Spiti District, Himachal Pradesh. The data included the general information about the horse owners, their source of earnings, family inventories, land holdings, equine herd strength, livestock herd strength, reproductive parameters, parameters of stallion selection, feeding and grazing practices followed, marketing and other constraints regarding the growth and development of the this enterprise in the region etc.

Results and Discussion

The Pin Valley is very cold as compared to the adjoining area of Lahoul Spiti division due to severe snow fall. Agricultural farming and animal husbandry are the major source of income for the people of Pin Valley. The people of this Valley rear Spiti horses for breeding purpose. The people engaged in Spiti horse production belong to mainly Buddhist community. Majority of them are poor, illiterate (36%), below matriculation (42%) and above matriculation (22%). Although, government has set up primary schools in almost every village, but there is only one high school in whole of the Spiti valley situated at Kaza. The children from distant villages are unable to attend this high school due to non-availability of hostel facility and it is impossible to commute in the difficult Himalayan terrain. The problem of girls is worse because of need of extra labour on the farms.

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Girls perform most of work of agricultural field and home. Hence, it is difficult for them to get education. All the respondents were maintaining Spiti horse as well as other livestock. Details of per cent owners maintaining various livestock and mean strength of livestock maintained by them is depicted Table 1. Mean herd size of horse was 1.88±0.16 (range 1to7) in the villages studied. Local breed of cow, yak, donkey, churu/churi (cross of yak and cow), sheep and goat are also maintained by the respondents. Local cow and churi are used for milk production. A very few horse owners (4%) are also maintaining crossbred cows for milk production. Sheep and goat are reared for wool and meat. Donkeys are mainly used as pack animals. Spiti horses are reared mainly for breeding and pleasure ride. The people of this Valley adore their horses and would never think of subjecting them to hard work. Yak, donkey and Spiti horse are also used in threshing operation. Both, the donkeys and yaks are maintained for the draught purpose. Oxen that are widely used in agricultural operations in plains were not being used in this valley as only two respondents were having an ox each.

In this study, mean family size of respondents was observed as 6.58±0.27 (range 3 to 12). Female members were observed more than male members in most of the families. Girl child after attaining the age of 8-10 years starts doing light household and farm works as well handling of donkeys. The average age of horse owners was 41.28±1.36 (range 26-70 year) indicating people of all age group take keen interest in owning Spiti horse.

All the equine owners were having agricultural land and average landholding was observed as 13.04±1.71 bigha (range 3-60 bigha) indicating farming as a major source of their livelihood, followed by horse husbandry. Tree plantation and vegetation in this region is very scarce. The major crops are barley (*Hordeum vulgare*), wheat, green pea and black pea (*Pisum sativum*). The green pea is purchased directly from the field by the merchants. Green pea production from one bigha of land is nearly of Rs ten to twelve thousand. Barley and black pea is the part of human and livestock diet. The production system of Spiti horses in the breeding tract is migratory as well as stationary. All the horses of different age groups are stationary during winter months (December to May) and are stall-fed. The foaling occurs mostly in April to May months (in the beginning of summers) followed by re-breeding usually in foal-heat during May. In summers, from June onwards all horses except young foals and advanced pregnant mares are shifted to highland alpine pastures for grazing for about next five months till heavy snow fall occurs and the horses are taken back home. The young stock is not send to alpine pastures for grazing because of the risk of wild animals. Chances of morbidity are higher when the equines are left uncared for grazing. Hence, all the horses of one village are looked after by two people of that village during the grazing in the pasture throughout the day and night. These two people are replaced by another two people of the village at fortnightly interval. Thus, the horses of the entire village are looked after only by two people at a time. During any emergency with the animal or in case of foaling, owner of that horse is called at pastures site to attend the animal.

The equine owners live in stoned walled *pucca* houses which are nicely white-washed, cleaned and decorated. The houses had flat thatched roots, prepared in traditional style, to keep the houses warm and safe from rain and snow. The ground and first floor of the house was a stable and family quarter, respectively. In the winter, horses were maintained inside the stables at night along with the other animals' *viz.*, cattle, yaks, donkeys, sheep, and goats. The animal sheds were very poorly ventilated and unhygienic. Dung had not been removed from the sheds throughout the year. But, some sand was spreaded in the shed at the place of urination to save the animal from wetness. By the month of October, the dung lying in the stables decomposed well and then it was removed from the stables by the women and girls of the family and dumped in the form of heap on the ground near the stables.

Then this decomposed dung was to be filled in gunny bags and donkeys carried these bags to the agricultural fields. All the activities *viz.*, removal of dung from stables, filling of gunny bags, loading on donkeys, accompanying the donkey to the field etc. were performed by women and girls of the family (Fig. 1 and 2). In the month of April, decomposed dung is scattered in the field and then sowing is done. During the day, women and kids collected dung cake of cows, yaks, donkeys and horses from the pastures in baskets on their back and that was used to burn in winter, since wood availability was scarce.

During the period of grazing in alpine pastures nothing extra is given to these horses as an energy source while during the stall-fed period, barley *busha*, pea *bhusa* and some other grasses are fed as hay to the equids. The owners generally prepared

Table 1: Per cent owners maintaining various livestock and mean livestock strength with them

Livestock	Horse	Donkey	Yak	Churi	Cow	Goat	Sheep
(%)	100	88	84	24	82	50	24
Mean ±SEM	1.88±1.14	2.05±0.89	1.14±0.35	1.16±0.58	1.73±1.01	3.80±2.64	4.08±2.02

Fig. 1: Compost being filled in gunny bags by women

Fig. 2: Donkeys carrying compost filled bags to agricultural farm

Fig. 3: Hay stored at the roofs of the houses

Fig. 4: Spiti horses

hay from available grasses and crop residues. They kept the fodder for hay making on the roof of house and hay could be seen on every roof of that locality (Fig. 3). The hay was provided to the animals during winter season when there is scarcity of green fodder due to heavy snow all around. Most of them offered black pea and barley as concentrate to their horses. Concentrate was provided in two equal doses of 1kg each in the morning and evening. The stallions were also occasionally fed other energy giving feeds like jaggery during the winter season. This is just to coup up the energy losses of the stallion engaged in covering the mares during the preceding breeding season. Almost all the stallion owners feed crushed grains rather than whole grains. The practice of feeding soaked grains was very common. Most of equine owners provide common salt to their horses at weekly intervals. The lukewarm water was provided once in a day during winter months.

They rear male and female horses till the age of three years and then breed indicating that equine owners breed their equines at an optimum age.

Eligible stallions of two to three villages which share common pastures are judged by a local selection committee compromising of elderly and experienced people of that area and the selected stallion is used for natural service of mares of the concerned villages. Generally, the stallion is selected on the basis of body confirmation, soundness, co-ordination of legs and against marking on the body. Young stallion of bluish grey colour without any markings is the most preferred which upon aging turns to white (Fig. 4). Height of the stallion is not considered an important criterion in stallion selection. As per the discussion with the equine owners, height at withers in Spiti horses has declined considerably which could possibly be due to inbreeding in the native tract and not considering height an important criterion for selection. People involved in committee constituted for stallion selection must include height as an important parameter of selection to avoid problem of reduction in height. The elite stallion is used for service only for one year and next year this stallion is also castrated and sold. Hence, they are not fully exploiting the superior stallion. The owners of brood mares are required to pay a nominal fee in cash or kind on account

of getting their brood-mares served by the selected stallion. The amount thus received is utilized by the owner for the upkeep of selected stallion. The mares freely roam with the stallion from April onwards and get covered through natural mating. Foaling generally occurs in the month of April and May and most of the mares conceive during the foal heat. Foals are reared till they become adult. The surplus male and female horses are taken to International Lavi fair at Rampur Bushar in Shimla District and Ladarcha fair at Kaza in Lahoul and Spiti District for sale every year and selling price ranged between Rs. 10,000 to 30,000 depending upon age, sex and physical condition of equids.

The traditional breeding practice followed in the Pin Valley include selection of the stallions for natural services and culling of the other eligible stallions by getting them castrated with the help of officials of the State Animal Husbandry Department. All the other stallions which could not be selected as stallion are castrated and sold. The castration of stallions is performed during the month of May at an age of three. Selling of surplus stallions after castration indicate that owners of Spiti horses are much aware and they don't want that Spiti germplasm should go outside the valley otherwise they will loose their monopoly. Spiti horses form an integral part of the society which is evident from the fact that before castrating any horse by the veterinarians, these people start reading Mantras from an ancient Buddhist chronicle of equines called Tar-dzung with the belief that reading these Mantras may cause less pain and post-operative suffering (http://ahdhp.nic.in/chamurthi.pdf retrieved on May 26, 2010). It was also observed that horse breeding is keeping pace through committee of elderly and experienced people involving all the stake holders and more or less on scientific lines like selection of young stallion for breeding, cost sharing of stallion, castration and sale of remaining adult males, removal of stallion from breeding on alternate years etc. Pal *et al.* (2007) cryopreserved the semen of true to breed Marwari stallions at farmers' door. It is a very good approach to conserve the true to breed stallions of any breed and the frozen semen could be used for AI in future. On similar lines, semen of true to breed Spiti stallions could be collected and cryopreserved. Also, stallions were being used for breeding only for one year in the home tract, this practice was not good. After using two years for service stallions should be shifted to other area for breeding to avoid inbreeding and exploitation of superior stallions.

At present the equine breeding services in the form of superior Spiti stallions and AI facilities at the field level are not available. Extension efforts need to be strengthened as to make equine owners aware about the merits of use of AI in mares and Animal Husbandry Department of State Government should also take initiative for AI in equines by getting their veterinarians trained through NRCE.

Colostrum provides passive immunity against various diseases during neonatal period. Most of the equine owners feed the colostrums to the foal within one hour of foaling. Average age of foal at weaning is 5 months (range 4-6 months). There is need to vaccinate the equines as prophylactic measures. But, none of the equine owners responded that they have got their equids vaccinated. While 10% are aware of prophylactic vaccinations but not get their equines vaccinated due to one or another constraints. The rest are not aware of disease requiring prophylactic treatment. It was also noted that deworming of equids was being done as curative measures in this region, whereas deworming of equines is a common practice among the equine owners of Haryana, Uttar Pradesh and Uttarakhand (Pal and Legha 2008). Importance of equine health is well understood by the equine owners, but the facilities they avail are mainly curative rather than preventive. The sick equines are taken to veterinary hospital for treatment. They were well satisfied with the services of veterinary hospital and the free supply of veterinary medicines they get. Equine are disposed to various diseases like tetanus, glanders, rabies, influenza, herpes etc. Most of the equine owners are ignorant about these diseases and their prophylactic control. Thus, there is need for public awareness of these diseases and their preventive measures. In general, as per most of the horse owners the problem of diseases is rare in Spiti ponies as they graze on Himalayas which is rich source of medicinal plants.

Although farmers of this valley at their own are maintaining and conserving true to breed Spiti horses to some extent. But, they expect some finance and technical support from the state/centre Govt. For breed conservation state Govt. of HP has already established a Spiti horse farm at Lari (Spiti) and ICAR also funded ad-hoc Network project on "Survey, characterization and conservation of Animal genetic Resources (Spiti horse)" for *in situ* breed conservation at farmers level. However, such efforts need to be sustained over a long period to achieve some tangible results.

Constraints: Most of the Spiti owners are small farmers. Lack of space for animal housing and non-availability of fodder particularly during winter months are the important problems in maintenance of horses. Increasing human population and family size have led to decline in space required for animal housing. Veterinarian are also not well trained for AI in equines, treatment, husbandry practices of equines as they were not exposed with equines during there basic degrees. Poverty and illiteracy is another factor, that they can't rear equines with scientific know how.

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URTICARIAL FORM OF SWINE ERYSIPELAS: A CASE REPORT

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Introduction

Swine erysipelas (SE) is worldwide in distribution caused by infection with *Erysipelothrix rhusiopathiae,* an important pathogen of swine. It occurs in most areas where domestic swine are reared. (Wood *et al*., 1999). The organism is believed to be transmitted directly via oronasal and faecal secretions or indirectly via environmental contamination. Pigs may be infected by ingestion of contaminated feed or water or contamination of skin wounds (Amass and Scholz, 1998). In swine, disease may be either acute or chronic, resulting in the development of urticaria, arthritis and endocarditis (Takahashi *et al.,* 1984). These forms may occur separately, in a sequence or together.

Case history and observation

A two-year-old sow, weighing about 70 kg, was brought to veterinary clinics of the university with a history of weakness, improper weight gain, reduced feed intake, skin discoloration and animal segregated from herd.

Clinical examination revealed prostration, stiff walk on toes, reluctancy to move and shifting of weight from one foot to another foot while standing. Erythematous and raised, polygonal dark red to purple lesions, on the skin of the snout, ear extremity, dorsum and belly region and fever (106°F), blood examination revealed (TEC: 5.5 x 10^6 per mm^3, Hb: 7.2g/dl, TLC: 11 x 10^3 mm^3, neutrophil: 43%, lymphocyte: 42%, monocyte: 1% and eosinophil: 14%). Based on history and pathognomic lesions and blood picture the diagnosis was arrived as swine erysipelas.

Therapeutic management

The sow was treated with a combination of procaine penicillin and dihydrostreptomycin at the dosage rate of 20,000 IU/kg b.wt. intramuscularly bid (Wabacha *et al.,* 1998; Brooke and Riley 1999), chlorpheniramin maleate @ 0.5 mg/kg i/m, bid, Meloxicam @ 0.5 mg/kg i/m, bid, for 10 days. The sow was monitored every day. The farmer was advised to place the animal under isolation to prevent spread of the disease. The condition of sow started improving after second day of treatment when temperature returned to normal. The skin lesions resolved and complete recovery was observed after day 10 of initiation of therapy.

Conclusion

Erysipelas is enzootic and is of considerable economic significance as infected pigs are rendered useless. *Erysipelothrix rhusiopathiae* is pathogenic for both animals and humans, causing erysipelas in swine and erysipeloid in human beings characterized by painful erythematous swelling at the site of entry. Treatment by oral and intramuscular penicillin is effective, however, containment and control procedures are far more effective ways to reduce infection in swines Therefore, it is critical to have an early diagnosis of *E. rhusiopathiae* infection.

Acknowledgements

Authors are thankful to Dean, College of Veterinary and Animal Sciences, Pantnagar for providing necessary facility.

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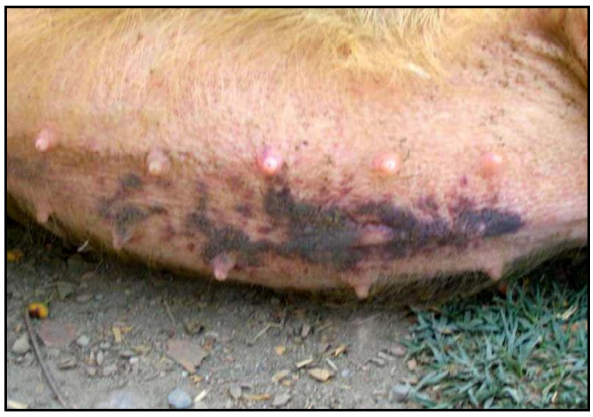

Fig. 1: Showing erythematous and raised, dark red to purple lesions, on the skin of the dorsum and belly region

Fig. 2: Showing lesions, on the skin of the snout, ear extremity.

NUTRITIVE EVALUATION OF THREE AQUATIC ANGIOSPERM PLANTS GROWING AS WEED IN THE BIKANER REGION OF THE INDIRA GANDHI CANAL

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ABSTRACT

In an attempt to find out the possibilities of utilization of aquatic angiosperms growing as weed in Indira Gandhi canal, plant sample of *C. demersum, E. crassipus and H. verticullata* were collected in lush green condition and subjected to determination of proximate principles, fibre fraction and carbohydrates contents present in them. The crude protein content were recorded to be 17.85% in *C. demersum* 13.67% in *E. crassipus* and 21.00% in *H .verticullata,* whereas, the total sugar and starch contents were recorded to be 132 and 9.8 mg/ g.d.w in *C. demersum* 25 and 7.34 mg/g.dw in *E . crassipus* and 165 and 5.25 mg/ g.d.w in *H .verticullata* . The data obtained indicated that aquatic plants growing in Indira Gandhi canal, Bikaner had sufficient amount of nutrient contents for their use as animal feed.

Key words: Aquatic angiosperm, weed

Introduction

One of the major limiting factors to animal production in India is nutrition. The acute shortage of feeds and fodders has stimulated a lot of research aimed at utilizing non-conventional feeds in animal ration. A number of reasons, including human population pressure on the land, scarcity of high cost concentrate feed and economic need to match livestock production system with available resources, justify the increased use of non-conventional feed resources for animal feeding. Feed costs, the single largest expense in animal production, may be reduced by using aquatic plants as animal feed. Numerous aquatic plants extensively grow throughout the year in India. Among them *C. demersum, E. crassipus* and *H. verticillata* commonly occur in ponds, canals and other water resources. But their usage in animal feed is limited due to poor understanding of their nutritional and economic value, as well as their proper use in animal ration. In fact, little is known about the nutritive value of this type of aquatic biomass for livestock due to paucity of the knowledge of their phytochemicals. To suggest possible method of using aquatic plants in animal feeding nutritional potentialities of these plants should be studied. Therefore, in the present study nutritive contents of *C. demersum, E. crassipus* and *H. verticillata* aquatic plants have been reported.

Materials and Methods

Aquatic plants *C. demersum, E. crassipus* and *H. verticillata* were collected from the natural habitat located, after survey work, along the IGNP canal at Birdhwal head. All the three plant samples were collected randomly in a lush green condition. Immediately after collection, the plants were separated and washed in tap water to make the plants free of soil, if any. The plants were dried at 100°C ± 5^{0}C.for 15 min. to inactivate enzymes and then at 60^{0}C for 18-24 hrs., till a constant weight was achieved. The dried plant material from each species were powdered using 20 mesh screen in Willy mill and then subjected to chemical analysis following the standard procedure of A.O.A.C. (1995).The method of Talpatra *et al.* (1940) was adopted for detecting calcium and phosphorus. Method of Georing and Vansoest (1970) was adopted for various fibre fractions.

Results and Discussion

Concentration of the proximate components in the three aquatic plant species are presented in Table 1. Crude protein concentration on dry matter basis was found to be higher in *H. Verticillata* (21%)when compared with those of *E. crassipus* (13.67%) and *C. demersum* (17.85%).The protein content was found to be considerably higher in all the above mentioned aquatic plant species when compared with those of crude protein content reported in various plant species of arid zone (twigs of *Corchous antichorchous* 7.76% (Mathur and Karwasra, 1967) above ground parts of *Panicum turgidum,* 5.12% (Purohit and Mathur, 1970) *Lasiurus sindicus* , 4.80% (Purohit *et al.,*1976) *Indigofera cordifolia*, 6.25% (Mathur and Purohit,1979) and different plant parts as roots, stems, leaves and seeds of *Tribulus alatus,* 2.19%, 4.69%,7.80%, 8.44% and *Tribulus terrestris* 3.44%, 3.13%, 6.25%,5.63% (Nag *et al.,*1979).

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Crude fibre concentration was also found to be more or less same in all three aquatic plants species. Amount of crude fat (ether extract) content did not show considerable variation in the plant species studied. Maximum ether extract fraction was found in *H. verticillata* (3.52%) and minimum in *E. crassipus* (2.93%).

Ash value showed considerable variation among the plant species investigated and the values include only endogenous plant minerals and do not reflect the amount of soil. Concentration of ash was found to be higher in *H. verticillata* (27.16%).

Nitrogen free extract was higher in the *E. crassipus* (47.91%).This was attributed in the low concentration of CP, CF, EE and ash of the plant studied. Mineral contents were approximately similar to those of land forages (Mathur and Karwasra, 1967; Purohit and Mathur.1970; Nag *et al.,* 1979; Harsh *et al.,* 1981; Grover and Nag, 1984; Kapoor *et al.,* 1988; Mathur *et al.,* 1988).However, the mineral contents were found to be higher in the aquatic plants studied by Khan *et al.* (2002).

Various fibre fractions found to be complementary to gross nutrients in quality assessment of vegetation as feed, the ADF, NDF and hemi-cellulose (HC) contents in *C.demersum* were found to be 39.62%, 60.86% and 21.24%, respectively, whereas in *E. crassipus and H. verticillata* these were found to be as 29.62%, 70.0%, and 40.38% and 39.02%, 54.16%, and 15.14% respectively. The HC and NDF content were found to be maximum in *E. crassipus* and minimum in *H. verticillata* while ADF contents were nearly same in *C. demersum* and *H. verticillata* and comparatively low in *E.crassipus* contrary to the trend of fibre fractions in aquatic plants recorded in present study. Shirley *et al*. (1973) studied the nutritive contents of some aquatic plants and reported that *Ceratophyllum, Eichhornia, Hydrilla, Potamogeton and Vallisneria* liberally supplied with many nutrients required by the livestock.

The data thus indicate that aquatic plants growing in Indira Gandhi canal, Bikaner had sufficient amount of nutritive contents which may be useful forage for the cattle. From the results, it could also be concluded that *E. crassipus* which is considered as aquatic weed could be utilized by cattle and can replace the other roughages to make the feeding of cattle economical. The reducing sugar was found to be maximum in *C. demersum* (32.5 mg/g.d.w.) and in a decreasing order in *H. verticillata* (20 mg/g.d.w.) and was lowest in *E. crassipus* (12.5 mg/g.d.w.) (Table 2).

The non reducing sugar showed a higher trend and amounted to be 145 mg/g.d.w. in *H.verticillata* and 100 mg/g.d.w. in *C.demersum* and very low in 12.5 mg/g.d.w.in *E. Crassipus.* The total sugar was higher in *H. verticillata* (165 mg/g.d.w.), followed by *C. demersum* (132 mg/g.d.w.) and lowest in *E. crassipus* (25 mg/g.d.w.) whereas, on the other hand the starch was found to be higher in *C. demersum* (9.8 mg/g.d.w.) than *H. verticillata* (7.34 mg/ g.d.w.) and lowest in *E. crassipus* (5.57 mg/g.d.w.). The present study reveals that not only terrestrial plants possesses sufficient amount of carbohydrate but aquatic plants also contain more or less equal amount of carbohydrate enabling them to be used for livestock feed.

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Table 1: Nutritive content of *Ceratophyllum demersum*, *Eichhornia crassipus* and *Hydrilla verticillata*

Plant	DM	OM	CP	EE	CF	NFE	TA	Ca	P	NDF	ADF	HC	ISA	ASA	Nitro-gen
C.demersum	4.40	73.09	17.85	3.29	18.32	33.63	26.91	1.46	0.18	60.86	39.62	21.24	9.80	17.11	2.85
E.*crassipus*	10.79	82.69	13.67	2.93	18.18	47.91	17.31	2.49	0.49	70.00	29.62	40.38	3.96	13.35	2.19
H.verticillata	6.05	72.84	21.00	3.52	20.29	28.03	27.16	3.32	0.22	54.16	39.02	15.14	12.52	14.64	3.36

Values expressed in percentage on dry matter basis

Table 2: Carbohydrate content (mg/g.d.w.) of *Ceratophyllum demersum*, *Eichhornia crassipus* and *Hydrilla verticillata*

Plant	Carbohydrate			
	Reducing Sugar	Non Reducing Sugar	Total Sugar	Starch
C.demersum	32.5	100.0	132.5	9.8
E.crassipus	12.5	12.5	25.0	7.34
H.verticillata	20.0	145.0	165.0	5.57

EFFECT OF BREED, PERIOD AND SOMATIC CELL COUNT CATEGORY ON SCC AND CMT STATUS IN A HERD

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ABSTRACT

The study here is undertaken to find out the effect of breed, period and somatic cell category on Somatic Cell Count and (SCC) California Mastitis Test (CMT) status in a herd. For this purpose total 325 animals of different category were selected and the status of subclinical mastitis was judged by performing CMT and SCC in individual animals. The data of infected animals were classified as per the breed, period, quarter affected and all these parameters taken into consideration as factors influencing occurrence of sub-clinical mastitis. The results revealed significant effect of breed and SCC class both on CMT status and SCC, however, period variation was not significant. That coefficient of correlation between CMT score and SCC for the individual animals across the breed category was found to be 0.73, 0.86, 0.91 and 0.34 in Karanswiss, Karanfriese, Sahiwal and Murrah, respectively.

Key words: SCC, CMT, mastitis, cattle, buffalo

Introduction

The major concern for dairy farmers in India and across the world is mastitis. It leads to reduced milk yield and an increased number of clinical treatments. Milk yield decreases with the increase in somatic cell count causing losses to buffalo milk producers (Ceron-Munoz *et al.,* 2002). One of the keys to prevent mastitis is to prevent the occurrence of the disease in a herd as a unit. In this endeavour Somatic Cell Count (SCC) and California Mastitis Test (CMT) based study can play a major role as mastitis itself can be characterized by an increase in somatic cell count and CMT results. The mean SCC of California mastitis test (CMT) positive quarter was significantly higher (P<0.01) than CMT negative quarters (Dhakal, 2006). Elevation of somatic cell count (SCC) is a clear indication of infection in the udder (Koivula *et al.,* 2005). Hence an attempt was made here to find out the relationship between SCC and CMT and how they are affected with factors like breed, period and SCC class.

Materials and Methods

The study involves 33 crossbreds of Karanswiss (KS), 150 crossbreds of Karanfries (KF), 89 Murrah (MU) Buffalo and 53 Sahiwal (SW) cattle. Animals were milked both by machine and hand milking three times a day i.e. in morning (5:00 AM to 7:00 AM), noon (12:00 to 2:00 PM) and evening (6:00 PM to 8:00 PM). Individual milk sample from all the four quarters were collected separately. For this purpose the quarters were termed as Left Fore (LF), Left Hind (LH), Right Fore (RF) and Right Hind (RH). About 20-30 ml of milk was collected aseptically in sterilized glass test tube after discarding the first 2-3 streaks of fore milk. The samples were brought to the laboratory immediately after collection and placed in refrigerator till use. For somatic cell count the slides were prepared within one hour of collection of milk samples. Modified California Mastitis Test was performed in the milking byre itself, by using a milk paddle.

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The total number of somatic cells (mainly neutrophils but also other leucocytes) secreted per ml. of milk were estimated using Prescott and Breed (1910) method which has been approved by B.I.S. (1981). A leucocyte count of more than 4,00,000 cells per ml of milk was considered as positive indication of subclinical mastitis as recommended by Narayanan and Iya (1953). For analysis of mastitis incidence the California Mastitis Test (CMT) developed by Schalm and Noorlander (1957) was modified according to Sastry (1978) in the present study.

For statistical analysis the test day milk yield of an animal has been considered to be the net product of several factors among which breed, replication(month), stage of lactation, parity and the somatic cell counts (as an index of mastitis incidence) provided the other variables including environmental/nutritional are well controlled. With this assumption the following least square model was used for analysis of variance in this study.

Y_{ijklmn}	=	$\mu+B_i+R_j+S_k+P_l+C_M+e_{ijklmn}$
Y_{ijkln}	=	test day milk yield of n_{th} animal in, m_{th} SCC class in l_{th} parity, k_{th} stage of lactation, j_{th} replication of i_{th} breed.
μ	=	population mean.
B_i	=	Effect of i_{th} breed where i = 1,2,3,5
P_j	=	Effect of j_{th} replication where j = 1,2
S_k	=	Effect of k_{th} stage of lactation where k = 1…3.
Ml	=	Effect of L_{th} parity where l= 1...3.
N_m	=	Effect of m_{th} class of somatic cell counts where m = 1…4.
e_{ijkln}	=	Random error N.I.D. (0, $\sigma^2 e$)
N.I.D.	=	Normally and independently distributed between 0 mean and σ^2 Variance

Duncan's Multiple Range test was used for testing the differences among Least-Squares means (using the inverse coefficient matrix). The difference was considered significant, if

$$y_i - y_j \quad \text{----------> } e.Z_p.n2 \sqrt{\frac{2}{C_{ii} + C_{jj} - 2C_{ij}}}$$

Where,

$y_i - y_j$	=	Difference between two constants.
C_{ii}	=	Corresponding diagonal element of ith sub class.
C_{jj}	=	Corresponding diagonal element of jth subclass.
C_{ij}	=	Corresponding element of ij th sub class.
Z_p n2	=	Significant ($p< 0.05$ and $p< 0.01$) standardized range value in Duncan's Table at p and n2 degrees of freedom.
P	=	Number of means in the range chosen, and
N2	=	Degree of freedom for error.

Results and Discussion

The results pertaining to breed period and quarter wise incidence of subclinical mastitis based on California Mastitis Test (CMT) results are presented in Table 1. Similarly incidence of subclinical mastitis based on SCC categories (<4 lakh, 4-10 lakh, 10-20 lakh and >20 lakh) was worked out twice in all the animals a month apart (Table 2). There was a close agreement between incidence based on CMT score and SSC. Whereas, there were appreciable breed differences for the incidence of subclinical mastitis, there were little variations between the two periods, which were a month apart. The overall incidence of subclinical mastitis varied between 31.17 and 35.33 % in KF for two periods. The corresponding figure in case of KS has been 34.85 and 37.88, respectively. Period wise overall incidence was 36.32% and 33.02% in SW cow for two periods. Incidence in case of Murrah was 9.27% for the period one and 11.24% for the period two. These results point towards a relatively much higher quarter infection rate in crossbred animals and Sahiwal as compared to buffalo, having very low incidence of sub clinical mastitis.

The overall means of somatic cell count in KS across the four somatic cell class averaged 136046.98 ± 26095.88 cells/ml for less than 4 lakh cells/ml category and 708881.10 ± 383123.31 in 4 to 10 lakh cells/ml category and 1573879.90 ± 53082.07 in 10 to 20 lakh cells/ml category and 4862905.00 ± 1266619.87 in more than 20 lakh cells/ml category (Table 3). In case of KF average somatic cell counts across the different four category of somatic cell count were found to be 137219.29 ± 12847.84, 528052.43 ± 157895.70, 1417704.62 ±

Table 1: Breed, period and quarter wise incidence of sub clinical mastitis based on CMT score

Breed	Period	Quarter	Mastitis incidence based on CMT class wise distribution				Overall incidence
			Healthy	Mild	Moderate	Severe	
KF	1	RF	97	12	24	17	
		RH	110	14	15	11	
		LF	105	17	15	13	
		LH	101	20	15	14	
		Total	413	63	69	55	31.17
		% Incidence	68.83	10.50	11.50	9.17	
	2	RF	110	11	10	19	
		RH	85	10	32	23	
		LF	105	12	20	13	
		LH	100	16	20	14	
		Total	400	49	82	69	33.33
		% Incidence	66.7	8.17	13.67	11.50	
KS	1	RF	23	1	9	0	
		RH	22	1	7	3	
		LF	25	2	4	2	
		LH	12	3	7	11	
		Total	82	7	27	16	37.88
		% Incidence	62.12	5.30	20.45	12.12	
	2	RF	21	2	10	0	
		RH	30	1	1	1	
		LF	23	0	8	2	
		LH	11	0	9	13	
		Total	85	3	28	16	35.61
		% Incidence	64.39	2.27	21.21	12.12	
SW	1	RF	31	5	11	6	
		RH	23	6	10	14	
		LF	36	3	8	6	
		LH	45	2	4	2	
		Total	135	16	33	28	36.32
		% Incidence	63.68	7.55	15.57	13.21	
	2	RF	26	6	14	7	
		RH	37	5	3	8	
		LF	39	2	5	7	
		LH	36	3	6	8	
		Total	138	16	28	30	34.91
		% Incidence	65.09	7.55	13.21	14.15	
MU	1	RF	77	5	4	3	
		RH	78	3	4	4	
		LF	76	4	5	4	
		LH	76	4	3	6	
		Total	307	16	16	17	13.76
		% Incidence	86.24	4.49	4.49	4.78	
	2	RF	78	3	3	5	
		RH	77	3	5	4	
		LF	77	4	4	4	
		LH	74	4	3	8	
		Total	306	14	15	21	14.04
		% Incidence	85.96	3.93	4.21	5.90	

Table 2: Breed, period and quarter wise incidence of subclinical mastitis based on SCC class

Cattle							
Breed	Period	Quarter	Somatic cell count (Lakh cells/ml) based on SCC class wise distribution				Overall incidence
			<4	**4-10**	**10-20**	**> 20**	
KF	1	RF	94	22	16	18	
		RH	96	12	22	20	
		LF	105	12	19	14	
		LH	93	19	13	25	
		Total	388	65	70	77	
		% Incidence	64.67	10.83	11.67	12.83	35.33
	2	RF	91	21	12	26	
		RH	96	20	22	12	
		LF	112	10	13	15	
		LH	98	15	11	26	
		Total	397	66	58	79	
		% Incidence	66.17	11.00	9.67	13.17	33.83
KS	1	RF	25	4	3	1	
		RH	21	2	6	5	
		LF	24	1	4	4	
		LH	16	2	5	9	
		Total	86	9	18	19	
		% Incidence	65.15	6.82	13.64	14.39	34.85
	2	RF	21	2	8	2	
		RH	22	2	5	4	
		LF	28	1	2	2	
		LH	13	4	6	10	
		Total	84	9	21	18	
		% Incidence	63.64	6.82	15.91	13.64	36.36
SW	1	RF	38	3	7	5	
		RH	28	5	9	11	
		LF	39	3	5	6	
		LH	35	4	6	8	
		Total	140	15	27	30	
		% Incidence	66.04	7.08	12.74	14.15	33.96
	2	RF	32	4	10	7	
		RH	40	5	4	4	
		LF	41	5	3	4	
		LH	29	7	8	9	
		Total	142	21	25	24	
		% Incidence	66.98	9.91	11.79	11.32	33.02

Buffalo						
Breed	Period	Quarter	Somatic cell count (Lakh cells/ml) based on SCC class wise distribution			Overall incidence
			<4	**4-10**	**>10**	
MU		RF	80	2	7	
		RH	80	2	6	
		LF	82	4	3	
		LH	81	5	3	
		Total	323	14	19	
		% Incidence	90.73	3.93	5.34	9.27
		RF	79	4	6	
		RH	80	5	4	
		LF	79	6	4	
		LH	78	2	9	
		Total	316	17	23	
		% Incidence	88.76	4.77	6.46	11.24

Table 3: Breed, period, quarter and SCC class wise means of somatic cell counts

Karan Swiss					
Period	Quarter	SCC class wise means of somatic cell counts			
		I<4 Lakh cells/ml	II (4-10 Lakh cells/ml)	III (10-20 Lakh cells/ml)	IV (> 20 Lakh cells/ml)
1	RF	108242.69±3334070	869433.31±771665.62	1196489.12±572273	4726357±204692
	RH	106746.26±3308.77	841499.75±750715.00	1445563.87±353955.62	4020443.50±1685641.50
	LF	117720.17±4736.21	643159.70±31425.50	1230778.87±224348.45	4073313.25±1017526.87
	LH	160119.39±50168.56	879908.44±71920.12	1873879.10±8291214	11446290.00±1825392.50
2	RF	159006.64±50890.03	512121.87±598244.62	1196489.15±572473.19	3906455.18±234406.28
	RH	106362.55±3308.77	535012.40±1988.70	1445563.17±353955.62	10333443.15±77273.93
	LF	117643.24±5115.04	622675.62±55472.63	1270978.87±242347.45	2373762.62±861895
	LH	110122.85±4483.68	8390434.62±531207.36	1873879±83430.14	2518219.5±1220015
Overall		136046.98±26095.88	708881.10±383123.31	1573879.90±53082.07	4862905.00±1266619.87
Karan Fries					
Period	Quarter	SCC class wise means of somatic cell counts			
		I<4 Lakh cells/ml	II (4-10 Lakh cells/ml)	III (10-20 Lakh cells/ml)	IV (> 20 Lakh cells/ml)
1	RF	127515.46±1658.65	715216.56±228488.7	135879.81±334936.44	4500500.9±712330.21
	RH	117695±1664.02	404634.61±307764.31	1141138.37±320314.53	6872620±788095.62
	LF	125360.55±15429.43	745678.31±180497.98	1580383.57±325954.84	2386572.00±648337.37
	LH	156871.07±23812.21	424823.53±203544	1473497.37±340535.71	5331243.50±792909.81
2	RF	110764.47±1784.80	650453.53±237005.56	1333115.62±326581.39	4411317±671026.37
	RH	118717.87±1781.50	488151.12±302151.37	1683697.62±343754.76	6619715±668537.68
	LF	126084.16±17589.18	566865.65±151393.26	1309387.5±362993.90	3421307.75±605187.81
	LH	114838.14±1315.06	616534.43±240175.50	1761911±360391.34	4979849.00±728267.62
Overall		137219.29±12847.84	528052.43±157895.70	1417704.62±243559.73	5150222.50±534702.50
Sahiwal					
Period	Quarter	SCC class wise means of somatic cell counts			
		I<4 Lakh cells/ml	II (4-10 Lakh cells/ml)	III (10-20 Lakh cells/ml)	IV (> 20 Lakh cells/ml)
1	RF	17457.27±287140.56	659577.14±585531	1232736±2605667	2966795±174586.00
	RH	328219.81±11550	921223.06±216423.23	1732428±281867.56	7117115±174585.26
	LF	294535±214561	601646±831112	1191642±217798	2269670±174584
	LH	312233.93±12867	676852±53277	1325183±212097	2967945±523755
2	RF	209302±24760	695058±206553	1244597±229150	2967945±174586.6
	RH	318360±18911	733459±319937	1831646±38344.12	3316711±174585
	LF	301217±16211	527634±24031	1014403±1778860	2269603±174584
	LH	351497±14441	810850±330267	1303568±182350	2755945±52755
Overall		291352.31±9398.77	731669.87±188583.4	1313084.87±136753.9	2967945.00±302390.87
Murrah					
Period	Quarter	SCC class wise means of somatic cell counts			
		I<4 Lakh cells/ml	II (4-10 Lakh cells/ml)	III (>10 Lakh cells/ml)	
1	RF	113107±12778	555354±162025	13740022±136201	
	RH	116303.44±10588	469929±62558	1513402±1566792	
	LF	13426±15368	708779±114062	5880037±217589	
	LH	141041±2343	775135±42381	13874337±15739	
2	RF	137262.94±17543	264109±85219	2329143±119922	
	RH	117741±10249	72274±20601	1442965±171562	
	LF	123402.48±10534	405037±22884.2	3180221±1872704	
	LH	148414.00±1678.38	378489±517147	1324956±1865089	
Overall		114680.9±15456.3	548480.4±34523.3	1838732.11±145323	

Table 4: Effect of breed, replication and SCC class on somatic cell counts and CMT status in a herd

Source of variance	Degree of freedom	Mean square of CMT status	Mean square of somatic cell class
Breed	3	5.4471**	1260264900000*
Period	2	0.0407	224957500000
SCC Class	3	332.77**	2051498500000**
Error	2280	0.7123	7486537500000

*Significant value
**Highly significant value

243559.73 and 5150222.50 ± 534702.50 in the category of less than 4 lakh, 4 to 10 lakh, 10 to 20 lakh and more than 20 lakh cells/ml, respectively (Table 3). The somatic cell count across different somatic cell count category in case of Sahiwal was found to be 291352.31 ± 9398.77, 731669.87 ± 188583.40, 1313084.87 ± 136753.93 and 2967945.00 ± 302390.87 in the category of less than 4 lakh, 4 to 10 lakh, 10 to 20 lakh and more than 20 lakh cells/ml, respectively (Table 3). But in case of buffalo due to less number of somatic cell found in milk it was categorized in three categories only i.e. 0 to 2 lakh, 2 to 10 and more than 10 lakh. The result regarding somatic cell counts across the category were 114680.9 ± 15456.3, 548480.4 ± 34523.3 and 1838732.11 ± 145323 less than 2 lakh, 2 to 10 lakh and more than 10 lakh cell/ml of milk category, respectively (Table 3).

The results of present study regarding somatic cell counts in crossbred revealed that there is marked variation in average somatic cell counts across the four categories of animals. The results of study agrees well with the results of various other research workers namely Narayanan and Iya 1953; Pape *et al*. (1963); Sudesh and Baxi (1975); Pednekar *et al.* (1992); Tuteja *et al.* (1993) and Samanta (1997). They have all reported that the mean count of animals effected from subclinical mastitis was more than 5,00,000 cells/ml of milk. Mukherjee and Dash (2003) have also reported in Haryana x Friesian that subclinical mastitis infected animals with Modified California Mastitis Test of 2 ranged from 7.77 to 8.79 x 10^5 cell/ml. The study conducted by Samanta (1997) on crossbreed also revealed such higher somatic cell counts in moderate to severally effected cow and data regarding normal quarters also matched his results. The results of the somatic cell counts on Sahiwal and Murrah also agrees with studies undertaken in previous years viz. Narayan and Iya 1953; Pape *et al.* (1963); Sudesh and Baxi (1975); Pednekar *et al.* (1992); Jha *et al.* (1993), Tuteja *et al.* (1993).The present study confirms that there is close relationship between Modified California Mastitis Test and SCC test; CMT test proves to be very good indicator of subclinical mastitis in a herd. The high correlation coefficient between SCC and CMT reveals that CMT can prove to be a good indicator of herd udder health status and it can be used as an excellent cow side test. Similar observations have been reported by Pednekar *et al.* (1992) who found that efficiency of detecting sub clinical mastitis with CMT as around 70-80 per cent.

Analysis of variance was applied to find out the effect of breed, period and SCC category on somatic cell count and CMT status in the herd. The mean squares of the ANOVA are presented in table 4. The result of ANOVA reveals significant effect of breed and SCC class both on CMT status and SCC. The period variation was not significant. The coefficient of correlation between replicate values of CMT score and somatic cell counts for the individual animals across the breed category was found to be very high (r=±0.9). In case of KS, KF, SW and MU it was 0.73, 0.86, 0.91 and 0.34, respectively.

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THERAPEUTIC EVALUATION OF IVERMECTIN AGAINST ENDOPARASITES OF DONKEY

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ABSTRACT

Therapeutic efficacy of ivermectin was evaluated in indigenous donkeys (14) purchased from farmer's field and reared on stall fed. Donkeys were naturally infected with helminth parasites *Strongyles, Strongyloides*, *Parascaris* and *Anoplocephala*. Ivermectin was given @ 0.2 mg/kg per orally. Mean EPG during control period on Day '0' (before the administration of ivermectin) was 1461±176, with range of 600 to 3150, which depicted very high level of infection. EPG was reduced to 39±16 on day 21 after treatment. Presence of helminth eggs on day 21st were mainly due to *Anoplocephala* which is not susceptible to ivermectin. Study illustrated efficacy of ivermectin against *Parascaris, Strongyloides, Strongyles* and *Anoplocephala* 100%,100%, 99.42% and no efficacy respectively. So it is suggested that ivermectin should be used in combination with any effective dewormer for tapeworms for effective deworming programme.

***Key words*:** Parasites, strongyles, anoplocephala, ivermectin, donkeys.

Introduction

Donkeys as a means of transport for men and material provide livelihood to a number of rural and semi-urban population of India. It is suggested that donkeys can play a great role in the frame works of food security and social equity of high food insecure countries. In areas away from roads, many people use donkeys to transport food and other supplies to villages. Even though donkeys have often been described as sturdy animals, they succumb to a variety of diseases and a number of other conditions. (Swedson, 1997).They suffers from a number of diseases. Parasitic infestation is a major cause of illness. (Shrikhande *et al*., 2009). Documentation of parasitic infestation of donkeys in India is lacking.

Materials and Methods

Fourteen adult male donkeys apparently healthy which were brought from Baran and Jhalawad district of Rajasthan were included in this study. Faecal samples were collected from the rectum of the animals on day '0' and the donkeys were treated with Ivermectin @ 0.2 mg/kg of b.wt., orally. Parasite eggs were differentiated with Stoll's method and EPG (Egg per gram) done with modified Mc Master Egg Counting Technique. Faecal samples were collected and examined again on 7th, 15th and 21st day of ivermectin administration to see the efficacy of drug.

Results and Discussion

Average rainfall in Jhalawar and Baran district of Rajasthan is about 150-200 cm annually and plenty of green grasses are available in this area. All the donkeys were reared absolutely on grazing before treatment and during the treatment all the animals were maintained on stall fed. Study depicted the prevalence of *Strongyles, Strongyloids westeri, Parascaris equorum*, and *Anoplocephala* in South eastern part of Rajasthan in donkey population. Mean EPG during control period on Day '0' (before the administration of ivermectin) was 1461±176, with range of 600 to 3150, which depicted very high level of infection. This EPG was reduced to 39±16 on day 21 after treatment. Presence of helminth eggs on day 21st was mainly due to *Anoplocephala* which is not susceptible to ivermectin.

Strongyles Species

The most common nematodes found in the large intestine of horses are *Strongyles vulgaris*, *S. edentus* and *S. equines*. Godara *et al*. (2009) and Ayele *et al*. (2006), depicted presence of Strongyles in all the

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Table 1: Range of egg per gram (Mean ± SE) of different parasitic species in donkey

Parasites	Day 0	Day 7	Day 15	Day 21
	EPG (Mean ±SE)	EPG (Mean ±SE)	EPG (Mean±SE)	EPG (Mean ±SE)
Strongyles (n=14)	1242.85± 177	0±0	3.57±3.64	7.14±7.15
Strongyloides westeri (n=2)	75±25	0±0	0±0	0±0
Parascaris equorum (n=5)	330±168	0±0	0±0	0±0
Anoplocephala (n=7)	71.42±34.26	35.71± 23.69	85.71± 41.85	85.71±14.29
Overall EPG	1460.714±176	17.85±12.4	42.85±23.5	39.28±15.88

samples collected from India and Ethiopia respectively. *Strongylus vulgaris* is most important parasites of horses and causes verminus arteritis and colic. Large Strongyles causes anaemia by direct blood sucking and by blood loss from feeding points once the worm detaches (Radostits *et al.,* 1996).

In the beginning (before treatment) of experiment all the animals were positive for *Strongyles* with mean of 1246±177 and range of 450 to 2850. On 7th day all 14 animals were found negative while on day 15th and 21st one animal was positive for *Strongyles*, all the animals were reared stall fed so there was no chance of reinfection. The study depicted efficacy of ivermectin 99.42% against *Strongyles* species based on mean EPG of day 0 and day 21st.

Parascaris equorm

Radostits *et al.* (1996) reported that foals are more seriously affected by *Parascaris* causes diarrhoea, poor coat and occasional colic. In addition convulsions, intestinal obstructions and perforations may occur. On day '0' five animals were positive for *Parascaris* eggs with mean of 330±168 (range of 100 to 1000). After ivermectin administration no *Parascaris* eggs were seen during entire study period. It depicted efficacy of ivermectin is 100% against *Parascaris* in donkeys.

Strongyloides westeri

Two donkeys were positive for *Strongyloides westeri* with mean of 75±25 (range of 50 to 100) during control period. After ivermectin administration no *Strongyloides* egg was seen during entire study period It depicted efficacy of ivermectin is 100% against *Strongyloides* in donkeys.

Anoplocephala

This parasite is prevalent world-wide and has been shown to be a significant cause of equine colic (Matthews *et al*., 2004). For many years, the equine tapeworm *Anoplocephala perfoliata* was thought to be relatively harmless (Soulsby *et al.,* 1968). While, there are many clinical case series and case reports (Barclay *et al.,* 1982, Beroza *et al*.,1983; and 1986; Coagrove *et al.,* 1986; Owen *et al.,* 1989) made association between the presence of tapeworms and certain types of colic arising from ileo-caecal junction. On day 0, 7th, 15th, and 21st mean value of *Anoplocephala* EPG were 71.42±34.26, 35.71±23.69, 85.71±41.85 and 85.71±14.29 respectively. No certain trend of Anoplocephala positive or negative was seen during the study period. This finding depicts that coprological methods for *Anoplocephala* diagnosis have lower sensitivity, it supports the findings of Proudmen and Edwards, 1992 and Meana *et al*.,1998 who reported that sensitivities of coprological examination is 11–61% for tapeworm *Anoplocephala perfoliata*. In the study no significant effect of ivermectin was observed on tapeworms.

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STUDIES ON INCIDENCE AND TRANSMISSION OF AMPHISTOMIASIS IN DOMESTIC AND WILD RUMINANTS OF UDAIPUR REGION

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ABSTRACT

A coprological survey to access the incidence of amphistomiasis in ruminants was carried out. 150 faecal samples comprising of 50 buffaloes, 30 cattle, 25 goat, 25 sheep and 20 *Neelgai* were collected and examined which revealed high overall infection (80%) of amphistomiasis. The maximum incidence was reported in buffaloes whereas it was minimum in sheep. The study revealed the sharing of grasslands and transmission of infection between wild and domestic animals, making its control difficult and spectrum wider. Amphistomes mostly are dominated by other trematodal infections like Fasciola and Schistosomes, so the economic losses and public health issues due to this parasite have still to gain some serious attention.

Key words: Amphistomiasis, buffalo, *Neelgai*, trematodal infections, Udaipur

Introduction

Amphistomiasis has been a neglected trematode infectious disease in ruminants, but has recently emerged as an important cause of productivity loss. Economic losses caused by amphistomes infection have not been estimated, but may be greater than those caused by many other parasites (Hanna *et al*., 1988). Adult amphistomes are the main parasites in the rumen and reticulum of water buffaloes, goats, cattle and sheep. Light infection dose not cause serious damage to the animals, but massive number of immature amphistomes can migrate through the intestinal tract causing acute parasitic gastroenteritis with high morbidity and mortality rates, particularly in young animals (Hanna *et al*., 1988). Mature amphistomes are responsible for ruminitis, irregular rumination, unthriftiness, loss of body condition, decrease in milk production and reduction of fertility (Zinsstag *et al*., 1997). The aim of the present study was not only to show the alarming presence of amphistomes in the region but to also to discuss the economic and zoonotic importance of the amphistomes.

Materials and Methods

A total of 150 faecal samples (50 buffaloes, 30 cattle, 25 goat, 25 sheep and 20 *Neelgai*) were randomly collected directly from the rectum or freshly passed by the animals. The samples were brought to the laboratory in labelled polythene bags and examined for the presence/absence of the amphistome eggs by the standard sedimentation techniques. (Soulsby, 1982)

Results and Discussion

The results (Table 1) of the study revealed that 120 out of 150 samples with an overall rate of 80% were found positive for amphistomiasis. The rate of incidence observed was high, which may be due to the rainy period of study (June to September) which facilitate the presence of the intermediate host (Al-Gaabary and Nasr, 1997), climatic and geographical parameters which affect the hatchability of amphistomes eggs (Dutta *et al.*, 1995 and Hirani *et al*., 1999) and also the small sample size. Incidence was found highest in buffaloes (92%) followed by goats (88%) whereas the incidence was reported

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Table 1: Per cent infection of Amphistomes in domestic and wild ruminants

S.No.	Species of the animals	Total samples	Positive	Per cent infection
1.	Buffalo	50	46	92%
2.	Cattle	30	21	70%
3.	Goat	25	22	88%
4.	Sheep	25	15	60%
5.	*Neelgai*	20	16	80%
	Total	150	120	80%

lowest in sheep (60%) followed by cattle (70%). This variation of the disease prevalence among different species may be attributed to the host specificity in addition to the rate of exposure where, cattle and buffaloes were exposed similarly compared to a little exposure of sheep (Al-Gabbary *et al*., 2009). The incidence in buffaloes was found higher due to the dwelling behaviour of the buffaloes in the water bodies which are infested with the snail intermediate host and thus pick up more infection as compared to rest of the animals. Keyyu *et al.* (2005) reported that the proportion of animals shedding the apmhistome egg is always higher in all zones, management, farms and age groups hence the infected animals serve as a potent source of infection even if their number is less. Khan *et al.* (2009) concluded that sheep and goats graze through out the year and sometimes due to reduction in pastures they are forced to graze in swampy areas thus exposing them to the vegetation infected with the metacercaria of flukes. Thus the sheep and goat pick up infection from the residual pastures near water bodies and may act as source of infection for large ruminants (Garg *et al.,* 2009). Amphistomiasis may be attributed to the damage and necrosis of gastro-intestinal mucosa which results from direct effect of the parasite which lead to impairment of digestion and absorption resulting in production loss. Submanidublar oedema may be observed due to hypoproteinaemia which resulted due to leakage of protein through the damaged mucous membrane of the duodenum. Decreased milk yield is seen due to decrease of volatile fatty acids (Amer *et al.,* 2002) which claims the economic importance of the disease. A considerable amount of infection was observed in *Neelgai* samples (80%). The sharing of grasslands was observed between the domestic and wild animals which may be the source of infection, similar observation was indicated by Shrivastava *et al.* (2005) and it was concluded that the transmission of infection between wild and domestic animals makes the control of disease difficult. The importance of amphistomiasis as a public health hazard, particularly in rural areas where a close association exists between man and domestic animal is well established (Kabir *et al*., 2010). Only a handful of literature is available on amphistomiasis because it always remained dominated by the other trematodal infections hence the economic losses due this particular infection has been underestimated.

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AMELIORATION OF CHICKEN INFECTIOUS ANAEMIA VIRUS INDUCED IMMUNOSUPPRESSION BY PROTEIN AND IMMUNOGLOBULIN SUPPLEMENTATION IN CHICKS

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ABSTRACT

The present investigation was undertaken to assess ameliorative potential of a protein and immunoglobulin supplement against chicken infectious anaemia virus induced immunosuppression. For the experimental study, total 60 SPF day-old broiler chicks were divided into three groups with 20 chicks each. Vaccination against NCD and IBD was performed on day 1st and 12th, respectively of the study period. All the chicks of groups II and III were challenged with CIAV intramuscularly with chicks of groups I and II serving as healthy and infected controls, respectively. The chicks of groups III, in addition received a protein and immunoglobulin supplement, in drinking water for the entire study period of 28 days. All the CIAV challenged birds showed a significant decline in cell count of erythrocytic and most leukocytic lineages, decline in antibody titres against NCD and IBD suggesting depressed HIR and immunosuppression. The intensity of immuno suppression was less severe in chicks supplemented with protein and immunoglobulins suggesting their use to minimize the economic losses due to CIAV to the poultry industry.

Key words: Chicken infectious anaemia, humoural immune response, protein, chicks, immunosuppression, immunoglobulins

Chicken infectious anaemia (CIA), an emerging disease mainly of young chicken and characterized by poor weight gain, severe anaemia, aplasia of the bone marrow, lymphoid atrophy, subcutaneous and muscular haemorrhages and increased mortality, has been responsible for considerable health problems and economic losses to the poultry industry (McNulty, 1991; Bulow and Schat, 1997; Hagood *et al.*, 2000; Dhama *et al*., 2008). CIAV is a potent immuno-suppressive agent for very young unprotected chicks thereby increasing their susceptibility to secondary infections, *viz.* viral, bacterial and fungal agents and depressing vaccinal immunity and production performance in the field situations (Van Den Berg, 1996; Adair, 2000; De Herdt *et al.*, 2001). Its etiological agent chicken infectious anaemia virus (CIAV) is the smallest DNA virus classified within the genus *Gyrovirus* under the virus family.

Circoviridae (Pringle, 1999) is now being recognized as an important avian pathogen worldwide (McNulty *et al.*, 1991; Farkas *et al.*, 1991; McIlroy *et al.*, 1992; Bulow and Schat, 1997). The virus seems to play a key role in the etiology of several multifactorial diseases, *viz.* haemorrhagic syndrome, haemorrhagic anaemia syndrome, infectious/aplastic anaemia, anaemia-dermatitis syndrome, gangrenous dermatitis and blue wing disease (Bulow, 1991a; Pope, 1991; Jorgensen, 1991; Toro *et al.*, 2000; Hagood *et al.*, 2000). Certain notable characteristics such as vertical transmission, detection in SPF eggs, its highly contagious, hardy and ubiquitous nature and the potential for inducing marked immunosuppression have attracted the global poultry production systems towards the CIAV infection (Todd, 2000).

As with other viral infections there is no specific therapeutic approach for the treatment of CIA. However, broad spectrum antibiotics to control or avoid secondary bacterial infections may be used. Birds in convalescent stages can be provided with immunostimulants and haematinics so as to boost the immune system and the process of blood formation, respectively (Dhama *et al.* 2002). Effective therapeutic regimen needs to be evaluated to counter the massive immunosuppression produced by the disease and reduce the economic losses. Therefore, a study was undertaken to study the immunomodulatory potential of a protein and immunoglobulin supplement in Chicken Infectious Anaemia Virus (CIAV) inoculated chicks.

Materials and Methods

A total of 60 day-old specific pathogen free (SPF) chicks were maintained in standard managemental conditions. Chicks were also given antibiotic for six days in prophylactic doses to prevent secondary bacterial infections. All the chicks were vaccinated with primary

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doses of Newcastle disease (NCD) and infectious bursal disease (IBD) on first and twelfth day, respectively. CIAV propagated in MDCC-MSB1 cells maintained in the avian disease section, Division of Pathology, IVRI, Izatnagar, UP (India) was used as challenge virus. The 0.5 ml of $10^{4.5}$ $TCID_{50}$/0.1ml of MSB1 cell culture passaged CIAV isolate was inoculated intramuscularly twice daily on day 15th and 16th of the study. Intermune*, a protein and immunoglobulin supplement, mainly consisting of all essential amino-acids like leucine, iso-leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, alpha lactalbumins, beta lactoglobulins, serum albumin, immunoglobulins and lactoferrines, was used as immunomodulator. It was given @ 1g and 2 g per 100 birds in drinking water, for 0-2 weeks and 2-4 weeks, respectively daily for 28 days.

The chicks were randomly divided into 3 equal groups. Group I chicks of this group were kept on normal basal ration and provided with plain drinking water. These chicks served as negative control. Group II chicks of this group were given normal basal ration and plain drinking water. It was also challenged by CIAV by the intra muscular route. No medication was given before and after the virus challenge. These chicks served as positive control. Group III chicks were given normal basal ration and were challenged with CIAV by intramuscular route. The chicks were given intermune in water for a period of 28 days. About 5 ml of pooled blood samples were collected from 5 birds taken at random from each group on day 14th, 21st and 28th. The blood was divided into two parts. One part was taken in heparinised vials (10-20 IU/ml) for haematological estimations and second part was taken in sterilized vials for serum collection and immunological assay. The blood collected in sterilized vials without heparin was allowed to clot for 20-30 minutes at room temperature. The clot was retracted by placing it under refrigeration followed by centrifugation for 10 minutes at 3000 rpm. The serum was harvested with the help of capillary pipette and stored at -20ºC for immunological assay.

Packed cell volume (PCV) and haemoglobin (Hb) were estimated using method of Jain (1986**)**. Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined as per method of Natt and Herric (1952) using poultry diluting fluid. Differential leukocyte count (DLC) was done by preparing blood smear from drop of uncoagulated blood and leucocytes were counted by zigzag method as described by Lucas and Jamroz (1961).

Humoural immune response against NCD and IBD in CIAV inoculated chicks were assessed with help of Haemegglutination Inhibition (HI) and Enzyme linked immunosorbant assay (ELISA). respectively. The initial screening of experimental chicks for CIAV antibodies was done using commercial ELISA kit (IDEXX Laboratories, USA) on day 14th of experiment. The chicks found negative were used in the experimental study. HI test was conducted using b-procedure as described by Allan *et al*. (1978). Two fold serial dilution of the test sera were made in NSS in round bottomed microtitre plates in 50 µl volume. Four HA units of ND virus in equal volume (50 µl) was added to each serum dilution, mixed by gentle shaking and the plates were kept at room temperature for 10 minutes. Freshly prepared 1% RBC suspension was added to each well in 50 µl volume. The negative controls were set up by taking NSS and 1% RBC alone, whereas virus control was set up by making serial two-fold dilution of 4 HA virus and adding 1% chicken RBC. Plates were incubated at room temperature for 30 minutes or till the RBCs in the control wells formed a clear button at the bottom.

The HI-titre of the serum was calculated as the reciprocal of the highest dilution of the test sera showing complete inhibition of haemagglutination of RBCs. The mean HI titre (log_2) for different groups was calculated for comparison.

ELISA was performed by single dilution method as per procedure described by Chandrasekhar (1994). The approximate concentration of viral antigen was determined by checkerboard titration. The antigen was diluted 1:50 in carbonate-bicarbonate buffer and 50 µl of this diluted virus was added to each well of 96 well ELISA plate (NUNC). The plate was incubated overnight at 4ºC. Next day, antigen coated plates were washed thrice with phosphate buffer saline-tris (PBS-T) for 5 minute each and tapped thoroughly. The unreacted sites were blocked by adding 100 µl of 5% skimmed milk powder to each well of plate. The plates were incubated for 1 hour at 37°C. The plates were again washed as mentioned above.

The single dilution of the test serum was made in another 96 well microtitre plate by adding 200 µl of dilution buffer and 2 µl of test serum per well resulting in dilution of 1:100. Positive control serum (against local isolate IBD virus) and negative serum were also diluted 1:100 in PBS-T.

The diluted negative serum was added in 100 µl to the first three wells of the first row and 100 µl of diluted positive serum was added to the first three wells of last six wells of last row of ELISA plate and last three wells of the last row are kept as blank. In remaining wells of antigen-coated plate, 100 µl of test serum from dilution plate was transferred to each of the corresponding three wells.

The plate was again incubated at 37ºC for 1 hour and washed thrice with PBS-T. Then 100 µl of rabbit anti-chicken horse radish peroxidase conjugate (Sigma) diluted 1:5000 in PBS-T was added to each well and plates were incubated at 37ºC for 1 hour. To each well of the plate then added with 100 µl of freshly prepared substrate solution ortho-diphenylenediamine dihydrochloride (OPD) in citrate buffer followed by H_2O_2 and then incubated at 37ºC for 30 minutes in dark. The reaction was stopped by adding 100 µl of stop solution

* Manufactured by M/s Interface Pharmaceuticals Ltd., New Delhi

(1N H_2SO_4) in each well of plate. The plate was read at 492 nm in an ELISA reader (ECIL Microscan MS 5605).

The average absorbance of positive and negative control was calculated from the absorbance value of ELISA plate and corrected positive control (CPC) value was determined by subtracting average negative absorbance from average positive absorbance.

The specific value (Sp. value) was calculated using following formula:

$$\text{Sp. Value} = \frac{\text{Average absorbance of test sample} - \text{Average absorbance of negative control}}{\text{Corrected positive control}}$$

The results were analyzed by standard method (Snedecor and Cochran, 1994).

Results and Discussion

The CIAV causes suppression of differentiation and proliferation of haematopoietic precursor cells. This drastically affects production of mature red blood cells (erythropoiesis) and myelopoiesis leading to transient destruction of erythroblastoid and granuloblastoid cell lineages in bone marrow resulting in hypoplasia, anaemia and panleukopenia (Dhama *et al*., 2008). In the present study, a significant (P<0.05) decline in the haemoglobin, PCV and total erythrocyte count was observed in all the CIAV challenged groups as compared to the control group. In leukocytic lineages also, a significant (P<0.05) decline in total leukocyte count, per cent lymphocyte count and percent basophil and eosinophil count was observed in all CIAV challenged chicks as compared to the control group at the end of study period (Table 1). This significant decline in erythrocytic and lymphocytic series cells can be attributed to depression of haemopoietic precursor cells due to CIAV.

It was observed at the end of the study that group III supplemented with Intermune showed a minimal decline in haematological parameters. Intermune have all essential amino acids and immunoglobulins and acts as an immunomodulator. The immunomodulatory potential of protein and immunoglobulin supplement has been reported by Kumar *et al.* (2008) and Chandrakar (2009).

CIAV is believed to be a potent immuno-suppressive agent by virtue of its pathological effects in susceptible chicks. Poor antibody response is observed after CIAV infection at day-old age which is a consequence of depressed T_h responses (Otaki *et al*., 1988a,b) reflecting the intensive inhibition of humoral immunity in the early phase of infection. CIAV exerts a destructive effect on both primary and secondary lymphoid tissues and especially suppresses the population of both helper (CD4+) and cytotoxic (CD8+) T-lymphocytes in the thymus (Hu *et al*., 1993a,b; Adair, 2000). Immuno-suppression so induced is caused at least in part by apoptosis induced by VP3 protein (apoptin). There is marked damage of haematopoietic and lymphopoietic tissue, *viz.* stem cells in bone marrow and precursor T-lymphocytes in thymus **(**Goryo *et al*., 1989a,b,c; Smyth *et al*., 1993).

In the present study, the humoural immune response (HIR) in chicks experimentally infected with CIAV was evaluated by assessing the antibody titre against NDV and IBD employing HI and ELISA, respectively. An interesting finding of the study was the higher levels of HI titres in groups supplemented with immunomodulators like Intermune before CIAV challenge. A significant decline in the antibody HI titre against NCD and ELISA titre against IBD was observed in all the infected chicks as compared to control group on day 21st which further declined on day 28th of the study (Table 1). Our findings are in agreement with several reports regarding marked depression of HIR in chicks experimentally infected with CIAV (Liu *et al*., 1997; Zheng *et al*., 1997; Zheng and Liu, 1997, 1998; Liu *et al*., 2001) leading to reduction in antibody producing cells. Similar findings were observed by Zheng and Liu (1996 a,b) who reported lower ND-HI titres in CIAV infected birds vaccinated at day old stage. Ragaland *et al.* (1998) showed an increase in CIAV detection co-related inversely with ND-HI titres while studying immune response to ND vaccine. Similar results have been reported earlier also, confirming that CIAV can cause decrease in antibody responses (Otaki *et al*., 1988 a,b).

The low antibody titre in CIAV infected birds have also been reported by many workers **(**Dhama, 2002 and Vacchani, 2005). The destructive effect of CIAV due to suppression of the population of both helper (CD4+) and cytotoxic (CD8+) T-Lymphocytes in the thymus as suggested by Hu *et al*. (1993a,b) and (Adair, 2000**)** could be the reason for poor immune response in CIAV inoculated chicks. The groups supplemented with protein and immunoglobulins showed a better HIR indicated by high antibody titres amongst CIAV inoculated groups. immuno-modulatory potential of protein and immunoglobulin supplement was reported by Kumar *et al*. (2008) and Chandrakar (2009) also. It can be concluded that the use of protein and immunoglobulin supplement for prophylaxis and therapeutics against CIAV can minimize the immunosuppression and clinical signs.

Acknowledgements

The authors are highly thankful to the Dean, College of Veterinary and Animal Sciences, Dean, Post Graduate Studies and Director, Experiment Station for providing necessary facilities to carry out this research work.

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Table 1: Effect of a protein and immunoglobulin supplementation on haematological profile and humoural immune response in CIAV inoculated chicks.

Parameters	Groups	Days of observation		
		14	21	28
Haemoglobin (g/l)	I	119.24±1.00	121.96±2.20^{C}	123.58±2.01^{C}
	II	119.14±1.67	101.30±1.10^{Ab}	67.66±1.72^{Aa}
	III	116.72±2.18^{b}	113.87±4.27^{Bb}	87.09±1.61^{Ba}
PCV (l/l)	I	0.33±0.01	0.34±0.01^{C}	0.34±0.00^{C}
	II	0.32±0.01^{c}	0.28±0.00^{Ab}	0.20±0.01^{Aa}
	III	0.32±0.00^{c}	0.30±0.00^{Bb}	0.27±0.00^{Ba}
TEC (x10^{12}/l)	I	3.36±0.05	3.43±0.06$_{C}$	3.43±0.09^{C}
	II	3.37±0.06^{c}	2.67±0.05^{Ab}	1.99±0.011^{Aa}
	III	3.30±0.10^{c}	3.05±0.05^{Bb}	2.50±0.02^{Ba}
TLC (x10^{9}/l)	I	22.17±0.76	23.16±0.31^{C}	22.68±0.60^{C}
	II	22.33±1.04^{c}	18.90±0.28^{Ab}	15.88±0.15^{Aa}
	III	22.50±0.50^{b}	21.17±0.70^{Bb}	18.27±0.51^{Ba}
PLC (%)	I	60.17±0.76	60.76±1.55^{B}	59.71±0.67^{C}
	II	60.83±1.61^{c}	52.66±0.69^{Ab}	48.50±1.36^{Aa}
	III	59.50±1.32^{c}	54.21±0.33^{Ab}	51.12±1.04^{Ba}
PHC (%)	I	29.13±1.31	30.08±1.22^{A}	30.83±0.45^{A}
	II	29.90±1.15^{a}	39.88±2.26^{Bb}	45.22±1.10^{Cc}
	III	30.17±1.04^{a}	38.17±0.33^{Bb}	40.18±0.74^{Bb}
HI titre (log_2) against NCD	I	10.32±0.00	9.99±0.58^{C}	9.32±1.00^{C}
	II	10.65±0.58^{c}	7.65±0.58^{Ab}	4.99±0.58^{Aa}
	III	10.99±0.58^{c}	9.32±0.00^{BCb}	7.65±0.58^{Ba}
ELISA antibody Titre against IBD	I	1.49±0.05^{Aa}	549.69±24.97^{Cb}	963.28±31.69^{Cc}
	II	1.66±0.10^{Aa}	22.04±1.59^{Ac}	16.72±1.84^{Ab}
	III	2.14±0.39^{Ba}	71.98±1.50^{Bb}	75.43±2.71^{Bc}

The values (Mean±SD) having at least one common superscript (Capital letters in columns and small letters in rows) does not differ significantly (P<0.05) for a parameter.

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EFFECT OF LACTATION AND PREGNANCY ON SERUM BIOCHEMICAL AND HAEMATOLOGICAL PROFILES OF SURTI BUFFALOES

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ABSTRACT

Blood samples were collected from the jugular veins of heifer (control), lactating and dry pregnant Surti buffalos to study the effect of lactation and pregnancy on serum biochemical and haematological profiles. Serum was separated and different biochemical parameters were estimated using commercially available kits. PCV, Hb and TEC were determined by standard methods. Total protein and glucose were significantly ($P<0.05$) higher in the pubertal heifer than dry and lactating groups. Blood urea, BUN, serum calcium, ALP, SGPT and SGOT were significantly ($P<0.05$) lower in the lactating buffaloes. Serum cholesterol, creatinine, total and conjugated bilirubin were found similar among the groups. PCV was significantly ($P<0.05$) higher in lactating as compared to other two groups while TEC and Hb did not vary significantly among the groups. Lower values of serum biochemical parameters in the lactating and dry pregnant groups may be imputed to their drainage for milk synthesis and fetal development respectively.

Key words: Serum biochemistry; hematology; heifer; lactating; dry pregnant; Surti buffalo.

Introduction

The blood biochemical and haematological profiles reflect the metabolic status and health conditions of animals. The estimates of biochemical constituents are the pre requisite to diagnose several pathophysiological and metabolic disorders in cattle (Chaffe, 1976; Mc Dowell, 1992). The normal serum levels of different metabolites and haematological attributes change at different physiological stages of animals. Hence the normal values of these parameters at different physiological stages are crucial before these can be utilized for assessing the disease conditions. Surti buffalo of Gujrat and western Rajasthan is known for its high fat content and disease resistance. Little information regarding normal serum levels of different metabolites and haematological parameters at different physiological stages is not well documented in this breed. Hence the current work was undertaken to estimate these parameters at different physiological stages in female Surti buffaloes.

Materials and Methods

Apparently healthy Surti buffaloes of Livestock Research Station, Vallabhnagar, Udaipur, Rajasthan, were used for this study. Three groups, each consisting of eight animals were made as pubertal heifer, lactating and dry pregnant. The blood samples were collected in the morning hours from the jugular veins into EDTA-K_3-coated vials and processed on the same day as per the method described by Benjamin (2001).

The serum biochemical estimations were done using the commercially available kits (SPAN diagnostics, India). The estimations of serum enzymes and bilirubin were done on the same day of collection while the other biochemical parameters were estimated by next 48 hrs. All the biochemical analysis was carried out using a Semi Auto Analyzer (Systronics, India). One-way ANOVA was used to compare the differences in the means using SPSS software version 14.0 and level of significance was set at 5%.

Results and Discussion

Serum biochemical parameters

The total serum protein was significantly ($P<0.05$) higher in the pubertal heifer (8.25± 0.93 g %) as compared to the lactating (4.76±0.34g %) and dry (4.65±0.37 g%) buffaloes. The results were much lower than those reported by Hagawane *et al.* (2009) in the lactating (7.5 ± 0.9 g%) and dry

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(8.19±0.69 g%) buffaloes and by Jani *et al*. (1995) in the lactating (9.88 ±0.3 g%) Surti buffaloes. The lower values of serum protein in the lactating and dry pregnant groups found in present study may be due to insufficient dietary supplementation to meet up the additional protein requirements during these physiological stages.

The blood glucose level was significantly (P<0.05) lower in the lactating group (37.27±2.16 mg/dl) than the pubertal heifer (55.9±6.27 mg/dl). The hypoglycaemia in the lactating animals might be due to excessive drainage of the blood glucose for the synthesis of milk lactose (Scultz, 1968 and Nale, 2003). Hagawane *et al.* (loc cit) and Jagatheesan *et al.* (2005) also reported similar blood glucose levels in the lactating and dry non-descript buffaloes. However, a much higher level (77 ±13.25 mg/dl) of the blood glucose was reported from the field lactating buffaloes by Jani *et al*. (loc cit).

Blood urea and blood urea nitrogen (BUN) were significantly (P<0.05) lower in the lactating group (12.19±0.55 and 5.6±0.25 mg/dl) than the pubertal heifer (21.41.39 and 9.84±0.64 mg/dl) and dry animals (18.48±1.58 and 8.49±0.73mg/dl). Similar results were also reported by Hagawane *et al*. (loc cit) and Jani *et al*. (loc cit). The lower blood urea level in lactating buffalo may be due to decreased protein catabolism and increased utilization of dietary protein for the synthesis of milk casein. While the higher level of blood urea in the pubertal heifer and dry pregnant groups may be due to higher intake and utilization of the dietary proteins and or increased catabolism of the body proteins (Mulei and Daniel, 1989; Oliva *et al*. 1991).

Serum cholesterol level did not vary significantly among the groups. However, it was found little higher in the heifer and dry animals as compared to lactating group. This may be due to enhanced endogenous synthesis of cholesterol in dry pregnant and pubertal heifer to work as precursor for biosynthesis of various steroid hormones such as oestrogen, progesterone and androstenidione (Parmer and Mehta, 1991). The cholesterol levels found in present study were in close approximation with those reported by Hagawane *et al*. (loc cit.) in non-descript buffalo and Jagatheesan *et al*. (loc cit.) in Murrah buffalo but were much lower than those reported by Parmar and Mehta (loc cit.) in Surti buffalo.

Serum creatinine, total bilirubin and conjugated bilirubin did not vary significantly among the groups (Table1). Serum calcium was significantly (P<0.05) lower in lactating group (8.59±0.2 mg/dl) as compared to heifer (10.99±0.39 mg/dl) and dry pregnant (10.270.56 mg/dl) animals. Similar results were also reported by Hagawane *et al*. (loc cit) and Jani *et al*. (loc cit). The lower calcium level in lactating group might be due to excessive drainage of blood calcium in milk and insufficient adjustment by the parathormone through mobilization of bone calcium.

Serum alkaline phosphatase (ALP) was significantly (P<0.05) lower in lactating group (1.92±0.23 KAU/dl) as compared to dry group (5.72 ±0.59 KAU/dl) or pubertal heifer (7.01±0.97 KAU/dl). A higher value of ALP (12.98±0.79KAU/dl) was reported in pubertal heifer by Kumar *et al*. (1991). Both SGOT (AST) and SGPT (ALT) were significantly lower (39.12± 2.05 IU/L and 23.5±2.08 IU/L) in lactating group as compared to pubertal heifer (78.75±5.43 IU/L and 32.87±1.8 IU/L) and dry pregnant (59.25±7.39 IU/L and 28.25±1.93 IU/L) animals. In contrast to the present results, higher values of serum transaminases were found by Jagatheesan *et al.* (loc cit) in lactating Murrah buffaloes. However, Kumar *et al.* (loc cit.) reported much lower values of these enzymes in buffalo heifer. SGOT varied significantly among all three groups. Lower level of serum transaminases in lactating animals might be due to diminished protein catabolism to compensate for the amino acids requirement for the synthesis of milk casein.

Haematological parameters

Total erythrocyte count (T.E.C, million/mm^3) and haemoglobin (g %) did not vary significantly among the three groups (Table1). PCV (%) however was significantly (P<0.05) higher in lactating (42.87±1.82 %) group than dry animals (35.75±3.29 %) and pubertal heifer (33.25±1.39 %). Jani *et al.* (loc cit.) reported much lower level of PCV (28±1.96 %) in lactating Surti buffalo. Gupta *et al.*(1995) also found similar TEC and Hb in lactating and dry buffaloes; however, Jani *et al.* (loc cit) reported little higher value of TEC (7.18±0.65 million/mm^3) in lactating group.

It was concluded from the above findings that most of the serum biochemical parameters studied here were found significantly (P<0.05) lower in the lactating group as compared to the pubertal heifer. Similarly comparatively lower values (though statistically non-significant) of these parameters were found in the pregnant buffaloes than the heifer. The reason behind this might be the excessive drainage of these components from the blood for the synthesis of milk in lactating animals and development of fetus in pregnant animals along with inadequate dietary replenishment.

Acknowledgements

The authors are thankful to the In-charge of Livestock Research Station, Vallabhnagar, Udaipur for providing the blood samples of Surti buffaloes to carry out the research**.**

Table1: Serum biochemical and haematological profiles of normal Surti buffaloes at different physiological stages (Mean±S.E.).

Parameters	Physiological stage of animals			
	Pubertal Heifer n=8	Lactating n=8	Dry Pregnant n=8	Over all n=24
Glucose (mg/dl)	55.9±6.27^{a}	37.27±2.16^{b}	44.02±5.06^{ab}	45.73±3.1
Total Protein (g/dl)	8.25±0.93^{a}	4.76±0.34^{b}	4.65±0.37^{b}	5.88±0.48
Blood Urea (mg/dl)	21.4±1.39^{a}	12.19±0.55^{b}	18.48±1.58^{a}	17.35±1.06
B.U.N.(mg/dl)	9.84±0.64^{a}	5.60±0.25^{b}	8.49±0.73^{a}	7.98±0.49
Serum cholesterol (mg/dl)	43.35±1.62	41.52±2.98	45.64±1.82	43.5±1.28
Serum creatinine (mg/dl)	2.01±0.17	1.92±0.06	2.16±0.15	2.03±0.08
Total bilirubin (mg/dl)	0.512±0.05	0.404±0.03	0.547±0.05	0.487±0.03
Direct bilirubin (mg/dl)	0.109±0.02	0.117±0.01	0.128±0.02	0.118±0.01
Calcium (mg/dl)	10.99±0.39^{a}	8.59±0.2^{b}	10.27±0.56^{a}	9.96±0.31
Serum ALP (KA unit)	7.01±0.97^{a}	1.92±0.23^{b}	5.72±0.59^{a}	4.88±0.58
S.G.P.T.(I.U/L)	32.87±1.8^{a}	23.5±2.08^{b}	28.25±1.93^{ab}	28.2±1.34
S.G.O.T.(I.U/L)	78.75±5.43^{a}	39.12 ±2.05^{b}	59.25±7.39^{c}	59.04±4.51
T.E.C.(X10^{6}/cumm)	5.55±0.21	5.57±0.13	5.7±0.23	5.60±0.11
P.C.V.(%)	33.25±1.39^{a}	42.87±1.82^{b}	35.75±3.29^{a}	37.29±1.54
Haemoglobin (g %)	10.82±0.37	11.05±0.55	11.27±0.79	11.05±0.33

n = number of animals. Means bearing different superscripts differ significantly (P<0.05) between groups.

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BRONCHODIALATORS IN MANAGEMENT OF PNEUMONIA IN GOAT

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ABSTRACT

A group of four diseased adult animals was put on for clinical examination recorded clinical signs of increase in temperature, dyspnoea, watery nasal discharge, inappetance, reduced ruminal movements and lachrymation without any observable effect on hair coat. Nasal swabs recorded presence of both kind of organisms mainly *E. coli*, *Staphylococcus* spp. *Proteus* spp. along with unidentified isolates. These animals were treated with Ampicillin plus plus cloxacillin (AC-VET FORTE, Intas) @ 10 mg/kg intramuscularly 8 hourly for 5 days, Etophylline and Theophylline (Deriphyllin) @ 0.75 ml intramuscularly 12 hourly for one day and Salbutamol 1.25 mg per animal per day orally for 3 days successfully.

Key words: Pneumonia, goat

Introduction

Among diseases of respiratory system, pneumonia appears to rank first particularly in sheep. The occurrence is influenced by many factors viz. region, season, age, management system and immune status. To record the clinical changes in naturally occurring cases in sheep in this region with varying season and to improve the therapeutic management, a study was carried out in outgoing cold season, February-March, 2010 on animals maintained at Central Sheep and Wool Research Institute, Avikanagar.

Materials and Methods

A group of four adult goat of Sirohi breed weighing around 25-40 kg was observed to be suffering from respiratory tract infection in November, 2010 during their clinical examination. These animals were carefully examined for recording physiological parameters, clinical symptoms and auscultative changes. The collected nasal swabs/samples were subjected to bacterial culture. The bacterial isolates were characterized by morphological, cultural and biochemical characteristics after their inoculation on primary, differential and/or selective media. These animals were treated with Ampicillin plus cloxacillin (AC-VET FORTE, Intas) @ 10 mg/kg intramuscularly 8 hourly for 5 days, Etophylline and Theophylline (Deriphyllin) @ 0.75 ml intramuscularly 12 hourly for one day and Salbutamol 1.25 mg per animal per day orally for 3 days. The clinical examination of all the animals was carried out two times in a day and progress in health status was assessed through improvement in clinical symptoms.

Results and Discussion

All the animals under treatment started showing improvement in their health status from next 12-18 hours of treatment from various symptoms such as dyspnoea, nasal discharge, high rise in temperature, lachrymation and abdominal respiration. Presence of dyspnoea may be a compensatory process of gas exchanges through lung parenchyma (Koneko, 1980) and high rectal temperature 105 to 105.5°F may be produced by bacterial, viral or chemical pyrogens. It is the result of a “resetting” of the thermoregulatory mechanism to function above the normal level. In many animals postaglandins are responsible for the readjustment as evident by use of prostaglandin inhibitors as antipyretic agents (Aiello, 1998) In acute infections, the temperature may rise several degrees above normal for a few days and this triggers the thought of many infections that cause fever by inducing production of several cytokines, of which ingterleukin-6 and tumour necrosis factor are most likely involved. Other symptoms like dullness, depression, inappetance reduced ruminal motility and scanty faeces persisted for longer period of time and marginal recovery could be noticed after 24-36 hours of onset of therapy. Auscultation of the lungs revealed quite harsh bronchovesicular sounds in some of the cases with abdominal friction rub indicative of inflammatory process but was not indicative of the extent or severity of respiratory disease. In a few cases anterior ventral consolidation was recorded in very narrow lung field indicative of lower respiratory disease.

Appreciable recovery was noticed especially at the time of withdrawal of treatment when only a few symptoms viz. reduced inclination to walk and intake particularly to water were seen. The symptoms such as dullness, depression, inappetance and high rise in temperature may be a reflection of the reaction of the body to its internal changes occurring due to propagation of a variety of micro organism subsequently release of pyrogens and other related intrinsic factors with in the system and dyspnoea, nasal discharge, may be the out come of and complex interaction of environmental factors producing stress, a variety of micro organisms working synergistically to damage the cells lining the respiratory tract allowing colonization and invasion of other organisms and a compensated host response. These relate to similar observations of Stevenson and Robinson (1970), Lehmkuhl and Cutlip (1984) and Robinson (1983). On bacterial cultural examination and characterization, both (Gram-positive and Gram-negative) kinds of organisms were obtained. Identified isolates were *E. coli, Staphylococcus*, *Proteus* spp. along with unidentified isolates. The therapeutic approach used in present treatment has shown a good recovery with Beta-blocker and bronchodialators. Deriphylline, is a combination of theophylline and etophylline which belong to methyl xanthine group of drugs used in the ratio of 1 :3. Etophylline is the hydroxy ethyl ester of theophylline (containing 80% of theopylline by weight). Low doses of theophylline have an anti-inflammatory or immunomodulatory effect *in vivo* (Tinkelman et a*l*., 1993; Reed *et al.,* 1998; Ward, *et al.,* 1993; Kidney *et al., 1995* and Kazuhiro, *et al.,* 2002) by way of inhibiting the activation of NF-kB and reducing the expression of inflammatory genes in a manner similar to corticosteroids (Tomita *et al.,*1999). In addition, eosinophil survival induced by IL-5 and GM-CSF is decreased by low concentrations of theophylline independently from PDE inhibition and changes in cAMP (Ohta *et al.,*1996; Yasui *et al.,*1997)). The theophylline can markedly reduce histone H4 acetylation at the GM-CSF promoter and now histone acetylation is thought to be an important area of regulation to produce bronchodilation by bronichial muscle relaxation and suppression of response of airways to stimuli.

Ampicillin and cloxacillin are broad-spectrum bactericidal antibiotic and they are well known to act synergistically against a variety of bacteria (Riviere and Papich, 2009) and accordingly has been evaluated in clinical cases of pneumonia in goats with supportive bronchodialators and beta blockers. These antibiotics are known to act by inhibiting the synthesis of bacterial cell walls. It inhibits cross linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-posiitive and negative bacteria. Synergistic antibacterial effects of mixtures of ampicillin and cloxacillin is well known (Bornside, 1968; Sutherland and Batchlor,1964). Use of Beta blockers and bronchodialators seem to be good enough in getting the desired results of gas exchange through respiratory tract in given dose time and schedule in goats. Though this is a very small study without screening the functions of vital organs during and after treatment but primarily indicate of beneficial effects of drugs individually and collectively in relieving the patients sufferings. Further studies are called for on larger scale with more scientific approach to reach the conclusion.

Acknowledgements

The authors are thankful to Director Central Sheep and Wool Research Institute Avikanagar and other supporting staff for providing necessary facility and support.

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EFFECT OF PROGESTERONE IMPREGNATED INTRA-VAGINAL SPONGES PLUS PMSG ON OESTRUS INDUCTION AND CONCEPTION IN ANOESTRUS BUFFALOES

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ABSTRACT

The aim of the present study was to determine the effect of intra-vaginal insertion of indigenously developed progesterone impregnated sponges in combination with two different doses of PMSG on induction of oestrus and conception rate in anoestrus buffalos. A total of 47 anoestrus buffaloes were selected from the herd of Central Institute of Research on Buffalo, Hissar and utilized for this study. The sponges were inserted intra-vaginally in all the animals and kept *in situ* for 15 days. After day 15 the sponges were removed and animals were divided in to two groups. The animals in Group I (n=19) were treated with the intramuscular administration of PMSG (600 IU per animal) while the animals in Group II (n=28) were treated with 1000 IU of PMSG on the day of sponge removal. The oestrus response of animals of both the groups was recorded. The oestrus response was 32% (6/19) and 57% (16/28) in Group I and Group II, respectively. In Group I all the six animals which exhibited estrus were inseminated using frozen thawed semen and in Group II seven out of 16 were inseminated. The conception rate based on per rectal examination was 33% (2/6) and 43% (3/7) in Group I and Group II, respectively. The results of this study indicate that the indigenously developed progesterone impregnated intra-vaginal sponges in combination with PMSG can be used for the induction of oestrus in anoestrus buffaloes with better results using 1000 IU PMSG compared to 600 IU PMSG.

Key words: Buffalo, Intra-vaginal sponge, progesterone, anoestrus, PMSG

Introduction

Anoestrus is a major problem in buffaloes responsible for delayed puberty and prolonged inter-calving periods, leading to its low reproductive efficiency. Higher incidences of anoestrus are due to inactive ovaries in buffaloes than in cows have been reported by Tanwar *et al.* (2003). Progesterone is one of the regulatory hormones for oestrous cycle and raising progesterone level for 10 to 14 days induces estrus in buffaloes (Singh *et al*., 1988 a; Singh, 2003; Murugvel *et al*., 2009; Zaabel *et al*., 2009; Nayak *et al*., 2009 a). Progestogens (synthetic analogs of progesterone) can be provided by feeding (MGA; melengesterol acetate), implants under the skin (Norgestomet-Synchro-Mate B), sponges inserted into the vagina (FGA, flurogestone acetate; MAP, medroxyprogesterone acetate; progesterone), or plastic delivery devices such as the CIDR-G (controlled internal drug releasing device) inserted into the vagina. Progesterone, the natural hormone, is the most desirable of progestogens because it is cleared rapidly from the body after withdrawal. However, in addition to the cost, the ability to incorporate adequate amounts of progesterone into suitable delivery devices that can maintain high concentrations in the animal throughout the treatment period has been a limiting factor. Efforts have been made to reduce the cost by reutilizing the delivery devices (Nayak *et al*., 2009 a, b; Dodamani *et al*., 2011).

In our laboratory, indigenously developed low cost progesterone impregnated intra-vaginal sponges have been developed and tested in buffaloes (Kumar *et al*., 2006) with 65.7% retention rate up to 15 days of insertion (Singh *et al.,* 2009), and elevating blood progesterone concentration (Singh *et al*., 2010), supporting the use of intra-vaginal sponges for drug delivery in buffaloes. In non-cycling true anoestrus animals, the primary effect of progesterone is enhanced by combining the effect of the progesterone removal and an injection of small dose of PMSG to stimulate follicular development (Singh *et al*., 1988b; Dodamani *et al.,* 2011). The present study was

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undertaken to determine the effect of indigenously developed sponges in combination with two different doses of PMSG on induction of estrus and conception rate in anoestrus buffalos.

Materials and Methods

A total of 47 anoestrus buffaloes were selected from the herd of Central Institute of Research on Buffalo, Hissar and utilized for this study. The speculum, plunger and sponges were fabricated at Central Sheep and Wool Research Institute, Avikanagar as per the needs of buffaloes (Singh *et al*., 2009). The speculum made up of plastic material having sufficient endurance thickness was 32.5 cm in length and had 3.5 cm slope at 29 cm with an internal diameter of 3.5 cm. The plunger was prepared from thick nylon material having the size of 55 cm length with 3 cm diameter. Vaginal sponges of 5 cm diameter and 5 cm thickness were prepared using a sheet of spongy foam and thread. A solution of progesterone (0.9 g) was prepared in ethanol. Each vaginal sponge was imbibed with progesterone solution by applying small aliquots so that it remained in the sponge and did not ooze out from the sponge. The sponges were then dried in hot air and kept in a neat and sterilized polythene bag.

Progesterone impregnated sponges were loaded in speculum through the anterior end by taking out the thread of sponge from its distal end. The external genitalia of buffaloes were cleaned with cotton swab soaked in dilute detergent solution just before insertion of loaded speculum in to the vagina. Liquid paraffin was applied on the outer surface of loaded speculum as lubricant for smooth insertion of speculum. After intra-vaginal insertion of loaded speculum, progesterone impregnated sponge was expelled out of the speculum into the vagina near os-cervix by pushing it through the speculum with the help of plunger, plunger was retained and speculum was withdrawn. After expulsion of sponge, the speculum and plunger were removed carefully. Progesterone impregnated sponges were kept *in situ* vagina for 15 days. All the buffaloes with inserted vaginal sponges were maintained under similar management practices. After 15 days, the sponges were removed by pulling the thread carefully and animals were divided in to two groups. The animals in Group I (n=19) were treated with the intramuscular administration of PMSG (600 IU per animal) while the animals in Group II (n=28) were treated with 1000 IU of PMSG on the day of sponge removal. The oestrus response of animals of both the groups was recorded. In Group I six animals which exhibited oestrus were inseminated using frozen thawed semen and in Group II seven animals were inseminated. All the inseminated animals were examined per rectally for pregnancy diagnosis at day 60 of insemination and conception rate was calculated accordingly. The data was statistically analysed as per Snedecor and Cochran (1964).

Results and Discussion

The results are presented in Table 1. Both the oestrus response and conception rate were higher in Group II as compared to Group I but the differences were statistically non-significant ($P<0.05$). The estrus response was 32% (6/19) and 57% (16/28) in Group I and Group II, respectively. The conception rate was 33% (2/6) and 43% (3/7) in Group I and Group II, respectively. A number of reports are available on the use of intra-vaginal sponges impregnated with varying amount of progesterone in cattle (Scanlon *et al*., 1971; 1972; Sreenan, 1974; Moore and Smith, 1980; Davis *et. al*., 1983), horses (Dinger *et al.*, 1981) and sheep (Robinson, 1965; MacDonnell and Crowley, 1978; Hamra *et al*., 1989; Crosby *et al.,* 1991; Naqvi *et al*., 1996) but to the best of our knowledge this is the first report on the use of indigenously developed progesterone impregnated intra-vaginal sponges for induction of oestrus and improving fertility in buffaloes. However, in our earlier report we have found high level of blood progesterone after intra-vaginal insertion of indigenously developed sponges in anestrous buffaloes (Singh *et al*., in press). Exogenous-

Table 1: Oestrus response and conception rate of anoestrus Buffaloes following treatment with progesterone impregnated intra-vaginal sponges and PMSG

Observations	Group I (Sponges + 600 IU PMSG)	Group II (Sponges + 1000 IU PMSG)
No. of animals treated	19	28
No. of animals expressed heat symptoms; n (%)	6 (32%)	16 (57%)
No. of animals inseminated	6	7
No. of animals conceived; n (%)	2 (33%)	3 (43%)

administration of progesterone exerts a negative feed back effect over the hypothalamus and pituitary and blocks the release of gonadotrophins (FSH/ LH). Upon withdrawal of progesterone the block is removed and as such larger quantities of gonadotrophins are released which in turn ensures growth and maturation of follicles and onset of oestrus.

Although, there is no report on the use of progesterone impregnated intra-vaginal sponges in buffaloes for induction of oestrus, but other progesterone impregnated intra-vaginal devices (CIDR, PRID or CRESTAR) have been effectively used to treat anoestrus buffaloes (Singh *et al*., 1988 b; Singh, 2003; Murugvel *et al*., 2009; Zaabel *et al*., 2009; Nayak *et al.,* 2009 a, b), which also support our results.

In conclusion, the results of this study indicate that the indigenously developed progesterone impregnated intra-vaginal sponges in combination with PMSG can be used for the induction of oestrus in anoestrus buffaloes with better results with 1000 IU PMSG compared to 600 IU PMSG. However, further research is required to standardize the dose of progesterone in intra-vaginal sponges for induction of estrus in anoestrus buffaloes.

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COENUROSIS IN SMALL RUMINANTS: AN OVERVIEW

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ABSTRACT

Coenurosis is a serious threat to sheep and goat production, particularly in the rural parts of Southeastern Asian and African countries which possess three-fourth of world's small ruminant population. The disease is caused by larval stage of *Taenia multiceps*, in sheep, goats and other ungulates including man. This paper reviews varied epidemiological factors, clinico-pathological effects and the developments and significance of diagnostic approaches for detecting the latent coenurosis, beside the prevalent control strategies and feasibility of developing a cost-effective vaccine have been discussed.

Introduction

The Southeastern Asian and African countries possess three-fourth of world's small ruminant population. In these countries, sheep and goat rearing is an important part of pastoral economy. Among the parasitic diseases affecting ruminants, coenurosis (gid or sturdy or stagger) has been a fatal disease, especially in sheep. It is caused by larval forms (metacestode) of *Taenia* (*Multiceps*) *multiceps*. The cystic larva (*Coenurus cerebralis*) essentially develops in the central nervous system (CNS) of sheep, goats and, sometimes, cattle, buffalo, horse, yak and wild animals and has also been reported in man (Backer and Jacobson, 1951; Toofanian and Ivoghli, 1976; Sanyal and Sinha, 1983; Gupta and Chowdhury, 1985; Samdup, 1993; Islam and Rahman, 1997; Aiello and Mays, 1998; Ing *et al*., 1998). In goats, occasionally aberrant sites of predilection of the metacestode, with an alternate name (*C. gaigery*) have been documented (Bhalla and Negi, 1962; Dey *et al*., 1988; Sharma *et al*., 1995; El-Sinnary *et al*., 1999; Godara *et al*., 2011).

The presence of cysts in the CNS gives rise to the neurological signs of coenurosis which are quite discrete in nature and that in a majority of cases result in the death of the animal from starvation after some weeks (Scala and Vercasia, 2006; Welchman and Bekh-Ochir, 2006). However, the animals in most cases remain normal without clinical symptoms when the size of the cyst(s) is too small to create pressure and hence to induce clinical signs (Parihar, 1988; Achenef *et al*., 1999). In such cases, condition is diagnosed only after the death of the animal. The cyst(s) present in muscles or subcutaneous tissues may impair functions of organ involved. In recent past, economic losses incidental to metacestode infections to the extent of US$ 2.6 million have been documented from Far Province of Iran (Oryan *et al*., 1994). This article presents an update on epidemiology and clinico-pathogenesis of the disease, and recent advances and prospects for coenurosis control.

Life-cycle

The adult *T. multiceps* is a parasite of small intestine of dogs and other canids like fox, jackal, coyote, etc. which act as definitive host and are continuous source of infection through the discharge of eggs in the faeces. The intermediate hosts (sheep and other ungulates including man) acquire infection by ingestion of food or water contaminated with dog faeces containing the eggs of *T. multiceps*. Onchospheres released in the intestine and through blood circulation reach to the CNS and also to intramuscular and subcutaneous tissues. As the parasite matures, it develops into a large, delicate, translucent fluid containing cyst (*Coenurus*) measuring about 5 cm in diameter after 6-8 months.

The *Coenurus* possesses unusual power of asexual multiplication giving rise to hundreds of protoscolices from the inner cysts wall. The protoscolices could get arranged either in clusters (*C. cerebralis* and *C. gaigery*) or in serial and straight rows (*C. serialis*) in the different intermediate hosts. The definitive host gets infection by ingesting the offals of slaughtered animals having mature *Coenurus* cyst. In the intestine of definitive hosts protoscolices result in the development of adult tapeworm (Soulsby, 1982).

Epidemiology

The authentic reports on the occurrence of coenurosis began to appear in the literature during the 17th century, although references to nervous disease with the symptoms of gid have been found in texts from the time of Hippocrates (Williams, 1977).

Coenurosis is worldwide in distribution but most commonly found in developing Southeastern Asian and African countries, where animals are mainly herded over open steppe grazing lands, beside sheep and goat population is kept in conjunction with dogs which help to control the flock. It accords ample opportunities for the transmission of tapeworm infections between two species (Welchman and Bekh-Ochir, 2006; Achenef *et al*., 1999). Poor awareness about the use of incinerators for disposal of contaminated carcasses left over further aggravates the situation. Thus intensity of infection of adult *T. multiceps* in dogs and larval (*C. cerebralis*) incidence in intermediate hosts in a particular area gives the real epidemiological picture of the disease. Although outbreaks of acute coenurosis in sheep and goats occur occasionally, the majority of cases are sporadic, chronic infections in animals of between one and two years of age. These animals may be potentially valuable members of the breeding stock as well as preferred for human consumption (Skerritt and Stallbaumer, 1984; Godara *et al*., 2011).

The epidemiology of coenurosis is also affected by: (i) age, although animals of all age groups (except 0-3 months due to a longer incubation period for cyst development) are affected, but the animals aged between 1 and 2 years are more susceptible (Sharma and Singh, 1997; Saikia *et al*., 1987; Achenef *et al*., 1999); (ii) sex, females show a higher prevalence than males (Karim, 1979; Sharma *et al*., 1998); (iii) season, prevalence of the disease is not affected by season (Sharma *et al*., 1998); (iv) host species, sheep are more susceptible than goats (Sharkhuu, 2001); (v) managemental practices, higher prevalence rate is observed in migratory sheep and goat flocks than those owned by permanent residents (Oryan *et al*., 1994; Jithendran and Katoch, 2003). The prevalence of coenurosis also depends on the agro-climatic zones, and may be affected by sociological and ecological factors (Sharma and Chauhan, 2006).

Clinico-pathological effects

It is difficult to identify the pathogenic effects of coenurosis and to quantify the economic losses it produces because of concomitant other metacestode infections. Economic losses resulting from coenurosis seems less apparent than those caused by other metacestode infections (i.e. hydatidosis or cysticercosis). Typically, the course of the disease is chronic, progressive and fatal. The animals suffering from coenurosis may present inappetance, dullness, frequent separation from the flocks, poor weight gain, lag behind, circling, torticollis, head pressing, feet stamping or walking in straight line, pain response on pressure over the cystic area and, sometimes, unilateral partial blindness (Ramdan *et al*., 1973; Karim, 1979; Gogoi *et al*., 1992; Nooruddin *et al*., 1996; Patro *et al*., 1997; Tafty *et al*., 1997). Superficial cysts may cause palpable rarefaction and pressure atrophy of the overlying skull, to the extent of perforation (Soulsby, 1982; Skerritt and Stallbaumer, 1984; Nooruddin *et al*., 1996). The presence of *Coenurus* cyst in lumbar region of the spinal cord causing posterior paralysis in an eight month old lamb (Bussell *et al*., 1997) and in a seven month old goat (Welchman and Bekh-Ochir, 2006) has also been documented. Sharma *et al*. (1995) and El-Sinnary *et al*. (1999) observed nodules or papillo-oedema of varying sizes over the skin and in different body parts in extra-cranial coenurosis in goats. Paliwal and Singh (1971) observed microscopic lesions of micro-abscesses in the brain of sheep.

The severity of the disease depends on the magnitude of the space occupied by the cyst and the lesions in the brain (Achenef *et al*., 1999). The affected animal is often easy to catch and appears to be depressed or unresponsive to sudden change of the events in the surroundings. The clinical signs develop intermittently and progressively become more overt prior to the death of the animal (Skerritt and Stallbaumer, 1984; Achenef *et al*., 1999; Godara *et al*., 2011).

The histopathological findings in cerebral coenurosis are focal pressure atrophy, congestion, hyperaemia, perivascular cuffing predominantly comprise of mononuclear cells, demyelination, liquidative degeneration and focal necrosis, beside neuronophagia, satellitosis and diffuse microgliosis leading to formation of microglial nodules (Gogoi *et al*., 1992; Tafty *et al*., 1997; Sharma *et al*., 1998; Achenef *et al*., 1999). The pathological lesions in hepatic coenurosis include compression of hepatic lobules, dilatations of sinusoids, vacuolar degenerative changes in the cytoplasm, karryorhitic changes in the nucleus and reduced size of hepatocytes leading to elongation of the cells (Godara *et al*., 2011).

The size, number and location of the cysts appear to be important in the pathogenesis of coenurosis. The size of the *Coenurus* cysts varies from 0.5 to 6.5 cm in diameter in the different intermediate hosts (Dinev *et al*., 1998; Sharma *et al*., 1998; Achenef *et al*., 1999; Biyikoglu *et al*., 2001). A large size *Coenurus* cyst weighing 145 g has recorded by Sharma *et al*. (1995) from subcutaneous tissue of a goat. The cysts can be found in different locations in the brain: the right and left hemispheres, median fissure, cerebellum, and the brain stem. Achenef *et al*. (1999) while studying coenurosis in Ethiopian highland sheep reported that 96% of the cases had cysts in cerebral hemispheres and remaining 4% in cerebellum. They observed negative correlations between cyst number and the

clinical course of the disease, the number and size of the cysts, the number of cysts and scolices, age and cyst size and age and cyst number, whereas a positive correlation was found between cyst size and number of scolices.

In a majority of *Coenurie* affected goats, the cysts anchor, develop and cause asymptomatic focal lesions in the extra cranial aberrant sites such as subcutaneous and intramuscular tissues and in the different organs of the abdominal cavity (Bhalla and Negi, 1962; Dey *et al*., 1988; Sharma *et al*., 1995; El-Sinnary *et al*., 1999; Godara *et al*., 2011). This may reflect a different host response to the parasite in goats or, alternatively, parasitism by larvae of another cestode species, *T. gaigery* (Clapham, 1942; Smyth and Heath, 1970; Soulsby, 1982; Smyth, 1994; Sharma *et al*., 1998).

Diagnosis

Diagnosis is based on clinical appearance, neurological examination, exploritative surgery or post-mortem examination. Although the clinical correlation of signs and locations of cysts is poorly developed (Palmer, 1976), interpretation of clinical signs remains the best method of diagnosis (Skerritt and Stallbaumer, 1984). Predilection of locations based on the direction of circling and head deviation had a success rate of 62% (Achenef *et al*., 1999) to 68% (Skerritt and Stallbaumer, 1984). The response to skin tests is inconsistent and specificity is low, probably because of cross-reactions with heterologous metacestode infections. Indirect ELISA was successfully used in the experimental *Coenurus* infection in sheep (Doganay *et al*., 1999), serological tests yet being unreliable. Computed tomography scan has shown encouraging results to diagnose coenurosis including exact size and location of the cysts (Diez *et al*., 1999; Gonzalo-Orden *et al*., 1999). As the presenting signs are so variable, knowledge of the endemic status and local epidemiological situation are helpful in making a presumptive diagnosis.

The differential diagnosis of the coenurosis with other similar clinical conditions includes listeriosis, nasal bots syndrome and cerebral echinococcosis have been discussed by many authors (Brewer, 1983; Clarkson and Faull, 1983; Achenef *et al*., 1999).

Treatment and control

Control of coenurosis has been a difficult task and unsatisfactory to date. Once clinical syndrome of coenurosis is established, the prognosis of the case becomes grave and case fatality rate is 100% (Ahmed and Ali, 1972). Earlier literature suggest simple aspiration of the cysts fluid through softened skull, preliminary investigations into the immunization of lambs against coenurosis, use of the various anthelmintics and other preventive measures, with surgery is only the feasible treatment.

Many anthelmintics (albendazole, praziquantel, bithionol, niclosamide, etc.) have been used against coenurosis with little or no success (Musakanov *et al*., 1991; Samdup, 1993; Aydin and Biyikoglu, 1996; Biyikoglu and Doganay, 1998). Surgery can be an effective form of treatment for coenurosis in cases which have skull softening, visual deficits, circling or a combination of these signs (Skerritt and Stallbaumer, 1984; Skerritt and Tadich, 1986; Kalita, 1997). The prognosis becomes grave where animal undergoes recumbency. Prophylactically, regular deworming of dogs is advised, besides destroying the cysts at slaughter, and separating dogs and livestock.

An alternative approach aiming development of acquired resistance in the susceptible host has been attempted by many workers (Laipanov, 1993; Kosmonkov *et al*., 1994; Talbani and Kosminkov, 1994). However, despite persistent efforts a vaccine with adequate protection against coenurosis has not yet been developed to the point of commercialization. Recently, Varcasia *et al*. (2009) gave promising results using recombinant proteins of *T. multiceps*, against naturally acquiring coenurosis in sheep.

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EPIDEMIOLOGY, TREATMENT AND MINERAL STATUS WITH DERMATOPHYTOSIS IN CALVES

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ABSTRACT

In the present study *Trichophyton verrucosum* was isolated from the 30 calves. The incidence of dermatophytosis was found in the age group of 1 to 24 months in calves. The incidence was found higher in male calves (70%) as compared to female calves (30%). Presence of ectoparasite along with *T. verrucosum* indicated the possible spread of fungi by ectoprasites. The occurrence of ring worm in age of 1-6 month, 6-12 months and more 12 months were 50%, 23.33% and 26.66%, respectively. There are rare reports of the possible role of zinc, selenium and copper concentration in the pathogenesis of cattle dermatophytosis. This study was conducted in veterinary hospital, Pannalal Gaushala, Jodhpur during the March 2007 to February 2008. After diagnosis confirmation by direct microscopic examination and fungi isolation inoculation on Sabouraud dextrose agar using skin scraps. Samples of infected calves, the zinc, selenium and copper concentration of plasma were determined by photometric stripping analyzer and atomic absorption spectrometry. Mean plasma concentration of zinc, selenium and copper in ring worm calves were 0.81±0.08, 0.10±0.008 and 0.48±0.03 mg/l, respectively. The corresponding values for healthy animals were 1.64±0.20, 0.72±0.006 and 0.82±0.04 mg/l. The average number of lesion were recorded 8.03 per calve. Results showed that plasma concentration of selenium and zinc in calves with dermatophytosis were significantly lower ($P<0.05$) than the healthy ones. Lesions were found more on face, dewlap, wither and on thorax region of the body. In conclusion, it seems that zinc, selenium and copper have a determinant role in immune status and the response of animals' immunity system to dermatophytosis. The result reported the higher infection rate in winter (76.67%) than spring (10%), autumn (6.67%) and summer (3.33%). The most effective treatment of ring worm was sodium Iodide and copper sulphate solution in concentration of 5 to 10% which were used as spray and was hundred percentage effective.

***Key words*:** Dermatophytosis, calve, zinc, selenium, copper, treatment, epidemiology, ringworm

Introduction

Ring worm infection medically known as dermatophytosis caused by dematophytes which are highly specialized group of fungi. They affect the superficial keratinized tissue (skin, hair and nails) of man and animals. It is a common superficial fungal infection found throughout the world (Kern, 1985).

It occurs in all species of animals including man. Ringworm is more common during the winter in stabled animals. It is a contagious and chronic primary skin disease. It is spore forming fungi spores are shed from the lesion by broken hairs or scabs from the lesion. The spores remain alive for years in a dry environment. Infected calves which confined to a barn is a source of infection. Spores germinate and attack the shaft of the hair and surface layer of the skins. Exudate oozes from the damaged skin and mixes with debris from skin and hair, thereby forming a crusty scab (Fig.1). The scab is grey white and noticeably higher than the surrounding skin. Infection of the skin and hair of cattle is most frequently due to *Trichophyton verrucosum.* It mainly occurs in calves. Transmission occurs primarily by ticks, infected animals, poor husbandry practice, close confinement, poor hygiene, contaminated fomites and soils (Barta, 1967). Dermatophytes grow only in dead keratinized tissues. Infection begins in growing hair. Ring worm has got zoonotic importance. In rural area, a sizeable percentage of human ringworm is attributable to animal ring worm.

Clinical examination

Calves of ward No.7 and 8 were affected in Panna Lal Goshala, Jodhpur. Calves were more than capacity of ward and in poor hygienic condition. Ticks infestation were found in ward. The animals health condition were not very good. Generally effected animals had normal body temperature, pulse, respiration and other body systems were also normal. Hairs in the infected areas breaks off or fall out. Skin lesions were thick, round, white, crusted plaques and expanded at the periphery. Lesions were present in different size at head, neck, thorax, abdomen, legs. There were no pruritis. The affected calves as suffered from alopecia and circumscribed greyish-white, crusty, raised lesions (Fig.1 and 2). This finding agreed with Radostits *et al*. (1997), Patel (1987) and Wabacha *et al*. (1998).

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Materials and Methods

Blood samples (15-20 ml approximately) were collected. All the samples were centrifuged at 3000 rpm for 15 min. Plasma was harvested and stored at -20^{0}C until assayed. Plasma samples were digested and minerals constituents were estimated using kits supplied by Bayer Diagnostics, Baroda, India. In the present study 30 cases of calves and heifers were selected for treatment. Animals were in the age groups of 1 month to 2 years. Determination of age was done by dentition and history of care taker. Complete general examination of all the animals were carried out. The lesions were recorded in relation to its anatomical distribution shape, size and the body region and time of the appearance of skin lesion were also reported. Skin scrapping was collected and examined microscopically after 10% KOH digestion. The surface of affected area was rubbed with a cotton scab impregnated with 70% ethyl alcohol to remove surface adhering organisms. Skin scales were collected by scrapping of the margin of the lesion using a sterile scalpel blade into Petri dish. Hairs were collected by removing dull broken hairs from the margin of the lesion as described by Cheesbrough (1992). Each sample collected was divided in to two portions. One portion was used for directed microscopic examination. The second portion was cultured on Sabarounds dextrose agar (SDA) with cycloheximide which were done at Microbiology.

Department at Medical College, Jodhpur. Microscopic examination for positive fungi culture was done using the lectophenol cotton blue wet mount method (Halley and Standard, 1973).

Diagnosis

Diagnosis based on typical clinical symptom and direct microscopically examination of skin scrappings. For staining of scrapping chlorazole black E solution were used.

Treatment

Many of the treatments appear successful because of spontaneous recovery shortly after treatment has been started. It occurs mainly in winter. Ringworm is frequently severe in confined cattle or calves during the winter (Ajello and Padhye, 1974; Ripon, 1974). Topical treatment, application of the medication directly onto the lesion, is the usual procedure. Medication cannot penetrate the crusts. The crusts were removed by scrapping or brushing. All the crusts were collected and burned to avoid contaminating the premises. Lesions were treated at least twice. Oral antifungal like griseofulvin may be used but the prolonged treatment and expense of the drug make it impractical in all the cases.

Different treatment methods were applied. Animals were divided in 2 groups each group having 15 animals.

Group A: For treatment 10% solution of sodium iodide were used for 5 days than weekly till recovery. Orally sodium iodide 1 gm/15 kg were given according to body weight for 7 days.

Group B: In this group of animals were treated by 5% solution of copper sulphate spray were done for 15 days. After treatment slowly hairs were growing.

Our results showed that rapid and effective cure of affected calves occurred with 7 applications of daily locally tropical application of sodium iodide 10% with 100% of complete recovery and this result was higher than that recorded by Pandey (1979). Lesions started to subside gradually and after 2 weeks the hair started to grow again. Within one month there was a complete recovery. The same recorded result was found after the application of copper sulphate solution, recovery cases, respectively. These results are in agreement with Jungerman and Schwartzman (1972) who shows that copper sulphate in concentration of 5 to 10 % is fungicidal and in contrast to Wabacha *et al*. (1998).

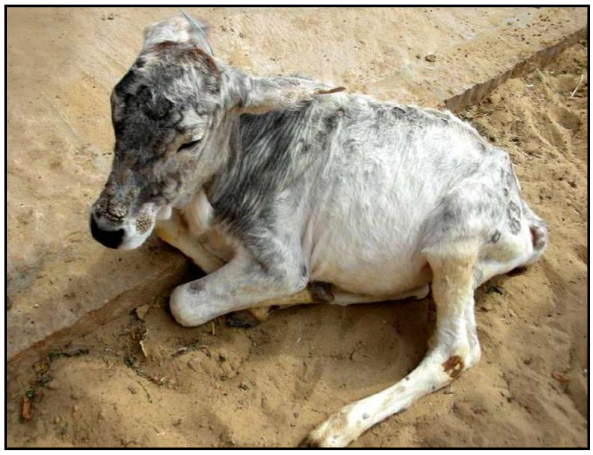

Fig. 1: Calf showing the lesion of ring worm

Fig. 2: Calf showing the lesion of ring worm

Results and discussion

Among calves, ringworm infection was common in winter season (76.67%) in compare to spring (10%), autumn (6.67%) and summer (3.33%). This result may be attributed to that the animals were stabled for longer periods of time during cold season as well as high humidity during winter season, which facilitate the growth of spores and increases the susceptibility of animals to infection (Nooruddin and Singh, 1987).

Sex distribution of ringworm infection among animals showed that more male than female were infected (70% and 30%, respectively). This result may be attributed to animals housed close proximity to each other for long periods in fattening period and the presence of infected one leads to spreading spores of fungus between animals so leads to greater infection rate in males than females.

Prevalence to infection was found amongst calves under the age of 1-6 months in compare to 6-12 months and more than 12 months (50%, 23.33% and 26.66%, respectively). This result showed that infection decreased with increase animal age. This result agreed with Acha and Szyfres (2003) who reported that dermatophytosis is more common when animals are immunosuppressed.

The average number of dermatophytosis was 8.03 per calve. The average numbers of lesions were high in comparison to other studies. It is probably due to the unhygienic conditions and close confinement. The average lesions on body part are presented in Tale 3. Edwardson and Andrews (1979) reported the average of 10.5 and 8.95 lesions per cattle. The lesion were of varying size 1 mm to 18 mm) with wide variation in shape of circular, ovoid to coalescing lesions with irregular in shape are in agreement with the reports of workers (Anon, 1970; Jungerman and Schwartzman, 1972; Radostitis *et al*., 1997 and Jani *et al*., 2000). The most common sites of ringworm lesions in the present were on and around eyes, ears, jaws, neck, thorax and in few animals on abdomen, hindquarter and tail. These are in agreement with Pal and Singh (1983); Thakur *et al*. (1983).

In this study was planned to determined plasma zinc, selenium and copper in ring worm calves. Mean plasma concentration of zinc, selenium and copper in ring worm calves were 0.81±0.08, 0.10±0.008 and 0.48±0.03 mg/l, respectively. The corresponding value for healthy animals were1.64±0.20, 0.72±0.006 and 0.82±0.04 mg/l. There were significantly lower plasma zinc, selenium and copper in ringworm calves ($P<0.005$). It was concluded that the deficiencies of copper, zinc and selenium either singly or in combination could be responsible for ringworm in claves and by improving the nutritional status the ringworm can be prevented in calves. Zinc and selenium have determinant role in immune status and helpful to increase immunity system to dermatophytosis (Kojouri *et al*., 2009).

In this study animals were treated by two different types of treatment. Treatment was effective in both treatment groups. The occurrence of dermatophytosis in cattle with the high incidence in young calves than in adult has been reported (Nooruddin and Dey 1984). The long confinement and overcrowding of calves predispose to dermatophyte infection (Thakur *et.al.,* 1983). Lesions were present commonly around eyes, ear, jaws, neck, thorax and few numbers of abdomen, hind quarter and tail. These are in agreement with Pal and Singh (1983), Thakur *et al.* (1983). The lesson were different size with variation in safe of circular, ovoid to coalescing with irregular in safe are in agreement with the reports of the workers (Anon, 1970; Jungerman and Schwartzman, 1972;). Plasma zinc, selenium and copper values were lowest. It has been suggested that deficiency of zinc, selenium and copper may by responsible for dermatophytosis.

The prevalence of dermatophytosis in cattle with the high incidence in calves than in adult. The long confinement and over crowding of calves predispose them to dermatophytosis. In the present study detection of ectoparasites (ticks) was found in the calves. The role of ectoparasites in the transmission of ringworm in cattle suggested that the ectoparasite might spread the disease in animals. Similarly, spreading of ringworm infection in presence of lice, fleas and mites have also been studied (Patel, 1987). The lesions were more diffuse covering wider area of body in those calves which are harbouring the ticks in the present study. The presence of trichophyton verrucossum in ectoparasites has been reported by many workers (Kral Schewarrtzman, 1964; Jenson and Mackey, 1971).

Acknowledgements

We are thankful to President, Panna Lal Gaushala, Head of Department, Microbiology, Medical Collge, Jodhpur and Dean, Veterinary College, Bikaner for their help and cooperation.

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Table 1: Age wise incidence of dermatophytosis in calves (n=30)

S. No.	Age	Affected no. of calves	Percentage
1.	1-6 months	7	23.33
2.	6-12 months	15	50.00
3.	More than 12 months	8	26.66

Table 2: Sex wise incidence of dermatophytosis in calves (n=30)

S. No.	Sex	Affected no. of calves	Percentage
1.	Male	21	70.00
2.	Female	9	30.00

Table 3: Body region wise mean, standard error, range and standard deviation of lesions (n=30)

S. No.	Body region	Right side	Left side
1.	Ear	3.03± 0.29 R1-7=6 SD=1.62	5.3± 0.32 R1-9=8 SD=1.80
2.	On and around eye	3.76± 0.31 R1-7=6 SD=1.71	2.1± 0.16 R1-4=63 SD=0.88
3.	Upper jaw and lip	8.03± 0.22 R 6-11=5 SD=1.24	7.76± 0.31 R4-11=7 SD=1.73
4.	Lower jaw and lip	6.9± 0.33 R3-12=9 SD=1.82	6.36± 0.27 R3-10=7 SD=1.51
5.	Neck and dewlop	7.03± 0.33 R3-9=6 SD=1.84	5.76± 0.33 R2-9=7 SD=1.85
6.	Wither and thorax	5.93± 0.26 R2-9=7 SD=1.43	4.56± 0.33 R1-8=7 SD=1.85
7.	Abdomen	1.93± 0.16 R1-4=3 SD=0.90	3.1± 0.29 R1-7=6 SD=1.62
8.	For quarter	1.66± 0.13 R0-3=3 SD=0.75	2.9± 0.25 R1-6=5 SD=1.39
9.	Hind quarter	0.3± 0.08 R1-1=1 SD=0.46	0.53± 0.10 R0-2=2 SD=0.57

Table 4: Period of sampling during the year (n=30)

S. No.	Season	No. of cases	Infestation rate (%)
1.	Winter	23	76.67
2.	Autumn	2	6.67
3.	Summer	1	3.33
4.	Spring	3	10.00

Table 5: Plasma mineral concentration in dermatophytosis

S. No.	Mineral	Value in diseased animal (mg/dl)	Normal value (mg/dl)
1.	Zinc	0.81±0.08	1.64±0.20
2.	Selenium	0.10±0.008	0.72±0.006
3.	Copper	0.48±0.03	0.82±0.04

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

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NITRATE AND NITRITE TOXICITY IN FARM ANIMALS

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Though nitrate toxicity among cattle is very common in our country, it is not diagnosed in the field condition due to various factors like lack of diagnostic laboratories, etc. Further, it is very difficult to diagnose by observing clinical signs alone since the animals suffering with nitrate toxicity will not exhibit any cardinal signs. The affected animals will show different set of clinical symptoms depending upon the percentage of methaemoglobinaemia. The toxic principle as it occurs in growing plants is always nitrate, usually as potassium nitrate, and may be ingested in sufficient quantities to cause gastroenteritis (Radostits *et al*., 2000).

Nitrates and Nitrites both are toxic. Nitrates act primarily as gastrointestinal irritants causing gastroenteritis (Sharma *et al*., 2010). Systemic absorption of nitrate as such is not reported but the toxicity is mainly due to nitrite (NO_2) ions but not nitrate (nitrite ion is 10 times more toxic than nitrate ion). Nitrate poisoning occurs when an animal eats forage material with high nitrate content (in excess of 0.35 to 0.45% nitrate in diet). Drinking of water contaminated with nitrogenous fertilizer also causes nitrite toxicity (Al-khafaji, 1996). The nitrite toxicosis also occur due to water in newly dug bore wells that contained nitrate levels of 1500 mg/ml (Sarathchandra *et al*., 1997). The environmental factors like soil nitrate being taken up but not used by plants because weather conditions are unsuitable for photosynthesis which would provide the energy to convert the nitrogen in to protein. Conditions which retard the photosynthesis include cloudy or cold weather, at night, herbicide application, disease in the plants, wilting of plants after a prolong drought (Pickrell *et al*., 1991). High level of nitrate accumulates in the soil during the drought and is not leached out because of the absence of rain. Plants absorb large amount when the drought ends. Accidental poisoning with commercial nitrate compounds occurs sporadically when nitrates used as explosives to blast out water holes used to store drinking water for cattle can be dangerous if the nitrate is left in the hole and the dam fills soon afterwards (Yong, 1990). The nitrate is converted into nitrite in the rumen by the rumen bacteria. Normally, the nitrite is converted to ammonia and used by rumen microbes as a nitrogen sources. If nitrate intake is faster than its breakdown to ammonia, however, nitrate ions will begin to accumulate in the rumen. Nitrite is absorbed rapidly into the blood system where it oxidizes haemoglobin into methaemoglobin, a substance which is incapable of transporting oxygen to body tissues. The animal dies from asphyxiation. The lethal dose of sodium nitrite in cattle ranges from 15.8 to 31.8 g/head and 47 to 108.5 mg/kg body wt. (Matsumoto *et al*., 1976). Nitrite poisoning is highly fatal as it forms methaemoglobin which is unable to transport oxygen. As a result of this, anaemic anoxia occur causing respiratory distress. Tissue anoxia is also there due to potent vasodilator action of nitrites causing peripheral circulatory fail-ure, but it is of little significance in causing death as compared to methaemoglobinaemia. Different species of animals have different threshold of continuing life with methaemoglobinaemia, in general, it is around less than 75 per cent of haemoglobin being converted to methaemoglobin.

The kinetics of nitrates and nitrites differ in dogs. There was signifi-cant salivary excretion of nitrates through saliva, whereas, in the bile large quantities of nitrates were excreted in dogs given nitrites indicating an endog-enous oxidation of the nitrite. In cattle and sheep nitrite poisoning was created by injecting sodium nitrite @ 110 mg/kg body weight (Barat *et al*., 1984).

Cumulative poisoning of nitrate is not reported but, Monge (1984) re-ported sudden deaths, lameness, low fertility, abortion, low productivity and retarded growth due to nitrate poisoning through drinking water (200-500 ppm), if accompanied by primary copper deficiency aggravated by excess sulphates and molybdenum. Some minerals such as molybdenum, copper, iron, magnesium, and manganese are involved in the complete reduction of nitrate to ammonia, which avoids nitrite accumulation. Cattle on some rations, especially all-forage diets in some areas, may lack some of these or other minerals that are important for normal rumen metabolism (Richard *et al*., 2010). The lethal dose for nitrate-nitrogen given as a drench is about 45 mg/kg of body weight for cow. However, given in forage the LD_{50} for nitrate is more than 5 times this amount. The toxic compound is nitrite, produced as bacteria reduce nitrate to ammonia. Slow intake and balanced rations reduces nitrate toxicity. Water containing less than 100 ppm NO3-N would be safe for livestock unless other adverse factors also exist (Crowby, 1985).

Prasad *et al.* (1984a) produced typical nitrite poisoning in buffalo calves by administration of sodium nitrite at 100 mg/kg body weight into the rumen through a stomach tube. Experimental poisoning of cattle calves @ 135 mg/kg body weight after over-night fasting has been produced (Bhikane *et al.,* 1990).

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All livestock are susceptible to nitrate toxicity. Pig, cattle, buffaloes, sheep and horses are more susceptible to poisoning (Sharma, *et al*., 2010). Animals under physiological stress (pregnant, lactating, hungry and sick) are more susceptible to nitrate toxicity than healthy animals. Sudden dietary change with introduction of suspected forage and excess carbohydrate feeding.

Factors influencing the nitrate accumulation in plants

1. Use of high level of nitrogenous fertilizers in the field
2. Cereals and weeds accumulate more than legumes and grasses.
3. Stage of forage development. More nitrate accumulates in young stage than mature plant.
4. Light intensity and temperature: Poor light intensity (shade) and high temperature will increase the nitrate content of plant. Higher concentration of nitrate accumulates in the plants during night.
5. Damaged due to frost or trampled fodder will produce more toxicity.
6. Drought affected fodders/crops will have very high content of nitrates.
7. After application of herbicides, Nitrates accumulation will be more.
8. Fodder grown by irrigating with sewage water.
9. Crops like millet, maize, *Bajara*, wheat, barely, oats, soya, mustards, sunflower, sorghum hybrids. the grasses like lucerne, stylo, kikyu, desmonthus, high bred varieties of fodders and the vegetables: carrot, radish, potato, beet root tops, turnip, sugar beets tops, cabbage, cauliflower, cucumber and all greens.

The toxic effects of nitrate may be caused by two separate mechanisms. (1) Acute irritation of digestive system and (2) formation of methaemoglobin in the blood. Depending upon the percentage of conversion of haemoglobin into methaemoglobin the symptoms will vary by affecting respiratory, circulatory, nervous and reproductive system. Leads to Vitamin A deficiency and thyroid deficiency in chronic cases.

Clinical manifestations

In peracute cases the death occurs without showing any symptoms. In acute cases the haemoglobin conversion 80-90%, cyanosis, respiratory distress, rapid weak pulse, weakness, recumbency and death. In sub-acute cases due to irritant effect of nitrates there is salivation, lachrymatioin, grinding of teeth, vomiting, colic, diarrhoea, weakness, ataxia, muscular tremors, convulsions, respiratory disorders, death. In chronic cases listlessness, lethargy, depressed feed intake, reduced milk production and infertility. The sub-clinical cases has 10-20% haemoglobin conversion with brownish mucosa, abortion/stillbirth, retention of placenta and cystic ovaries. Fatal affected animals go down, show severe depression and terminal clonic convulsions, and death occurs from a few minute to an hour after onset. Frequent urination and abortion are other recorded signs, and in some outbreaks in cattle the principal problem is abortion a few days after exposure (Norton and Campbell, 1990).

Diagnosis

Nitrite poisoning is difficult to diagnose unless the blood samples are collected during life and their contents of nitrite, or more commonly nitrate, is estimated by the diphenylamine test (Bhikane and Bhoop Singh, 1990). Diagnosis of nitrate toxicity is based on observed clinical signs and assessing the possibility of exposure to toxic plants or water. Field practitioners can suspect nitrate toxicity when the blood is dark red to coffee brown on venipuncture or on post-mortem. Suspected plants/water for the presence of nitrate can be tested. To confirm a field diagnosis of nitrite poisoning, a laboratory should receive, not only the samples usually recommended, but also blood diluted in 20 volumes of phosphate buffer (pH 6.6) and 1-2 ml of urine. Methaemoglobin in blood is stable for 48 hours after dilution in buffer. Nitrite is detectable in the urine for 24 hours (Watts *et al.,* 1969). Diphenylamine blue test (DPB) on blood, plasma, urine, CSF, rumen liquor and aqueous humour show a strong positive reaction at the peak of nitrate toxicity. The CSF nitrite appeared to be diagnostic since it reflected the nitrate toxicity (Mondal and Pandey, 1999). For testing of forage, cut in the lower stem of the fodder or plant and apply one drop of test solution to the cut site. If an intense blue colour appears in few seconds, the fodder contains potentially dangerous level of nitrate. For testing in water, take a small white cup or an eggshell and put one ml of reagent into the cup and add one or two drops of suspected water by placing in the sides of cup. A blue colour diffusing out of water into the reagent is regarded as positive for nitrates. Do not stir. A positive reaction reaches greatest intensity within 1 to 2 minutes.

The use of a commercial reagent strip (Combur 9), produced for urine analysis, is ten times more sensitive than DPB in detecting nitrate in urine may be equally sensitive in aqueous humour (Montogomery and Hum, 1995). The chemical tests include detection of methaemoglobin by examination of the blood in rever-sion spectrometer. It is not diagnostic of nitrate poisoning. Other test is estimation of nitrite or nitrate content of fodder. Filter paper strips (Rivanol paper strip test) impregnated with rivanol citrate solution was used to detect natural and experimental cases of nitrite poisoning in pigs. Results were identical with laboratory findings. This is claimed to be highly specific and sensitive (Lin *et al.,* 1987) and anion chromatographic method was used to simultaneously determine nitrate and nitrite ions in bio-logical samples. Ultrafiltration was used to produce a pro-tein free filtrate. Chloride interferences were eliminated by precipitation as the

nitrite ions in bio-logical samples. Ultrafiltration was used to produce a pro-tein free filtrate. Chloride interferences were eliminated by precipitation as the silver salt. Detection limit and average recoveries were 0.5 mg/litre and 102 per cent for nitrate and 0.2 mg/litre are 78 per cent for nitrite, respectively.

Treatment

One per cent methylene blue solution, 4 mg/lb body weight, i/v Repeat only once, if necessary. Methylene blue is an oxidising agent and is converted by NADPH2-dependent system to leuco-methylene blue, which then reduces methaemoglobin to haemoglobin. Laboratory grade methylene blue is not to be used for intravenous use to any animal. Use pharmaceutical grade only for i/v use.

Injection of 2 per cent methylene blue solution @ 9 mg/kg as an anti-dote at the first appearance of clinical signs rapidly overcame methaemo-globinaemia (Barat *et al.,* 1984).

During gastrointestinal symptoms, the antihistamine Injection of Tripelennamine hydrochloride (Pyribenzamine) @ 0.5 mg/Kg Body weight at 8 hourly interval (maximum dose should not exceed 240 mg). For removal of irritants, drench 250 ml of liquid paraffin orally. In all cases of chronic and subclinical toxicity, injection of Vitamin A 3000 IU/ 100 lb body weight i/m daily for one week.

At the time of collapse due to fall of blood pressure 2 to 4 ml 1:1000 adrenaline to ruminants. It is ideal to choose the Injection of etamiphyline camsylate @ 3 mg/kg body weight is suggested. Do not give calcium injection i/v.

Vitamin C @ 20 mg/kg was infused intravenously 1-2 hours after ni-trite poisoning. The clinical symptoms pertaining to heart rate, respiration, urination and inappetance improved within a period of 4-24 hours. The methaemoglobin level dropped significantly by 8 hours (Prasad *el al.,* 1984b).

Oral administration of sodium tungustate might be of use in preventing acute nitrate-nitrite poisoning in calves (Bicudo, 1987).

Sheep given selenium or vitamin E had lower methaemoglobin values after experimental sodium nitrite poisoning (Popeseu *et al.,* 1986).

Infusing L-cysteine with nitrate reduced nitrite production in the ru-men and methaemoglobin formation and consequently higher rates of the gas exchange and metabolism occurred. It is concluded that L-cysteine or a treat-ment utilising a similar effect or mechanism, has prophylactic potential against nitrite poisoning in ruminants (Takahashi and Young, 1991).

Tolonium chloride (1%, 20 mg/kg body weight) was superior to methylene blue and ascorbic acid for treatment of nitrate toxicity in goats (Mondal and Pandey, 2000).

General guidelines for handling nitrates

A. Blend high-nitrate forages with low-nitrate feeds, such as grain.
B. Feed adequate energy, vitamins, and mineral mixtures. Have all feeds analyzed, and balance rations accordingly.
C. Do not feed hungry animals feeds that are high in nitrates. Offer low-nitrate feeds first and then high-nitrate feeds.
D. Do not free choice feed forages containing more nitrate.
E. Regulate intake of feeds so that changes are gradual. Feed limited amounts several times a day rather than free choice or only once or twice a day.
F. Introduce questionable feeds over a period of two to three weeks so that rumen microbes can adapt to higher nitrate levels.
G. Administer Lixen Bolus (Cephalexin) or Amoxirum Bolus (Amoxycillin) orally to ruminants to prevent the conversion of nitrate in to nitrite

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ROLE OF MICRONUTRIENTS IN REPRODUCTION: AN OVERVIEW

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In farm animals reproductive efficiency is very important for their economical rearing. If cows do not produce a calf every year, the cost of production will increase due to cost of keeping open/non-productive cows. Reproduction is influenced by many factors like hormone levels, disease, mycotoxins, nitrates and other plant toxins and nutrition. As the production potential of animal has increased over the time through careful genetic selection, various reproductive parameters have shown a decline. Conception rate at first service for cows declined from 66% in 1951 to 40-52% in 1986 (Butler and Smith, 1989). The decline in reproductive performance is related to the dramatic improvement in milk yield and the increased nutrient demands placed on the cow. Energy and protein are the most limiting nutrients in early lactation and severe deficiency of these two over an extended period leads to delayed conception. Apart from macro-nutrients, minerals and vitamins are essential for optimum production and reproduction. In the present discussion, emphasis will be only on those vitamin and minerals which influence reproduction directly i.e. vitamins A, D and E which are considered to be important for reproduction. Similarly minerals like Ca, P, Se, Zn, Cu, Co etc. also play important role in reproduction (Hurley and Doane, 1989).

Functions of minerals

Four broad types of functions exist for minerals:

1. **Structural:** Those minerals which can form structural components of body organs and tissues viz. calcium, phosphorus, magnesium, fluorine and silicon in bones and teeth and phosphorus and sulphur in muscle proteins.
2. **Physiological:** Those minerals which occur in body fluids and tissues as electrolytes, concerned with the maintenance of osmotic pressure, acid base balance and membrane permeability viz. sodium, potassium, chlorine, calcium and magnesium.
3. **Catalytic:** Those minerals which can act as catalysts in enzyme and hormone systems, as integral and specific components of the structure of metallo-enzymes or as less specific activators within those systems viz. manganese, molybdenum and selenium.
4. **Regulatory:** Those minerals which have been found to regulate cell replication and differentiation viz. calcium influences signal transduction and zinc influences transcription.

Vitamin A

It is most commonly deficient vitamin in cattle. Ganguly *et al.* (1980) studied the role of vitamin A on reproduction in rats. They reported that in male rat the classic symptoms of vitamin A deficiency include inhibition of spermatogenesis, reduction in testicular size, and decline in testicular steroidogenesis. The vitamin A deficiency in females is characterized by keratinization of the vaginal epithelium and a failure to conceive. Even in mild deficiency of vitamin A, the germinal epithelium contains mostly spermatogonia with few spermatocytes, and no spermatids. The rats raised on a vitamin A-deficient diet supplemented with retinoic acid show normal growth but their testes appear more like those of vitamin A deficient rats. Similarly mostly spermatogonia, with a few spermatocytes but no spermatids, are found in the seminiferous tubules of the testes of these rats. This shows that even the initial steps of mitosis leading to the formation of spermatocytes from the spermatogonia are arrested in these rats.

The effect of vitamin A deficiency is less visible in rat ovaries except that the ovaries are smaller in size. Retinoic acid supplemented female rats appear normal and conceive but pregnancy is terminated by gestation resorption around day 14 of pregnancy (Juneja *et al.,* 1964). The rate of cell division in both placenta and foetus of retionate-fed pregnant rats is markedly reduced around day 14 of their pregnancies. It has been shown that when rats are subjected to unilateral ovariectomy, the consequent compensatory growth of remaining ovary is markedly less in the retionate-treated animals compared with normal controls. The activity of enzyme 3-b-hydroxy-D^5-steroid dehydrogenase which is a key enzyme in the steroidogenesis that converts pregnenelone to progestrone is lower in retionate fed rats. The ovaries

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of retionate fed rats are also less responsive to gonadotropin stimulus. Jayram *et al.* (1973) demonstrated that the activity of the cholesterol side chain cleavage enzyme, which is a rate limiting enzyme in the biosynthesis of steroids, is markedly lower in the testes and ovaries of the retionate-fed rats.

Vitamin A is essential for embryogenic development of cardiovascular and nervous system. Several developmental genes regulated by vitamin A during early embryogenesis have been identified (Zile, 1998). Retinoic acid has been reported to affect expression of transforming growth factor (TGF-b) which is a major modifier of extracellular matrix and adhesive molecules (Schmid *et al.,* 1991). Reproductive efficiency of cows is maintained when diets provided 0.18 mg of b-carotene/kg BW (72 IU vitamin A/kg b.wt.). *In vitro* rumen studies found that 67 to 72% retinol was destroyed within 12 h of incubation when rumen fluid was obtained from cattle fed 50 or 70% concentrate diets. The *in vitro* destruction was 16 to 20% in rumen fluid obtained from cattle fed high (> 75%) forage diets (Weiss, 1998). Talavera and Chew (1987) reported that retinoic acid and b-carotene stimulate the utilization of low-density lypoproteins for progesterone synthesis in luteal cells.

Vitamin D

Receptors for 1, 25-dihydroxy D_3 have been found in number of reproductive tissues including ovary, uterus, placenta, testes and pituitary. Vitamin D is thought to function in regulating intracellular Ca and Ca--binding proteins in these tissues. Placental tissue metabolizes 25-hydroxy D_3 to 1, 25-dihydroxy D_3 and then to 24, 25-dihydroxy D_3. Specific receptors for the latter metabolite have been identified in several skeletal tissues but not in reproductive tissues. Tsang and Grunder, (1984) reported that deficiency of vitamin D affects synthesis and catabolism of estradiol in the hen. *In vitro* cultures of rat pituitary cell line can be stimulated to synthesize prolactin by 1, 25-dihydroxy D_3 (Work and Tashjian, 1982). A role of vitamin D in skeletal growth of the foetus may be mediated by vitamin D-dependent Ca-binding proteins in the placenta (Warembourg *et al.,* 1986). Plasma concentration of < 5 ng/ml indicates a vitamin D deficiency and values between 20-50 ng/ml indicate adequate vitamin D status in dairy cows (Horst *et al.,* 1994). Current recommendation is 30 IU vitamin D/kg b.wt.

Vitamin E and selenium

Vitamin E and Se act as antioxidants and are essential for cell integrity. Free radical catalysed peroxidation is a continuous biological process causing damage to cellular and intracellular structures. The superoxide dismutase, glucose-6-phosphate dehydrogenase, and glutathione peroxidase are important enzymes involved in disposing of reactive oxygen species. Se is the component of the enzyme glutathione peroxidase which functions in cellular oxidation - reduction reactions to protect the cell from oxidative damage by free radicals. It is located in cytosol. Vitamin E also functions as an antioxidant. It is lipid soluble and primarily associated with cell membranes. Abortion, retained placenta, early embryonic death and infertility have been associated with Se deficiency. Several measures of Se adequacy have been used. These include serum or plasma Se, whole blood Se, liver Se, plasma glutathione peroxidase and blood glutathione peroxidase. Buchanan *et al.* (1969) reported no differences in fertility in ewes fed purified diets sufficient or deficient in Se provided vitamin E was adequate. Vitamin E and Se supplementation increased the uterine contractions moving towards oviduct in ewes. Glutathione peroxidase of which Se is a component may be protecting ovum from oxidative damage prior to ovulation, thereby, affecting subsequent fertilization.

Vitamin E deficiency in the male rat does not impair LH and testosterone or FSH and inhibin feed back loops, but causes testicular degeneration at intratesticular level. Vitamin E may affect germ cell development through some mechanism other than as a cellular antioxidant. Selenium concentration of various tissues and semen increases following administration of Se to bulls, but supplementation does not influence content or viability of sperms (Segerson and Johnson, 1979). Se deficiency prevails in areas where plant Se content is < 0.5 ppm. Vitamin E in dry cow diet is reported to reduce the incidence of clinical mastitis in periparturient cow (Weiss *et al.,* 1990).

In addition to general antioxidant, Se and vitamin E may be involved indirectly in prostaglandin synthesis where peroxy radicles are a normal part of metabolic pathways. Vitamin E has been implicated in control of phospholipase A_2 activity which is responsible for cleaving arachidonic acid from membrane phospholipids. Arachidonic acid is common precursor for all prostaglandins. Selenium preferentially accumulates in the placentome, ovary, pituitary and adrenal glands suggesting specific requirement for Se in these tissues (Harrison and Conrad, 1984). Selenium also accumulates in testes and atleast two selenoproteins have been identified in the rats that do not have glutathione peroxidase activity. Selenium in bovine spermatozoa is associated with low molecular weight protein (Niemi *et al.,* 1981). Despite their potential role in reproduction, identification and functional characterization of specific selenoproteins in reproductive tissues have not been done extensively.

Vitamin B-complex

Folic acid supplementation of sows has been reported to increase litter size and embryo survival (Trembley *et al.,* 1989). Oestrogen and progesterone replacement therapy do not prevent foetal loss in pantothenic acid or folic acid deficiency, suggesting that steroid action on target tissues is impaired (Leathem, 1966). Pyridoxine (B_6) deficiency in rat results in loss of oestrus cycles and embryonic resorption. Administration of oestrogen and progesterone to pyridoxine-deficient rats maintains pregnancy, suggesting that hormonal inadequacies are induced in the pyridoxine-deficient state. Pituitaries of pyridoxine-deficient rats have elevated gonadotropin contents and subnormal follicle development and atrophy of ovarian interstitial tissue. This suggests that there is decreased gonadotropin release from pituitary. In ruminants, the deficiency of B-vitamins is generally not there, but in high producing cows during early lactation there may be requirement of some B--vitamins. Biotin is reported to decrease the incidence of lameness and consequently improved reproductive efficiency. Niacin is also reported to improve the production performance but its impact on reproduction is very limited.

Calcium and Phosphorus

Calcium deficiency is a problem of stall-fed animals, where as phosphorus deficiency is more common in grazing ruminants. Phosphorus is commonly known as fertility mineral.The adult animal contains 0.6 to 0.75% P on fresh basis. The average weight of P in 600 kg cow, 100 kg sow, 50 kg sheep, 20 kg dog and 2 kg hen are 3600, 460, 280, 135, 13 g, respectively. Phosphorus is one of the main structural elements of the body. It is part of nucleic acids and ATP. Phosphoric acid is a component of a large number of coenzymes. These are coenzyme A, transaminase (pyridoxal phosphate), carboxylases (lipothiamide pyrophosphate) and ATP that is present in all body cells and acts as donor of energy. It is well established that c-AMP, a derivative of ATP, acts as a second messenger in a large number of protein hormones. In deficiency, there is decrease in the blood level of inorganic and lipid phosphorus and cholesterol. The conversion of b-carotene to vitamin A is reduced resulting in lower liver vitamin A. The activity of alkaline phosphatase in blood decreases sharply. Impairment of the reproductive function by P deficiency is a reflection of overall metabolic disturbances due to lower feed intake and an insufficient supply of phosphorus. Milk production has preference in phosphorus utilization over reproductive cycle. Earlier studies showed that P supplementation improves the reproduction in farm animals. Black *et al.* (1943) showed that calf crop was improved by individually supplementing phosphorus to beef cows in the gulf coast region of the Texas. Supplemented cows annually produced 70.3 kg more calf per cow than controls (181.9 vs 111.6 kg). Read *et al.* (1986) reported that cows supplemented with dicalcium phosphate provided as a free choice lick had a 71% weaned calf crop compared with a 48% weaned calf crop for un-supplemented cows. Wu *et al.* (2000) fed three levels of phosphorus viz. 0.3%, 0.4% and 0.5% for entire lactation. They observed that P level of 0.4% in diet is optimum for normal reproduction in dairy cattle.

Ca-dependent mechanisms are involved in steroid biosynthesis in testes, adrenal glands and ovaries. Although Ca is not necessary for basal testosterone production by Ledig cells, maximal *in vitro* LH-stimulated testosterone production requires Ca (Janszen *et al.,* 1976*).* This involves a Ca and phospholipid dependent protein kinase. Ca may also have a role in steroidogenesis by influencing delivery or utilization of cholesterol by mitochondria. Ca also stimulates the conversion of pregnenelone to progesterone. The action of Gonadotropin-releasing-hormone on pituitary to release LH is mediated through Ca. LH is not secreted in the absence of Ca or in the presence of Ca blocking agents. Reduced blood Ca may delay uterine involution and increase incidence of dystocia, retained placenta and prolapsed uterus (Risco *et al.,* 1984).

Micro-minerals and their importance

It is generally agreed that micronutrients have an effect upon fertility of cattle. The mineral requirements for reproduction in mammals are usually equated to the mineral content of the foetus and products of conception and therefore increase exponentially to reach a peak in late gestation. There is also a small additional requirement for growth of mammary tissue and the accumulation of colostrum prior to parturition. For those minerals where the generosity of maternal nutrition determines the size of fetal reserve viz. copper and selenium, it may be prudent, but not essential, to allow for maximal foetal retention (Nocek *et al.,* 2006). Most micronutrient deficiencies exert their effects upon reproduction through depression of the activity of rumen micro-flora, reduction in enzyme activity affecting energy and protein metabolism and the synthesis of hormones. Of the 15 essential trace elements, Zn, Cu, Co, I, Mn, Fe and Cr play an important role in reproduction and hence production.

Zinc

Zinc is known to be essential element for proper sexual maturity, reproductive capacity and onset of oestrus. The average concentration of zinc in plasma of animals is 0.1 – 0.2 mg/100 ml, which varies due to specie and age. Although zinc is not a part of insulin but it increases the hypoglycemic effect of

insulin and protects this hormone from decomposition by insulinase. The beneficial effect of zinc on the reproductive function may be direct or indirect. The concentration of zinc complexes with a specific ligand in the gonads and in the prostate gland is high (Kuhlman and Rompala, 1998). Fertility of sheep has been improved when both rams and ewes received zinc supplementation. Poor fertility of ewes on low zinc ration is due to early embryonic death. The fertilized ovum of ewes fed low zinc diets does not implant in uterine mucosa (Hidiroglou, 1979). In lambs fed zinc deficient diet (2.4 ppm), there is atrophy of seminiferous tubules accompanied complete cessation of spermatogenesis (Underwood and Somers, 1969). In camels a diet with 17 ppm of zinc supported satisfactory somatic growth but was insufficient for normal testicular growth and spermatogenesis compared with lambs fed a similar diet containing 32 ppm zinc. Male accessory sex glands are also dependent on zinc for normal function. The prostate contains high concentration of zinc and its uptake by this gland is regulated by testosterone. Exact mechanism of zinc in reproduction is not fully understood. It has been postulated that zinc prevents destruction of spermatozoa DNA by inhibiting the DNAase activity. FSH and LH in serum of zinc-deficient cows are not altered by zinc supplementation.

Copper

Copper is component of several enzymes like cytochrome oxidase, cerruloplasmin, diamine oxidase, monoamine oxidase etc. Deficiency of Cu affects all the cells of body including reproductive organs due to alterations of enzyme system. Many cases of impairment of the reproductive function in cattle due to copper deficiency in the diet have been reported. Anaemia due to Cu deficiency suppresses ovarian functions. A deficiency of this element results in weakened sexual desire and silent estrus. Cows grazing on grass containing 5 mg copper/kg or less become acyclic. In such cows daily supplementation of 1-2 gm copper/animal or even a single intravenous injection of 100-300 mg copper increases the fertility (Kuhlman and Rompala, 1998). A low copper content in the diet of the ewe either prevented implantation or induced embryonic loss and foetal death (McChowell, 1968). In goats copper deficiency lowered the conception rates and 50% aborted. Abortion takes place between 2nd and 5th month of pregnancy. The foetuses were mummified and placenta was degenerated with haemorrhagic or necrotic lesions. In copper deficient herds of cows, a single administration of 400 mg of copper glycinate improved fertility. The conception rate for copper treated cows was 72% versus 53% for the untreated cows. Treated cows required 1.00 and untreated 1.15 services per conception (Hunter, 1977). Copper in blood plasma of ewes falls during pregnancy and rises after parturition. However, in pregnant cows, cerruloplasmin and plasma copper increase during pregnancy. Cows with retained placenta have lower serum copper than normal cows. Diethylstilbestrol (oestrogen) administration increases copper in the rat serum and humans. But this interaction is not there in ruminants. In bulls administration of copper improves semen quality. The improvement was associated with increased sperm mobility and fewer dead spermatozoa (Hidiroglou, 1979). Copper requirement in dairy cattle generally ranged between 9 and 12 ppm depending upon stage of life cycle and dry matter intake (NRC, 2001).

Cobalt

Cobalt is a structural part of vitamin B_{12} (Cynocobalamine) which contains 4.5% of trivalent cobalt. Vitamin B_{12} is essential for haemopoiesis and acts as coenzyme in transmethylation and oxygen transfer (methyl-melonyl CoA mutase). It has been shown that CO^{2+} is required for haemopoiesis independent of vitamin B_{12} requirement. The involution of uterus is complete by 3 weeks after calving in cows treated with cobalt but require 6-9 weeks in cobalt deficient cows. A shorthorn herd grazing a cobalt deficient pasture had a conception rate of 53% at first service compared with 67% when given copper therapy and 93% when given cobalt as well as copper therapy (Hidiroglou, 1979).

Iodine

Deficiency of iodine during pregnancy impairs fetal thyroid functions and results in high incidence of aborted, stillbirth and week calves. Iodine is a constituent of thyroid hormone, which controls BMR, oxidation rate and plays an active role in development of foetus. Thyroidectomized dairy heifers cease to exhibit oestrus at regular intervals. Beneficial effect of iodine is believed to involve stimulation of the anterior pituitary gonadotropin secretion mediated through thyroid gland. Intrauterine injections of iodine in cows caused distinct lengthening of the estrus cycle (Grunert *et al.,* 1973). The iodine deficiency can be alleviated by salt containing 0.07% iodine. Allcraft *et al.* (1954) reported that subnormal protein bound iodine (PBI) in cows has been associated with infertility. In chronic repeat breeders, the average PBI was 37 mg/litre, significantly below the average of 46 mg/ litre in normal cows. There was a significant inverse relation between serum PBI and interval between first breeding and conception as well as number of services per conception. Lennon and Mixner, (1959) reported that for every 10 mg/litre increase in PHI, there was a decrease in service interval of 7.4 days

and decrease of 0.2 services per conception. Low serum PBI has been associated with abortions in cows. Grazing on pastures containing plants of Brassica species reduces the iodine utilization for thyroid hormone synthesis.

Manganese

Manganese deficiency causes infertility, delayed estrus and reduced conception rates. The pituitary and ovary are relatively rich in manganese (5.9 and 5.2 mg/g). Ovarian content of manganese is sensitive to dietary deficiency of this mineral. Large ovarian follicles and corpora lutea of ewes have taken up radioactively labeled manganese to a greater extent than other ovarian or extra-ovarian reproductive tissues. Uptakes also have differed in relation to the oestrus cycle. Maximal uptake occurred in the corpora lutea and this is greatest between day 4 and 11 of the estrus cycle when luteal progesterone production is maximum. This suggests that manganese is involved specifically in luteal metabolism and activity (Khillare *et al.,* 2007). Lack of Mn in diet can suppress conception rates, delay estrus in both postpartum females and young pre-pubertal heifers.

Iron

Iron is the most abundant trace mineral in the body. Iron primarily functions as a component of haeme found in haemoglobin and myoglobin. Enzymes of electron transport chain, cytochrome oxidase, feradoxine, myeloperoxidase, catalase and cytochrome P-450 enzyme also require iron as cofactor. Thus, iron functions in transport of oxygen to tissue, maintenance of oxidative enzyme system and is also concerned with ferritin formation. The relationship of iron level and the stimulation of hypothalamus, pituitary and adrenal cortex could explain the reason of iron deficiency leading to abnormal reproductive functioning (NRC, 2001). Iron deficiency affects response of ovarian receptors to hormones.

Chromium

Naturally occurring Cr also known as glucose tolerance factor (GTF) which comprises of Cr^{+3}, nicotinic acid, glutamic acid, glycine and cystine can potentiate the effect of insulin on tissue. It is vital for glucose or carbohydrate metabolism. It is present in high concentration in nuclear proteins. Thus, it must be required for gametogenesis and healthy fetal growth. The deficiency symptoms include lower sperm count and decreased fertility (Tuormaa, 2000).

In intensive animal production the animals are bred for maximum production performance. The requirement of such animals is also more for all type of nutrients. So, for maximum production and reproduction supplementation of micronutrients (minerals and vitamins) at appropriate levels is a must. Mineral supplementation depends on their concentration in feed which is largely determined by the mineral status of the soil.

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THERAPEUTIC MANAGEMENT OF PYREXIA WITH MYOSITIS IN SHEEP-A CLINICAL APPROACH

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ABSTRACT

Sixty five adult and lambs showing clinical signs of dullness, depression respiratory distress with rectal temperature varying between 103°F-105.5°F, inappetance to anorexia, occasional lachrymation, rough and dull hair coat, and slight lameness were suspected to be suffering from myositis and treated with long acting tetracycline along with acetaminophen with muscle relaxant and heavy doses of slow releasing nervine tonic and antioxidant successfully.

Introduction

Pyrexia alone or in combination with or sequel of other diseases is a well known problem in all the animals including sheep. The occurrence is influenced by many factors viz. season, age. region. transmitters, etiological agents, management system and above all immune status of the animals. Sporadic cases are taken of routinely but occurrence in the larger population draws attention of researchers to look in to seriously and record the clinical changes with due care particularly in naturally occurring cases and correlate them with varying precipitating factors so as to arrive at diagnosis. treat the disease effectively and improve future therapeutic management.

Materials and Methods

A group of sixty five adult and lambs sheep of Magra breed maintained at Central Sheep and Wool Research Institute. sub campus Bikaner was reported to be sick with sudden onset of high rise of temperature and lameness. These animals were put on exhaustive clinical examination with recording of their rectal temperature, pulse and respiratory rates. Mucous membrane colour at the conjunctiva. gums and vulva. Examination at the neck for any large, firm swellings and of rumen for doughness, gases and contractions, assessment of abdominal contour and auscultation of the heart, lungs was carried out. The- hair or wool around the perineum was inspected for the presence of wet or dry faecal material. The mammary gland was visually inspected noting contour, skin coloration, swelling, and/or shape. Palpating the gland for firmness, tenderness, and/or fibrosis performed a manual examination. Patency of the teats at was assessed during the manual examination and then milk was evaluated. A careful examination of legs and feet was carried out to be certain that a suspected locomotor problem is not, in fact secondary to a neurologic, metabolic or infectious disease. Musculoskeletal examination was carried out to record the symptoms of lameness. Representative clinical samples (blood) were collected in EDTA on pre and post treatment for haematological (Hb, PCV, TLC, TEC, DLC) investigations (Jain,1986). Therapeutic management was carried out using technical experience with long acting Tetracycline hydrochloride @ 10 mg/kg i/m, chlorpoxazone with paracetamol 2-3 ml i/m 8 hourly, Hivit 2-3 ml i/m once a day and Evion 400 mg once a day for 5 days. Recovery of animals from illness and quick and effective response to therapy made the basis of successful diagnosis and treatment after a follow up study to record relapses if any for a week.

Results and Discussion

Sixty five affected cases were thoroughly examined clinically in two days. Majority animals (40) were showing rectal temp from 103-105.6°F. Pulse rates range from 70-90 and resting respiratory rates range from 12-20 per minute. In the excited patient, rates were elevated 30-42, which came with in limit after the animals were put in comfortable position and were with out coughing and open mouth breathing. Rectal temperature, respiratory rate, and heart rate are easily elevated in the anxious small ruminant. Normal rectal temperatures range from 101.5 - 104.0°F and may reach up to 104.5°F in a full fleeced animal on a hot day. As the animal becomes more anxious the temperature will elevate. Exertion, excitement, or prolonged exposure to warm or humid environments may result in increase in temperature of several degrees. Comparison to herd mate helped in examination and do not seem to have remaining position except in a few cases. Though, the pyrexia due to reduced heat loss infection may seriously affect normal functions and may reduce their food

intake and loss weight and milk production and heat loss is associated with water loss, a care able point for a clinician while addressing the disease. In 1961 in man kind Petersdorf and Beeson suggested the criteria for fever: Fever higher than 38.3°C (101°F) on several occasions, persisting without diagnosis for at least 3 weeks, at least 1 week's investigation in hospital have been partly revised from time to time but still pertinent with addition of points like: Classic FUO referring infections (abscesses, endocarditis, tuberculosis, and complicated urinary tract infections), neoplasms (Lymphoma, Leukaemia), connective tissue diseases (temporal arteritis and polymyalgia rheumatica, Still's disease, systemic lupus erythematosus, and rheumatoid arthritis),miscellaneous disorders (toxic hepatitis, granulomatous conditions), and undiagnosed conditions and Nosocomial refers to associated factors such as, surgery, use of urinary catheter, intravascular devices. Thouh as yet in case of sheep no such well defined criteria seem to have been formulated by any renowned internationally accepted agency but primarily efforts were made to rule out the possibilities of above listed causes to best possible level through physical exam of the head which did not yield any observable change of clinical importance. Mucous membrane colour assessed at the conjunctiva, gums, or vulvas were not suggestive of any critical condition in most of the cases. Pale to white colour observed in a few cases were suggestive of mild anaemia and may be correlated with reduced intake (Radostis *et al*., 2000). Examination at the neck for any large, firm swellings and auscultation of the heart, revealed high pitched continuous murmurs that decrease with forced held expirations indicative of hepatic venous hum and in one case 3119 increased pulsus paradoxus was noticed and presently unexplainable and no any such report seem to be available to substantiate present findings. Others did not apparently yield any detectable abnormality. Auscultation of the lungs revealed quite harsh bronchovesicular sounds in some of the cases with abdominal friction rub indicative of inflammatory process but was not indicative of the extent or severity of respiratory disease. In a few cases anterior ventral consolidation was recorded in very narrow lung field indicative of lower respiratory disease and presence of a bit high risen rectal temperature 105 to 105.5°F triggers the thought of many infections that cause fever by inducing production of several cytokines, of which ingterleukin-6 and tumour necrosis factor are most likely involved. It is unlikely that the cytokines cross the blood-brain barrier, but act on the organum vasculosum of the laminia terminalis, a circumventricular organ outside the brain, which results in activation of the preoptic area of the hypothalamus to induce the production of prostaglandins. A fever is produced by bacterial, viral or chemical pyrogens. It is the result of a "resetting" of the thermoregulatory mechanism to function above the normal level. In many animals postaglandins are responsible for the readjustment. In acute infections, the temperature may rise several degrees above normal for a few days, sometimes with a superimposed diurnal variation. In a few cases, the depth of respiration was a bit short and shallow but not associated with obvious abnormal lung sounds. Eliciting a cough by tracheal palpation was not a detectable parameter in any of the case ruling the possibility of severe lung involvement. Assessment of abdominal contour did not show any detectable abnormality of immediate clinical significance, Bloat, advanced pregnancy, and ruptured bladder etc, the possible causes of a distended abdomen were ruled out. Ballotment was helpful in determining the presence of excess gas or fluid within the rumen and dorsally it was quite soft and indentible but as progressed ventrally the rumen becomes doughier in consistency. Rumen contractions occur at the rate of 1-2/minute recorded in the left para lumbar area. It was interesting to record abdominal bruits in three cases 3523, 3381, 3119 may be indicative of fibromuscular hyperplasia The hair or wool around the perineum inspected for the presence of wet or dry faecal material did not indicate the presence of diarrhoea. The mammary gland was visually inspected noting contour, skin coloration, swelling, and/or shape. A manual examination of udder performed by palpating the gland for firmness, tenderness, and/or fibrosis did not reveal any thing of clinical importance. Patency of the teats was assessed during the manual examination and then milk was evaluated but fortunately nothing clinically abnormal was diagnosed in any of the examined case. A careful examination of legs and feet was carried out to be certain that a suspected locomotor problem is not, in fact secondary to a neurologic, metabolic or infectious disease. Musculoskeletal examination began with observing the animal move about. There were symptoms of mild lameness in forelimbs during first few walks with out any detectable lesion in any of the leg and feet. On palpation of affected area muscle the animals were not cooperative and elicited signs of pain on mild force touch and leg movement that indicate inflammatory changes in leg muscles. The excited animals appeared sound and only when the animal settled down and moved slowly had a subtle lameness. When standing still, the animals had swing or hold the affected limb off the ground. The skin was examined for the presence of ectoparasites as well as other skin disorders and urine through history and physical examination but nothing abnormal could be detected. Twenty five affected cases (11 adult males and 14 females)

showed slight lameness and dullness with rectal temperature around 105°F. Some of them were showing inhalation/exhalation problem, laboured breathing, respiratory rates and a few animals were anaemic and weak. Haematological investigations of representative blood samples for Hb (9-13 g%), PCV (26-40%), TLC (6500-12000 th/cumm), TEC (7-11 millions/cumm), DLC (N 35-45%, L 37-51%, M 4-8%, E 5-10%, B 0-2%) did not highlight for any serious infectious, allergic disease and values remain nearly same in post treated cases.

An appreciable clinical recovery was noted in all the treated animals from second day onwards from various symptoms such as dullness, depression, high rise in temperature and it was observed that interest to intake and movement has improved marginally. Nearly complete recovery was noticed in animals after 4-5 days of treatment with notable changes especially at the time of withdrawal of treatment. The symptoms such as dullness, depression, inappetance and high rise in temperature may be a reflection of reactions of the body to its internal secretions/changes in an attempt to maintain normal physiological functions and/or to nullify the adverse effects various etiological agents including a check on propagation of a variety of micro organism subsequently release of pyrogens and other related intrinsic factors with in the system and in a few cases dyspnoea, respiratory rates may be the out come of a complex interaction of environmental factors producing stress, a variety of micro organisms working synergistically to damage the cells lining the respiratory tract allowing colonization and invasion of other organisms and a compensated host response. These relate to similar observations of Stevenson and Robinson (1970), Lehmkuhl and Cutlip (1984) and Robinson (1983). Among used antimicrobial, Tetracycline hydrochloride is a broad-spectrum antibacterial agent effective against both spectrum of (gram-positive and gram-negative) bacteria including Chlamydia, Mycoplasma and some methicillin resistant strains (Riviere and Papich, 2009) and therefore, was put in use in these clinical cases support of antipyretic and muscle relaxant and antioxidant which might have acted in one or the other way (by inhibiting the synthesis of bacterial cell walls by way of a check on cross linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-positive and Gram-negative bacteria) individually or collectively to produce desired therapeutic effect on ailing animals. Drug resistant among pathogens is an increasing problem apart from toxicity and residual effects and therefore, antibiotics and other drugs should be used with caution and repeated clinical field trials must be carried out covering large number population in different seasons and geographical area.

Acknowledgements

The authors are thankful to Director, Central Sheep and Wool Research Institute, Avikanagar and other supporting staff for providing necessary facility and support.

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RINGWORM (*MICROSPORUM GYPSEUM*) INFECTION IN EQUINE -A CASE REPORT

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Ringworm is an infectious disease of animals caused by different species of keratinophilic fungi. It is a major public and veterinary health problem reported from different parts of the world and causes great economic loss (Calderone, 1989). The disease appears to be more common in tropical than temperate climates particularly in countries having hot and humid climatic condition (Pascoe, 1976). It has been reported that animals housed in close proximity to each other for long periods and the presence of infected debris in buildings account for both the higher incidence and the greater infection rate in winter (Radostits *et al.*,1997).

The present report describes the occurrence and successful treatment of *Microsporum gypseum* infection in equine. A one year old mare was presented to Teaching Veterinary Clinical Complex of Apollo College of Veterinary Medicine, Jaipur with history of generalized skin infection with itching Fig.1. Based upon history and clinical signs, skin scrappings from the mare was collected from the neck region as per the standard protocol (Quinn *et al.,* 1994).

Wet mount of each skin sample was prepared and examined for the presence of macroconidia and mycotic hyphae, using standard techniques (Rippon, 1988). Simultaneously, the scrapping was examined by fluorescent microscopy, using Calcofluor white technique (Quinn *et al*., 1994). Microscopic findings were finally confirmed by inoculating the material over Sabouraud Dextrose agar (S.D.A.) culture plate (Hi-media), containing antibacterial compound (Penicillin and Streptomycin). Dermatophytes were identified using standard keys (Mackenzie *et al*., 1986)**.**

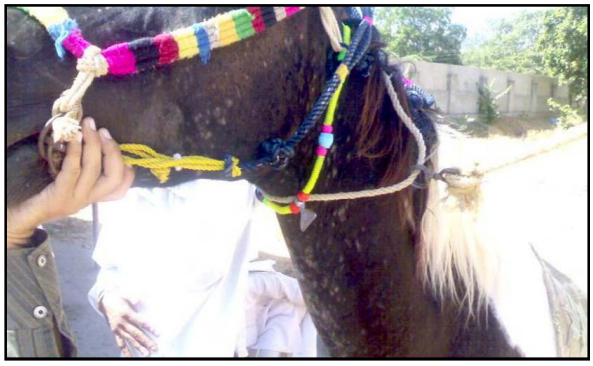

Fig. 1: Lesions of *Microsporum* infection on skin

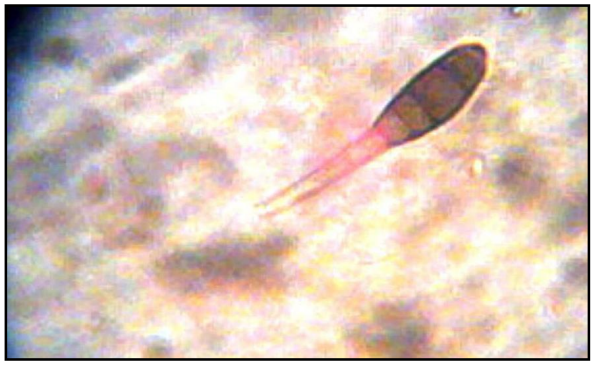

Fig. 2: *M. gypseum* from skin scrappings of Equine

Results

Microscopic examination of skin scrappings from the mare was positive for dermatophyte. Culture of the scrappings on S.D.A. revealed *M. gypseum* (Fig. 2). The mare was treated with griseofulvin orally @ 20 mg/kg b.wt., once a day, Micodin® shampoo bath weekly and Wokazole® lotion locally for fifteen days. After treatment the mare was fully recovered.

Acknowledgments

Authors thanks to Dean and Head of the Department, Apollo College of Veterinary Medicine, for providing necessary facilities and support during the study.

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BLOOD BIOCHEMICAL PROFILE OF STRONGYLE AND EIMERIA SPECIES INFECTION IN CROSSBRED CATTLE- A COMPARATIVE STUDY

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ABSTRACT

The present investigation was designed to determine the comparative blood biochemistry of cattle affected by strongyles and *Eimeria* spp infection. The blood and faecal samples were collected from crossbred cattle of 1-3 years of age belonging to farmers' stock. The faecal samples were examined for presence of eggs/ova by standard qualitative as well as quantitative methods of faecal examination. Animals having strongyle epg >500, Eimeria opg >2000 and mixed infection of strongyle and *Eimeria* spp both were considered infected and divided into different groups whereas clinically healthy animals negative for any ova/cyst served as control. The result of present investigation revealed a significant ($P<0.05$) effect of strongyle and strongyle - *Eimeria* spp mixed infection on total serum protein and albumin in cattle whereas other biochemical components remained unaltered. The *Eimeria* spp. infection did not affect the different biochemical components of the cattle. This can be concluded from the study that the strongyles are more pathogenic to cattle of 1- 2 years of age in comparison to *Eimeria* spp infection.

Key words: Biochemical profile, strongyle, Eimeria, cattle

Introduction

Gastrointestinal parasite infection in cattle is very common in Jammu and Kashmir State. Gastrointestinal parasites may lead to physio-pathological changes like digestive disturbances, enteritis, anorexia and improper nutrient utilization. Prolonged inappetence and improper nutrient utilization further leads to retarded growth, depressed reproduction, reduced resistance of animal and heavy losses to diary industry. The present investigation was, therefore, undertaken to record the changes in blood biochemical constituents of cattle naturally infested with gastrointestinal parasites.

Materials and Methods

The present study was conducted on cross bred cattle randomly selected from field. Faecal samples were collected from selected animals of 1 to 3 years age (irrespective of sex) in polythene bags and blood of each animal was collected by venepunture in heparinized vials. The samples were carried to lab in ice cold condition for further analysis.

The faecal samples were subjected to standard qualitative method of faecal examination by standard salt floatation technique (Soulsby, 1982). The animals found positive for eggs of strongyle and oocysts of *Eimeria* spp. were further subjected to quantitative faecal examination using modified Mcmaster technique. Animals having strongyle epg >500 and Eimeria opg >2000 were considered infected. These infected animals were further divided into three groups as II, III, and IV infected with strongyle, *Eimeria* oocyst and both, respectively. Nine clinically healthy cattle which were negative for any ova/cyst served as control and placed in group I.

The plasma of blood samples were separated by centrifugation at 2500 rpm for 10 minutes and stored at (-) 20^0C until analyzed. Blood samples were analyzed for plasma glucose, total plasma protein, albumin, globulin, A:G ratio, sodium potassium, magnesium, and chloride by using commercially available diagnosis kits on chemistry analyzer (RT-1904C). The data were analyzed using One-way ANOVA (Snedecor and Cochran, 1994).

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Results and Discussion

The serum biochemical profiles of cattle infected with gastrointestinal parasites have been depicted in Table 1.

In infected animal the total plasma protein was lower in all the groups than healthy control animals indicating hypoproteinaemia (Benjamin, 1985 and Radostits *et al.*, 2000) which might be ascribed to leakage of proteins from damaged gastrointestinal mucosa and decreased feed intake as mentioned by Holmes (1987). This fall in total protein might also be due to selective loss of albumin which is having smaller size and osmotic sensitivity to fluid movement (Tanwar and Mishra, 2001). The present study substantiate the findings of Jas *et al.* (2008) in goats and Bharti and Prasad (2001), Singh *et al.* (2006) and Ritanath (2007) in bovine infected with gastrointestinal parasites. The total proteins in animals having mixed infection were observed to be higher than the group infected with stronglyle but were significantly lower than control group animal.

The level of albumin was observed to be lower in all infected group, but, the level was significantly lower in animals infected with strongyle as compared with other groups. Similar findings were also been reported by Kumar *et al.* (2005) in goats with hemonchosis and cocciodiosis and Bharti and Prasad (2001) in cattle and buffalo.

Though, the plasma glucose values were within normal range yet observed slightly lower in all the infected animals. This slight lower plasma glucose values corroborate the findings of Singh *et al.* (2006) in cattle and buffalo and Kumar *et al.* (2005) in goats. The fall in glucose values in infected animals might be due to reduction in dry matter intake and absorption through the damaged gut caused by the parasites and also because the parasites thrive on carbohydrate available in the gastrointestinal epithelium depleting the glucose level in blood (Coop, 1981; Sena *et al.*, 1997). The concentration of plasma sodium, potassium magnesium and chloride showed no difference in all infected group than normal healthy group.

The present study indicates that parasitic infection such as strongyle and mixed infection (Strongyle and *Eimeria spp.*) in cattle of 1-2 years of age severely affects the blood biochemical parameters where as *Eimeria spp.* infection alone do not have the significant deleterious effect on health of cattle. Sustainable control of strongyle parasite infection can improve the production significantly.

Table1: Biochemical profile of cattle naturally infected with different gastrointestinal parasites

Parameter	Group I (N=9)	Group II (N=9)	Group III (N=10)	Group IV (N=12)	P- value
Glucose	55.23±5.60^{a}	42.04±9.37^{a}	54.80±3.25^{a}	41.62±4.27^{a}	0.05
Total protein	6.37±0.21^{a}	5.73±0.33^{b}	5.66±0.33$^{a\,c}$	6.12±0.17^{c}	0.00
Albumin	2.55±0.39^{a}	1.78±0.20^{b}	2.32±0.22^{a}	2.25±0.23^{b}	0.01
Globulin	3.82±0.30^{a}	3.95±0.45^{a}	3.34±0.44^{a}	3.86±0.30^{a}	0.85
AG ratio	0.75±0.16^{a}	0.55±0.13^{a}	0.84±0.14^{a}	0.67±0.13^{a}	0.33
Sodium	111.60±9.39^{a}	115.60±6.80^{a}	96.22±9.85^{a}	112.77±11.54^{a}	0.52
Potassium	3.11±0.35^{a}	3.04±0.28^{a}	3.68±0.34^{a}	3.41±0.38^{a}	0.58
Magnesium	1.86±0.24^{a}	2.44±0.10^{a}	2.52±0.19^{a}	2.25±0.17^{a}	0.09
Chloride	78.93±6.14^{a}	72.66±9.76^{a}	73.23±6.80^{a}	77.80±6.82^{a}	0.91

Values with different superscripts differ significantly (P<0.05) from each other.

Acknowledgement

The authors are highly thankful to the Dean, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agriculture Sciences and Technology of Jammu, RS Pura, Jammu, for providing necessary facilities to execute the research.

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AMBIENT TEMPERATURE ASSOCIATED VARIATIONS IN SERUM ARGINASE AND ALDOLASE IN *MARWARI* GOAT

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ABSTRACT

To determine ambient temperature associated variations in serum arginase and aldolase enzymes, 540 apparently healthy *Marwari* goats of either sex, between 5 months to 4 years of age were screened during moderate, extreme hot and cold ambient temperature periods. The mean values of serum arginase and aldolase were higher during extreme hot and cold ambient temperature periods than moderate period. The present study indicated that extreme ambience stimulated the liver of the animals of both the sexes and all age groups.

Introduction

Animals are drastically affected at all levels of their organisation by any change in their thermal surroundings and the process of adaptation of organisms to seasonal changes involve variation in every aspect of physiology (Al-Bassam *et al*., 2007). Before reaching to any conclusion, it is important for a clinician to consider variations in clinical values due to ambient temperatures. Any ambient factor affecting the metabolism will definitely bring about changes in physiological functions of liver as it is the hub of metabolic activities. These changes can be noticed by measuring enzyme activities associated with metabolic functions. Serum arginase and aldolase are two such important enzymes bearing significance in clinical physiology.

Arginase is a cytosolic enzyme which catalyses the final step in Krebs-Henseleit urea cycle and present mainly in the liver. It cleaves arginine to yield urea and ornithine. Arginase is present in significant concentration in the liver of mammals (Dittrich *et al*.,1974) and is an indicator of active liver cell damage in ruminants (Harvey and Obeid, 1974). The enzyme aldolase or fructose-1, 6-biphosphate aldolase catalyses a reversible aldol condensation in glycolytic cycle. Aldolase has a wide specificity but is usually connected with the splitting of fructose-1,6-diphosphate. Liver damage can be predicted on the basis of variation in the levels of serum aldolase (Alemu *et al.,*1977).

Studies on serum arginase and aldolase have not been well documented for *Marwari* breed and obtaining of base line data can be used for diagnosis of diseases, for criteria of adaptability as well as to elucidate some physiological mechanisms in the *Marwari* breed of goat. Further it is important to find out the stress, if produced, on the basis of variations in the serum enzyme activities due to variations in ambient temperatures. Looking towards the lack of research on serum aldolase and arginase in *Marwari* breed of goat the present investigation was planned to determine variations in their levels during extreme hot and cold ambient temperatures.

Materials and Methods

To determine ambient temperature associated variations in serum arginase and aldolase, 540 apparently healthy *Marwari* goats of either sex, between 5 months to 4 years of age were screened during moderate, extreme hot and cold ambient temperature periods.

Blood samples were collected from private slaughter houses (Bikaner, Rajasthan) during moderate (October-November), extreme cold (December-January) and extreme hot (May-June) ambient temperature periods. Blood was collected directly into the clean, dry test tubes without any anticoagulant to harvest the serum. Only non-haemolysed serum samples were used. The serum samples were stored at 0°C - 4 °C in the refrigerator until analysis, as freezing of serum was reported to cause partial inactivation of some enzymes (King,1965).

All the animals were kept in well ventilated area and fed with sole roughage diet of dry *pala* (*Ziziphus nummularia*) leaves and watered *ad libitum* during the period before slaughtering. In each ambient temperature period 180 blood samples were collected and the animals were grouped into male (90) and female (90). Further each group was divided according to age as 5-10 months (30 male and 30 female); 1-2 years (30 male and 30 female) and 2.5-4 years (30 male and 30 female). Serum arginase

(ARG) was determined by the method of Manning and Grisolia (1957). Serum aldolase (ALD) was determined by the colorimetric method as described by Sibley and Lehninger (1949).

The main parameters of the present investigation were serum enzymes and the main effects were classified as ambient temperatures, sex and age groups. The subsets of ambient temperatures were moderate, hot and cold periods; of sex were male and female; and of age groups were 5-10 months, 1-2 years and 2.5-4 years. For each subset data were expressed as mean ± SE of mean. The changes in the means were measured as the differences between the moderate in ambient temperatures; male in sex; and 5-10 months in age groups and the respective value at a particular subset whether each mean change was significantly different from control was assessed by 't' test (Snedecor and Cochran, 1967).

Results and Discussion

Mean ±SEM values serum ARG and ALD during different ambient temperature periods, sex and age groups are presented in Table 1.

Arginase

The results of present study for serum ARG were more or less similar to the reported values by Jain *et al.* (1995) in goats; Kaneko *et al.* (1999) in sheep and Khinda *et al.* (2004) in buffaloes. As arginase is present in significant concentration in the liver, the serum arginase elevations have been demonstrated in progressive hepatic necrosis with unfavourable prognosis (Tennant, 1999); in naturally occurring liver diseases of goats (Adam *et al*., 1974); hepatocellular damage in goats (Braun *et al*.,1986), making it a useful tool for diagnosis of liver diseases.

Effect of hot and cold ambient temperatures on serum ARG

The mean value of ARG was significantly ($P \leq 0.05$) higher during hot and cold ambient temperature periods in comparison to overall moderate mean value. All the enzymes of urea cycle are synthesized at a higher rate during starvation or in animals with high protein diet and animals on protein free diet produce lower level of enzymes of urea cycle (Lehninger *et al.*, 1993). Scanty vegetation or low feed intake in the summer season and higher availability and feed intake could be the possible causes influencing the urea cycle thereby showing increased concentration of arginase.

Effect of sex on serum ARG

In each ambient temperature period the sex effect was significant ($P \leq 0.05$). The mean values in all the ambient temperature periods were significantly ($P \leq 0.05$) higher in male animals than female animals. Higher concentration of total proteins in the diet of male goat could be the possible cause of higher ARG activity. Earlier studies have implicated arginase as a controlling factor in both male erectile function and female sexual arousal, and is therefore a potential target for treatment of sexual dysfunction in both sexes (Moody *et al.*,1997).

Effect of age on serum ARG

In each ambient temperature period the age effect was significant ($P \leq 0.05$). Age effect showed a significant ($P \leq 0.05$) increase in the mean values being lowest in the animals of 5-10 months of age. Lower arginase activity in younger stock resulted in lower urea concentration.

Interactions of ambient temperatures with sex and with age

Age X sex; age X ambient temperature; and sex X ambient temperature interactions were significant ($P \leq 0.05$). These results showed that serum ARG values were affected by variations in ambient temperature, sex and age. Each effect influenced the activity irrespective of other effect and well within the other group. This showed the dominance of effects which was significant in statistical terms.

Aldolase

The range of ALD values was more or less similar to the reports of Alemu *et al.* (1977) in sheep. However. Jain *et al*.(1995) reported a very high activity of serum aldolase than earlier reports (Zimmerman *et al.,* 1965 and Sundaravadanan *et al.,* 1989). Elevated levels are useful predictors of liver damage (Alemu *et al.,*1977); acute viral hepatitis or hepatic necrosis due to chemicals or drugs (Sibley and Fleisher, 1954); acute and chronic hepatic damage (Katz and Ducci, 1958); and myocardial infarction in animals (Chazov and Savina, 1958).

Effect of hot and cold ambient temperatures on serum ALD

The mean value of ALD was significantly ($P \leq 0.05$) higher during hot and cold ambient temperature periods in comparison to overall moderate mean value. Probably it was related with higher utilisation of glucose through glycolysis to generate energy and also in the synthesis of glucose by gluconeogenesis in the stress period. Further higher ALD activity also helped in the generation of more glyceraldehydes -3- phosphate which can also be utilized for the synthesis of fat in the body (Lehninger *et al*., 1993). Aldolase is one of the seven glycolytic enzymes, which function reversibly for gluconeogenesis, an important mechanism for generation of glucose in ruminants

Table 1 : Mean ± SEM values of serum arginase (ARG) and aldolase (ALD) in *Marwari goats.*

S.No.	Effects	Enzyme Activity, U/l	
		ARG	ALD
1.	Overall (540)	14.26 ± 0.12	10.21±0.15
2.	Ambient temperature period		
(A)	Moderate overall (180)	9.6±0.12	9.61±0.14
I	Sex		
(i)	Male (90)	12.8±0.13	10.12±0.12
(ii)	Female (90)	6.4±0.12[c]	9.11 ±0.16[c]
II	Age		
(i)	5-10 months (60)	6.7±0.12	10.9±0.13
(ii)	1-2 Years (60)	9.2±0.11[d]	9.8±0.15[d]
(iii)	2.5-4 Years (60)	12.8±0.13[d]	8.1±0.14[d]
(B)	Hot overall (180)	18.5±0.13[b]	11.1±0.15[b]
I	Sex		
(i)	Male (90)	19.6±0.15	12.1±0.12
(ii)	Female (90)	17.4±0.12[c]	10.2±0.18[c]
II	Age		
(i)	5-10 months (60)	16.5±0.11	13.0±0.14
(ii)	1-2 Years (60)	18.0±0.15[d]	11.2±0.19[d]
(iii)	2.5-4 Years (60)	20.9±0.15[d]	10.1±0.12[d]
(C)	Cold overall (180)	14.2±0.12[b]	10.0 ±0.15[b]
I	Sex		
(i)	Male (90)	16.3±0.11	11.3±0.17
(ii)	Female (90)	12.1±0.13[c]	8.6±0.13[c]
II	Age		
(i)	5-10 months (60)	12.1±0.17	12.6±0.15
(ii)	1-2 Years (60)	13.8±0.10[d]	10.0±0.19[d]
(iii)	2.5-4 Years (60)	16.9±0.11[d]	8.4±0.11[d]

(i) Figures in the parenthesis indicate number of animals.
(ii) In ambient temperature effect mean values of all the parameters of hot and cold ambient temperature periods have been compared with respective mean values of moderate temperature period.
(iii) Superscript 'b' indicates a significant difference ($P \leq 0.05$) according to ambient temperatures.
(iv) In sex effect mean values of all parameters of female animals have been compared with respective mean values of male animals within ambient temperature.
(v) Superscript 'c' indicates a significant ($P \leq 0.05$) difference according to sex within one ambient temperature period for a parameter.
(vi) In age effect mean values of all the parameters have been compared from 5-10 months age group.
(vii) Superscript 'd' indicates a significant ($P \leq 0.05$) difference according to age within one ambient temperature period for a parameter.

(Abdel-Fattah *et al*., 2002). Role of aldolase in carbohydrate metabolism explains its functional significance. Regulated glycolysis and glucone-ogenesis prevent the futile cycling with accompanying loss of ATP energy (Lehninger *et al*., 1993).

Effect of sex on serum ALD

In each ambient temperature period the sex effect was significant ($P \leq 0.05$). The mean values in all the ambient temperature periods were significantly ($P \leq 0.05$) higher in male animals than female animals. Increased ALD activity in males was probably the indication of higher metabolic status.

Effect of age on serum ALD

In each ambient temperature period the age effect was significant ($P \leq 0.05$). Age effect showed a significant ($P \leq 0.05$) decrease in the mean values being highest in the animals of 5-10 months of age. Higher ALD activity in younger stock was indicative of higher metabolism and higher turnover of glucose.

Interactions of ambient temperatures with sex and with age

Age x sex; age x ambient temperature; and sex x ambient temperature interactions were studied and found significant ($P \leq 0.05$). These interactions indicated the effect of ambient temperatures on the serum ALD activity in all the groups of animals. Sex

and age also influenced the activity in all the ambient temperature periods studied, irrespective of each other, and within each group.

The present study indicated that extreme ambience can stimulate the liver of the animals of both the sexes and all age groups, which was reflected in the form of increased activity of both the enzymes. It was clear that the changes in the activity could also occur in health conditions, as a means of developing the strategies, which make the survival of the animal possible, in otherwise hostile environment. These results pointed out towards adaptability of the animals and modulation of physiological mechanisms.

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THERAPEUTIC EFFICACY OF POLYHERBAL GEL (MASTILEP GEL®) AND ANTIOXIDANT-MINERAL FORMULATION (UNISELIT®) AGAINST CLINICAL MASTITIS IN GOATS

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ABSTRACT

Therapeutic efficacy of topical polyherbal gel (Mastilep®), oral antioxidant-mineral formulation (Uniselit®) with and without antibiotic was evaluated in eighteen (n=18) mastitic goats divided in to three groups (Group A, Group B and Group C) of six each. The mastitic goats of Group A (n=6) were subjected to antibiotic (Amoxicillin-Cloxacillin) routinely used in the field, while Group B (n=6) with topical polyherbal gel on udder and oral antioxidant-mineral trace mineral formulation. The mastitic goats of Group C (n=6) were administered antibiotic as well as topical herbal medicine and antioxidant-mineral therapy. The six apparently healthy goats (n=6) constituted Group D and served as healthy control. Following therapy, clinical recovery, restoration of milk yield and reversal of SCC and E.C values in clinical mastitic goats was maximum in Group C (Antibiotic+topical herbal gel+ oral antioxidant-mineral) followed by Group B (Topical herbal gel + oral antioxidant mineral) and Group A (Antibiotic alone) therapy. The present trial indicated that therapeutic regimen comprising herbal medicine, antioxidant-mineral formulation and an antibiotic is highly efficacious and attains faster recovery in mastitic goats.

Key words: Antibiotic, antioxidant-mineral formulation, clinical, electrical conductivity (EC), herbal medicine, somatic cell count (SCC).

Introduction

Mastitis is a multifactorial and costliest disease of all milk producing ruminants. Lack of scientific health care and managemental practices has been repeatedly highlightened for cause of mastitis. Early detection and a holistic approach is a bare need of days to combat mastitis in milch animals.

The maintenance of hygienic environment, udder health and enhancement of udder immunity has been recommended for control of mastitis (Radostits *et al.*, 2007). The herbal medicine can be successfully employed for maintaining udder hygiene score and bear added advantage of being safe, effective and economical (Buragohain and Dutta, 1998). Antioxidant like Vitamin E and selenium are known to play an important role in host defence mechanism and conferring udder immunity. Similarly macro and micro minerals like calcium, phosphorous, copper, cobalt, manganese maintains homeostasis (Upadhyay and Dwivedi, 2004) maintain homeostasis thereby regulating inflammatory process. Hence the present investigation was undertaken with the objective to access efficacy of herbal medicine (Mastilep®) and antioxidant-mineral formulation (Uniselit®) in the treatment of clinical mastitis in goats and to evaluate comparative efficacy of different therapeutic regimen against caprine mastitis.

Materials and Methods

Goat population of private goat farms in and around Parbhani (Maharashtra) and those presented to Department of Veterinary Medicine, COVAS, Parbhani were screened using Modified California Mastitis test (MCMT) described by Pandit and Mehta (1969) for diagnosis of the mastitis in goats. A total of 126 goats were screened of which, 21 goats were positive for clinical mastitis. The mastitic goats were randomly divided in three treatment groups (A, B and C) of six animal each while group D (n=6) served as healthy control. The mastitic goats of group A (n=6) were subjected to routine antibiotic therapy of inj. amoxicillin+cloxacillin @ 10 mg/kg b.wt. by intramuscular route with supportive NSAID for 5 days. Mastitic goats of group B (n=6) were subjected to topical polyherbal medicine (Mastilep gel®) topically on the udder by gentle messaging after milking bid for 5 days and

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an oral antioxidant-mineral (Uniselit®) formulation @ 5 gm O.D orally for 5 days. Group C mastitic goats (n=6) were administered antibiotic Amoxicillin-Cloxacillin @ 10 mg/kg b.wt. i/m for 3-5 days as well as topical herbal medicine (Mastilep gel®) twice daily for 5-6 days and oral antioxidant/mineral (Uniselit powder®) formulation @ 5 gm OD for 5-6 days. The efficacy of different therapeutic regimen was evaluated on the basis of reference diagnostic test, improvement in somatic cell count (SCC) and electrical conductivity (EC), increase in milk yield, quarter cure rate, clinical recovery and period required for restoration towards normalcy. The data was analyzed as per statistical methods given by Snedecor and Cochran (1994).

Results and Discussion

The prevalence of caprine clinical mastitis in and around Parbhani (Maharashtra) was 16.66%. The comparative efficacy of antibiotic and non-antibiotic therapeutic regimen against clinical mastitis in goats is depicted in Table 1.

In Group A, mastitic does were treated with antibiotic inj. Amoxicillin+Cloxacillin and out of 7 affected quarters (QIR-58.33%), 5 recovered indicating per cent efficacy of antibiotic therapeutic regimen the drug as 71.42% against caprine mastitis. In Group B, i.e in non antibiotic group, among 6 affected quarters (50%), 5 recovered indicating therapeutic efficacy as 83.33 %. Clinically mastitic goats of group C had 8 affected quarters (QIR-66.66%) and following treatment all the four quarters completely cured indicating efficacy of therapeutic regimen of Group C (Antibiotic + Mastilep gel® + Uniselit® powder) as 100% in treating mastitis in goats (Table 1).

The somatic cell count (SCC) was significantly higher in mastitic goats of Group A,B & C before therapy (Table 2). Increase in somatic cell count is most sensitive index of inflammation of udder tissue and is indicative of arrival of defence cell at the site of infection (Pednekar *et al.*, 1992). Somatic cell count (SCC) was significantly ($p < 0.01$) elevated in all the three groups of mastitic goats and following

Table 1. Efficacy of antibiotic and non-antibiotic therapeutic regimen against clinical mastitis (CM) in goats.

Group	Name of drug used	No of goats	Quarter infection rate (QIR)	Quarter cure rate	% Efficacy of drug	Average days of therapy
A	Inj Amoxicillin+ Cloxacillin	6	7/12 (58.33%)	5/7	71.42 %	4.66 ± 0.49
B	Mastilep® topically and Uniselit orally	6	6/12 (50.00%)	5/6	83.33 %	4.83 ± 0.30
C	Inj Amoxicillin+Cloxacillin Mastilep® topically + Uniselit® orally	6	8/12 (66.66%)	8/8	100 %	3.50 ± 0.22

Table 2: Somatic cell count (SCC) and electrical conductivity (EC) values before (BT) and after treatment (AT) with different therapeutic regimen against clinical mastitis (CM) in goats.

GROUPS	SCC ($x10^5$)				EC($mscm^{-1}$)			
	BT		AT		BT		AT	
	LT	RT	LT	RT	LT	RT	LT	RT
A) Inj Amox+Clox	11.18 ±1.16	10.54 ±0.75	8.35** ±0.61	8.48** ±0.73	6.92 ±0.51	7.15 ±0.50	5.70** ±0.19	5.83** ±0.14
B) Mastilep+Uniselit	11.33 ± 0.84	9.84 ± 0.29	8.69** ± 0.68	7.65** ± 0.09	6.95 ±0.45	6.37 ±0.02	5.68** ±0.17	5.35** ±0.05
C) Inj. Amox+Clox and Mastilep+ Uniselit	11.28 ±0.43	12.08 ±0.34	8.00** ±0.20	8.10** ±0.12	6.93 ±0.35	7.17 ±0.43	4.86** ±0.06	5.00** ±0.09
D) Healthy Control	7.25 ± 0.15	7.32 ± 0.15	…….	…….	4.93 ±0.09	4.99 ±0.09	……..	……..

** Statistically Significant $p < 0.01$ as compared to their before treatment values

therapy the SCC was significantly ($P \leq 0.01$) reduced to near to normal scores. The improvement in SCC was maximum in Group C and after treatment SCC was almost at par and well comparable with that of healthy control (Group D).

Topical polyherbal gel diffused well through out udder surface and eliminated udder microbial load. Herbal ingredients of the Mastilep gel® also enhanced and maintained integrity of teat canal thus elevating udder immunity. Antibacterial, anti-inflammatory, analgesic and immunomodulatary properties of Polyherbal gel-Mastilep® has been well established. Sudden elevation in the levels of sodium, chloride, potassium in milk due to inflammation of mammary parenchyma has been already documented by Oshima *et al.*, 1974 and Muley, 2003. Increased electrical conductivity (E.C) of milk therefore possesses diagnostic importance in mastitis. Following treatment Electrical conductivity of the affected milk was restored due to ceasation of inflammation and stoppage of electrolyte secretion in the affected milk. The superior efficacy of herbal medicine (Mastilep® gel) is attributed to its herbal constituents namely *Eucalyptus globulus, Curcuma longa, Paedaria foetida, Glycyrrihza glabra, Cedrus deodara* in treating mastitis has been documented by Nath and Dutta (2005), Buragohain and Dutta (1998). Similar findings on therapeutic efficacy of Mastilep gel in treating subclinical mastitis are reported by Maini *et al.,* 2008. Antioxidant–mineral formulation boosted udder local immunity and prevented entry of invading pathogens. The role of antioxidant radicals (Vitamin E, Selenium) enhancing GSH-Px activity and in turn reducing the severity and the duration of inflammation has been well documented by Radostits *et al*. (2007). In the present trial therefore, polyherbal gel (Mastilep®) topical application and oral antioxidant-mineral formulation (Uniselit®) therapy showed remarkable efficacy (83.33%) in treating caprine mastitis. Based on the present investigation, it can be concluded that polyherbal medicine (Mastilep gel ®), antioxidant-formulation (Uniselit®) is a safe, effective and economical combination for the treatment of mastitis in goats. While use of herbal medicine (Mastilep gel®), antioxidant-mineral formulation (Uniselit®) with suitable antibiotic is highly efficacious and promising therapy against clinical mastitis in goats.

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VETERINARY PRACTITIONER

Volume 12 No. 1 June 2011

HALF YEARLY JOURNAL DEDICATED TO THE PRACTICING VETERINARIANS

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BIKANER-334 003 (Raj.)
Email : drapsinghbikaner@yahoo.co.in

Published and edited by Dr. S.N. Sharma and printed by Man Mohan Kalyani at Kalyani Printers, Kalyani Bhawan, 2nd Entrace of Railway Station, Alakh Sagar Road, Bikaner, published from Care of Gahlot Kuteer, B-3-A, Karni Nagar, Nagneniji Road, Bikaner

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(Half yearly publication-June and December every year)

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