The Blue Cross Book

For the advancement of the veterinary profession

MSD Animal Health

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From Editor's Desk

MSD –Animal Health and Editorial Board of the "Blue Cross Book" delighted to bring about the 34th edition to enlighten its readers about the latest research findings, alternative opinion about the veterinary treatment and latest happening in the livestock sector of India.

As we received a good monsoon this year, almost entire Nation may go for bumper harvest and hopefully bring down the prices of agricultural products as well as overall growth of the Nation. At the same time more rainfall may also bring some ailments to the animals like bacterial, viral or high parasitic load in the livestock. This edition brings articles and other useful information to overcome these conditions by addressing different approaches to control parasites and judicious use of antibiotics in livestock

Managing the productivity of cow is very important for the farmers as well as for the dairy Industry. This will also reduce number of cows illegally going for the slaughter. This edition addresses the issue by publishing articles about the estrus synchronization of cows with hormonal treatment, good management practices, effective management of hypocalcaemia conditions and also controlling of disease spread through the artificial insemination.

Treating Mastitis condition with correct antimicrobials is important for mitigating the recurrent problem faced by the farmers. Performance of different antibiotics for effective treatment of mastitis is explained in this issue may give good inputs to the Veterinarian for selection of the right antimicrobials for mastitis treatment.

Handling of Obstetrics and Gynecological problems in the ruminants especially dystocia conditions is very important to save both calf and dam. Articles addressing different dystocia conditions especially in dicepahlic monsters will give good insight for the Veterinarians to handle these types of cases in their dispensaries or hospitals.

Editorial board be grateful to Dr. Ram Krishna Bauri, Dept. of Parasitology, Ranchi Veterinary College and Dr. N. Krishnaiah, HOD, Dept. of Veterinary Public health & Epidemiology, College of Veterinary Science, Hyderabad for their valuable contribution in reviewing the articles of this volume.

Dr. Yash Goyal
Managing Director,
MSD Animal Health

Dear Veterinarians,

I am very much delighted that MSD Animal Health is releasing the 34th edition of "The Blue Cross Book" that is dedicated to all the practicing Veterinarians, who are in need of knowing the new treatments, technicalities for addressing the issues concerned to health, productivity and management of animals. I personally would like to convey our sincere thanks to all the contributors of research, review, clinical and general articles for this issue, whose continued support is essential for the benefit of all Veterinarians.

Food safety and supply are the current issue related to public health that world is facing now. MSD Animal Health is committed for its vision as "Science for Healthier animals", with its continued research in developing new products, solutions and services to the well-being of livestock. MSD Animal Health is committed for the scientific progress in the fields of health and management of animals.

I am really happy to see good monsoon this year after a long gap of drought. This will definitely help the needy farmers who thrive on the productivity of their livestock apart from agriculture source. This will help the current mission of National Dairy Plan, which is focusing on increasing the productivity of milch animals and thereby increasing milk production to meet the rapidly growing demand for milk.

Keeping dog or cat as a companion animal is the trend emerging in urban and semi urban area, which give lot of love and pleasure to the animal lovers. Due to the close association of these pets with public, it is rather more necessary for keeping these pets always healthy and safe. MSD Animal Health has many solutions for the health and management of companion animals, who are the close associates to the people.

I am also happy to bring to your notice that MSD Animal Health, has placed all the issues of Blue Cross Book on its website and can be accessed from www.msd-animal-health.co.in.

I wish all the Veterinary professionals and our customers a happy festive season.

SWINE

Increasing meat supplies relies on a more intensive, large-scale approach to farming. Larger animal groups need to be carefully protected from disease. Ecological upheavals, and the migration of parasites and wild animals, can all encourage the spread of zoonoses – which is a particular threat for our swine farmers.

To help them control the swine influenza virus (SIV) we have developed MAXIVAC Excel 5.0. It is the first pentavalent inactivated vaccine to control most common strains of swine influenza.

Similarly, in the fight against *Escherichia coli,* Merck Animal Health offers producers PORCILIS Porcoli DF.

Other important swine vaccines include PORCILIS PCV and CIRCUMVENT PCV/MHyo which prevent the spread of porcine circovirus type 2, a cause of growth retardation in swine. PORCILIS PRRS provides protection against the virus responsible for porcine reproductive and respiratory syndrome, and PORCILIS APP, a subunit vaccine, reduces pulmonary lesions caused by *Actinobacillus pleuropneumoniae infection.*

Apart from vaccines, the swine pharmaceutical range includes the antibiotics NUFLOR (florfenicol), COBACTAN (cefquinome) and ZUPREVO (tildipirosin) as well as PANACUR AquaSol (fenbendazole), a potent antiparasitic.

Our new IDAL vaccinator, which allows needleless intradermal administration, will make vaccination easier and more efficient for swine producers.

SPOTLIGHT ON PARTNERSHIP

AT MERCK ANIMAL HEALTH WE BELIEVE COLLABORATION IS A BETTER WAY TO TACKLE CHALLENGING DISEASES. RESPIG, FOR EXAMPLE, IS OUR DEDICATED SWINE HEALTH SERVICE FOR VETERINARIANS, PRODUCERS AND OUR OTHER SPECIALIST PARTNERS. IT CONTAINS CUTTING-EDGE INSIGHTS ON MANY COMPLEX RESPIRATORY DISEASES, AND MAKES IT EASY FOR EVERYONE TO CONTRIBUTE TO SOLUTIONS.

Review Articles

Research Articles

*The information on Parasitic diseases of animals has been accessed through Internet, articles published in various journals and reports submitted by government Institute.

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Bovine Semen Transmittable Diseases

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Abstract

Artificial insemination is used for effectively utilizing the germplasm of superior quality breeds to improve milk yield and other characteristic as bovine semen. A few major advantages of AI are a continuous genetic improvement, more superior calves from fewer bulls and prevent or eliminate sexually transmittable diseases. However, a major disadvantage of AI is a risk of spreading genetic defects and semen transmittable diseases in bovine population. Therefore, AI may be boon or bane for livestock depends on monitoring semen production station. The article focuses on the risk of specific diseases which may be transmitted through bull semen if a bull or its semen is infected. Appropriate measures may not only prevent the risk of dissemination of diseases in animals, but it also prevents spreading many diseases to the human population.

Key words : Semen, artificial insemination, infectious agents.

Introduction

Artificial Insemination (AI) is a technique by which semen collected from male is introduced into the female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring. The introduction of semen into the female reproductive tract could be possible by using diluted fresh or frozen semen under most hygienic condition. The regular testing of frozen semen produced by the bull stations or the semen production unit is mandatory mostly by the Government agencies or committees worldwide. These agencies / committees supervise or monitor the semen quality in order to avoid spreading of infectious diseases and ensure the supply of disease free semen for animal breeding programmes. The standards for semen production and distribution are usually based on regulatory programmes to ensure that important diseases are identified and appropriate tests are applied to breeding bulls entering and residing in semen production units. Monitoring committees/ agencies emphasize testing programmes need to be continually improved and updated based on new scientific developments in order to monitor emerging new infectious diseases or pathogens (Truyen et al, 1995). At international level guidelines are published and revised by the Office International des Epizooties (OIE). This article focuses on information on infectious agents known to be transmitted by the bull semen, causing infectious diseases and their prevention.

Infectious Bovine Rhinotracheitis (IBR) is a highly contagious, infectious disease caused by a DNA virus, Bovine herpesvirus-1 (BHV-1). In addition to causing respiratory disease, this virus can cause conjunctivitis, abortions, encephalitis, and generalized systemic infections. IBR was initially recognized during the early 1950s in feeder cattle in the western US. The IBR virus can persist in clinically recovered animals for years.

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The virus remains inactive until the animal is placed under stress. The virus is shed in secretions from the eye, nose and reproductive organs including semen. The clinical diseases caused by the virus can be grouped into: 1) respiratory tract infections 2) eye infections 3) abortions 4) genital infections 5) brain infections 6) generalized infections of newborn calves. Bull plays an important role in the dissemination of the disease because the virus is excreted in semen (Van der, 1995). Incidence of IBR was first recorded in India during the year 1976 (Mehrotra, 1977). Subsequently IBR has been reported from the states of Orissa (Mishra and Mishra, 1987), Karnataka (Mohan et al., 1997) and Gujarat (Singh et al., 1986), and moreover widespread serological evidence due to respiratory as well as genital tract infections is reported from most of the states in India (Samal et al., 1980; Suribabu et al., 1984). Although clinical findings of respiratory disease, abortion or Infectious Pustular Vulvovaginitis (IPV) may be highly suggestive of IBR, there is no definitive clinical diagnosis for IBR. Methods of BHV-1 detection currently used by diagnostic veterinary laboratories include virus isolation, examination of tissues by the fluorescent antibody (FA) technique, polymerase chain reaction (PCR), serological testing viz.; serum neutralization (SN) test or enzyme-linked immunosorbent assay (ELISA). Another method is the 'Cornell semen test', in which pooled samples of semen are inoculated into susceptible calves or sheep which are then monitored for neutralising antibodies to BHV-1 (Schultz et al., 1982), but presently this method is not generally used widely as several other new techniques are currently available. The traditional method for detection of BHV-1 in bovine semen is virus isolation and identification in cell culture of bovine origin (Straub, 1990). IBR is not a zoonotic disease. As no treatment for the virus is available, supportive care should be provided. Antibiotics in the feed and water are used to treat secondary bacterial infections.

By the year 1997-98, BAIF had developed a specific field diagnostic kit for identifying infected animals. Intervet India (Ex-BAIF) Laboratories, based in Pune is well known for its contribution in the promotion and development of animal healthcare products. It had launched Ibrivax-IBR oil adjuvant vaccine developed by BAIF Development Research Foundation for the prevention and control of viral Infectious Bovine Rhinotracheitis (IBR) disease. Preferably only sero-negative bulls should be maintained in the semen stations, however, semen from sero-positive bulls housed in the semen station should be tested for the presence of IBR virus and semen negative for virus should be used for insemination.

Bovine Viral Diarrhoea is a viral disease of cattle and other ruminants that is caused by the bovine viral diarrhea virus (BVDV) that is world-wide distributed. However, some countries have recently eradicated the virus. It causes a variety of clinical outcomes that range from the unapparent (sub-clinical) to the more severe (clinical manifestation) including abortion, infertility, an immuno-suppression that underlies calf respiratory and enteric diseases, and most dramatically, the fatal mucosal disease (Baker, 1995).Infections of the breeding female may result in conception failure or embryonic and fetal infection, which results in abortions, stillbirths, teratogenic abnormalities or the birth of persistently infected (PI) calves. PI animals shed BVDV in their excretions and secretions throughout life and are the primary source of transmission of the virus. These animals can usually be readily detected by virus or viral antigen detection assays (RT-PCR, ELISA), except in the immediate post-natal period where colostral antibodies may mask the presence of virus (Lanyon, 2014). The virus can be detected by using molecular techniques (RT-PCR) (Hoffmann et al., 2006). BVDV is an RNA virus and the disease is not zoonotic in nature. The

prevalence of BVDV antibodies in Indian cattle was reported as 15.29% (50/327) in 16 states compared to 23.21% (26/112) in buffalo in 9 states, with an overall prevalence of 17.31% (76/439) in 17 states (Sudharshana et al., 1999; Mishra et al., 2007).Treatment of BVD is limited primarily to supportive therapy. Once identified, persistently infected(PI) animals should be culled. Strategic vaccination and high-quality colostrum could also decrease the proportion of susceptible cattle.

BVDV vaccines are used primarily for disease control purposes. BVDV eradication programmes are being undertaken in many countries. Controlling BVDV infections by the vaccine can be challenging due to antigenic variability of the virus and the occurrence of persistent infections. On-going maintenance of the virus in nature is predominantly sustained by PI animals. Traditionally, BVD vaccines fall into two classes: modified live virus or inactivated vaccines. Bovidec is the tried and trusted BVD vaccine, and has a long track record of BVD control in both beef and dairy herds throughout the UK and Ireland. Similarly, Bovilis BVD, an inactivated vaccine containing cytopathogenic BVD virus strain C86 produced by the MSD Animal Health, New Zealand. However, BVD vaccine is not available in India.

Bovine Brucellosis is a contagious disease of livestock with significant zoonotic importance. Brucellosis causes economic losses to the tune of Rs 350 million/year in India (PD-ADMAS, 2012). In cattle it is mostly caused by *Brucella abortus* less frequently by *B. melitensis*, and rarely by B. *suis*. In cattle, the disease is characterised by abortion and is often associated with retained placenta, metritis and a subsequent period of infertility. It affects many animal species viz.; cattle, buffaloes, sheep, goat and also humans. It is zoonotic and affecting 5% population of livestock worldwide. The highest incidence is observed in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico (Chitupil et al., 2015; Mangen et al., 2002). Currently, countries in central and southwest Asia are seeing the greatest increase in cases. A serological survey of brucellosis was performed in 23 states of India. A total of 30,437 bovine sample was screened with Rose Bengal plate test(RBPT) and standard tube agglutination test (STAT) which reveals 1.9% prevalence in cattle and 1.8% in buffaloes (Isloor et al 1998). The Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS) conducted long-term serological studies which indicated 5% of cattle and 3% of buffaloes are infected with brucellosis (Renukaradhya et al., 2002), which was higher than previous studies. In one of his presentations, Singh (2007) observed prevalence rate of brucellosis was 8.58% in cattle from Rajasthan and Bihar states. A number of reports on brucellosis in India reveals a high incidence of the diseases (Aulakh et al., 2008; Jagapur et al., 2013; Shome et al., 2014).

All abortions in cattle in late gestation, starting from the fifth month, should be treated as suspected brucellosis and should be investigated and may be confirmed by laboratory examination. The diagnostic tests are applied with different goals, such as national screening, confirmatory diagnosis, certification, and international trade. Unequivocal diagnosis of *Brucella* infections can be made only by the isolation and identification of *Brucella*, but in situations where the bacteriological examination is not practicable, diagnosis must be based on serological methods. There is no single test by which a bacterium can be confirmed as *Brucella spp.* A combination of growth characteristics, serological, bacteriological and/or molecular methods is usually needed. *Brucella* is Gram negative, non-motile and smooth, usually does not show bipolar staining.

Cultural isolation and identification is one of the confirmatory method of diagnosis which is usually performed by culture on solid media. A wide range of commercial dehydrated basal media is available, e.g. Brucella medium base, tryptose (or trypticase)–soy agar (TSA). The addition of 2–5% bovine or equine serum is necessary for the growth of strains. On suitable solid media, Brucella colonies are visible after a 2–3-day incubation period. For the diagnosis of animal brucellosis by cultural examination, the choice of samples usually depends on the clinical signs observed. The most valuable samples include aborted fetuses (stomach contents, spleen and lung), fetal membranes, vaginal secretions (swabs), milk, semen and arthritis or hygroma fluids. From animal carcasses, the preferred tissues for culture are head, mammary and genital lymph nodes, spleen, uterus and the udder.

Identification of Brucella organisms can be carried out by a combination of the following tests: organism morphology and Gram or Stamp's staining, colonial morphology, growth characteristics, urease, oxidase and catalase tests, and the slide agglutination test with anti-Brucella polyclonal serum. Species and biovar identification requires elaborate tests; phage lysis and agglutination with A-, M- or R-specific antisera. The serum samples can be analysed by Rose Bengal Plate Test (RBPT) according to standard protocol (Alton et al. 1988). The Rose Bengal test (RBT) is a simple, rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum however, interpretations of the RBT results can be affected by personal experience (Cho et al., 2010).

The advent of molecular techniques allowing identification of *Brucella* has been developed (Bricker et al.2003) and are widely used in certain diagnostic laboratories. Several PCR based methods have been developed. The best validated methods are based on the detection of specific sequences of *Brucella* spp., such as the 16S-23S genes, the IS711 insertion sequence or the *bcsp*31 gene encoding a 31-kDa protein (Baddour et al., 2008; Ouahrani-Bettache et al., 1996).

This disease can be serologically diagnosed by RBPT, complement fixation test (CFT), standard tube agglutination test (STAT), lateral flow assay (LFA) and using various types of ELISAs. Milk ring test (MRT) can be used for screening the pooled milk for brucellosis.

The most widely used vaccine for the prevention of brucellosis in cattle is the *Brucella abortus* S19 vaccine. It is used as a live vaccine and is normally given to female calves between 3 and 6 months of age as a single subcutaneous dose at a concentration of 5-8 × 10^{10} viable organisms. Whereas, a reduced dose (@ cons. of 3×108 to 3×10^{9} organisms) can be administered subcutaneously to adult cattle. *Brucella abortus* S19 vaccine induces good immunity to moderate challenge by virulent organisms. Since 1996, *B. abortus* strain RB51 vaccine has been used for prevention of brucellosis in cattle in some countries but each country is using different concentration of viable strain. Vaccines using *Brucella melitensis* and *Brucella abortus* strain 19 are produced by many companies in India.

Leptospirosis is an economically important zoonotic bacterial infection of livestock that causes abortions, stillbirths, infertility, and loss of milk production in chronic cases. Leptospirosis is worldwide distribution and occurs in man, cattle, buffaloes, pig, sheep, goat, dog, horse, etc. The epidemic presents an increasing incidence in both developing and developed countries (Meites et al., 2004). It manifests as an acute or chronic disease or as a clinical in apparent contagious disease of domesticated and wild

animals as well as man. It is caused by spirochetes belonging to genus *Leptospira. Leptospira* are ubiquitous spirochetes and are spiral shaped bacteria and posses a Gram negative like cell envelope consisting of cytoplasmic and outer membranes. In 2007 meeting of the Subcommittee on the Taxonomy of *Leptospiraceae* held in Ecuador, some of the previously described genomospecies were given the status of species resulting in a family comprising 13 pathogenic *Leptospira* species with more than 260 serovars and 6 saprophytic species comprising more than 60 serovars. Serotype *hardjo*, the predominant in most cattle populations (Bokhout et al., 1989), has been divided into two genotypes: *hardjo-prajitno* and *hardjo-bovis* (Thiermann and Ellis, 1986). Persistent infection of the male and female genital tract is also a prominent feature of serovar Hardjo infections. It is expected that additional new species exist and will add to this ever expanding taxa (Adler and Moctezuma, 2010). Survival of the leptospirae depends on the variation in soil and water conditions in the contaminated area. They are susceptible to drying, pH lower than 6 or greater than 8, ambient temperatures lower than 7°C or higher than 34°C. They can survive for as long as 183 days in water saturated soil, but survives for less than 30 minutes when soil is air dried. It can survive for very long periods in free surface water (WHO, 2006). Carrier animals, domestic or wild, maintain and propagate *leptospires* within the population. Infected animals may excrete leptospires intermittently or regularly for months or years, or even for their lifetime. The leptospires dwell in the renal tubules of their animal host. Vaccinated animals may still shed infectious organisms in the urine. Main transmission among animals can be sexual contact or by suckling milk from infected mother.

Although, Leptospirosis in Indian cattle is reported from the early 20th century, first report of leptospirosis among bufflaoes was from Uttar Pradesh by Adinarayanan et al. (1960). Most outbreaks of leptospirosis in India are reported from the coastal regions of the states of Gujarat, Maharashtra, West Bengal, Orissa, Kerala, Tamil Nadu, Karnataka and the Andaman Islands. Highest rates occur during October to November which coincides with the monsoon season in these parts. A significant outbreaks of leptospirosis have been occurring in the past few years in different parts of India; Orissa (Faine, 1994; Sehgal et al., 2001), Mumbai (Karande et al., 2002) and the Andaman archipelago (Sehgal et al., 1995; Singh et al., 1999), Karnataka (http://www.gideononline.com). Reports from the Southern part of Gujarat revealed 130 deaths of infected animals within a two month period because of leptospirosis. Many deaths due to leptospirosis were reported from Kochi (Kerala), Surat and Valsad (Gujarat) (Patel et al., 2014; Himani et al., 2013). Most leptospiral infections are subclinical and infection is more common than clinical disease. Subsequently, the prevalence of the disease in animals from various parts of the country has been reported by Rajasekhar and Nanjiah (1971), Srivastava et al. (1983, 1991), Varma et al. (2001), Piramanayagan et al. (2002) and Sivaseelan et al. (2003). Consequent to an outbreak of bovine leptospirosis in Chennai, serological evidence of leptospirosis was evident among human subjects (Ratnam et al., 1983). Prevalence studies being carried out by Indian Veterinary Research Institute during the last 15 years have showed an overall prevalence of 10.1% during 1975-90. During 1991-2000 the overall sero-positivity marginally increased.

Diagnosis of leptospirosis in cattle is relatively straightforward. In general, infected animals develop high antibody titers to the infecting serovar; an antibody titre>1:800 at the time of abortion is considered evidence of leptospirosis. Leptospires can be demonstrated in the placenta

and the fetus in some cases by immunofluorescence, PCR, and immunohistochemistry. Diagnosis of serovar Hardjo infection is more difficult and requires a combination of approaches. Serology alone often fails to identify animals infected with serovar Hardjo, because seronegative shedders are common in infected cattle herds. The recommended diagnostic testing strategy includes the primary use of a test (immunofluorescence or PCR) to detect the organism in the urine from a sample of cattle in the herd followed by serologic testing to provide insight into the likely infecting serovar of *Leptospira*. The microscopic Agglutination Test (MAT) is considered to be the "gold standard" for the laboratory diagnosis of leptospirosis where the serospirosis where the serovars involved can also be determined.

Cattle with acute leptospirosis can be treated with the label dosage of tetracycline, Oxytetracycline, penicillin, ceftiofur, tilmicosin, or tulathromycin. Leptospires are also highly susceptible to erythromycin, tiamulin, and tylosin, although these antibiotics cannot be relied on to remove the renal carrier state. Injectable, long-acting Oxytetracycline (20 mg/kg) and sustained-release ceftiofur have been shown to effectively eliminate shedding in cattle infected with serovar Hardjo. Vaccination can be combined with antibiotic treatment in the face of an outbreak of leptospirosis, but vaccination alone will not reduce urinary shedding. Leptospirosis in domestic animals can be controlled through vaccination with inactivated whole cells or an outer membrane preparation (Palaniappan et al., 2002). However, vaccine for leptospirosis is not available in India and infected animals are usually treated with antibiotics to eradicate the disease.

Bovine Genital Campylobacteriosis (BGC) is a sexually transmitted bacterial disease caused by *Campylobacter fetus subsp venerealis*. It is a gram-negative, non-spore forming bacterium, which is microaerophilic, fragile, and survives for only 6 hours under normal atmospheric conditions. The disease is subclinical nature in bulls with no overt clinical signs, but they are carriers and can infect females at service (Hum, 1994). The disease is characterised by temporary infertility of cows manifested by subacute diffuse mucopurulent (composed of mucus and pus) cervicitis (inflammation of the cervix), endometritis (Inflammation and infection of the endometrium (lining of the uterus) and salpingitis (inflammation of a Fallopian tube). Abortion occurs in a small percentage of infected cows, months after initial infection (Clark, 1997; Hoerlein, 1981). *C fetus* subsp *fetus* is similar to *C fetus* subsp *venerealis* that affects cattle and other species; sheep, birds. Although it has been known to cause sporadic abortions in cattle, it is not usually associated with infertility (Eaglesome and Garcia 1992).

No disease reported in India, however, it is present in other countries (OIE reports). A definitive diagnosis of genital vibriosis can be difficult and the results of laboratory tests are often disappointing (Andrews and Frank, 1974). Four laboratory tests – serum agglutination, cervical mucus agglutination, fluorescent antibody and culture have been used extensively in the past; however, each of these tests has a number of limitations. For laboratory diagnosis, the bacteria may be isolated and identified from preputial scrapings and semen from bulls, and cervicovaginal mucus and aborted fetuses, placentas from females. Infected bulls produce IgA in their preputial secretions. The multiplex PCR described by Hum *et al.* (1997) is currently the most cited PCR. Thus, the performance of this multiplex PCR allows differentiation of the two subspecies (*C. fetus* = one amplification product vs *C. fetus* subsp. *venerealis* = two amplification products).Vaccination should start

as soon as genital campylobacteriosis is diagnosed. Infected cows and cows at risk should be vaccinated. However, vaccine for BGC is not produced in India.

Bovine Trichomonosis is a sexually transmitted disease both in bovine and human. Human trichomoniasis is caused by *Trichomonas vaginalis* and bovine trichomoniasis is caused by *Tritrichmonas foetus.* Bovine trichomoniasis has resulted in reproductive failure. Fetal loss occurs most often late in the first trimester or early in the second trimester, although late term abortions are also seen. Although the parasite can survive in diluted semen and through the freezing process, the probability of transmitting infection through AI is not known. The human infection is also associated with adverse outcome of pregnancy, including preterm birth, premature rupture of the membranes and low birth weight infants (Cotch et al., 1997; Minkoff et al., 1984; Petrin et al., 1998; Schwelke, 2002). Trichomonosis occurs world-wide, particularly among range cattle (Pefanis et al., 1988; BonDurant et al., 1990; Riley et al., 1995).There are no reports of trichomonosis available in Indian bovine as De et al (1982) did not find infected animals among 13 well-managed herds in West Bengal, India. Only one cow from a rural herd was diagnosed as being infected.

Since these organisms tend to be present only in small numbers, a vigorous scraping of the preputial epithelium is recommended. A diagnostic kit has been developed which consists of a clear plastic pouch with two chambers of selective media. This is inoculated on-site with the sample and is then used for both transport and culture (Thomas et al., 1990). Similar diagnostic kits are also currently being used in several animal disease diagnostic laboratories in India. The organism obtained in the growth media may be further confirmed by DNA extraction and PCR detection of protozoan parasite. The inoculated culture media are examined microscopically for motile trichomonads for up to seven to ten days (Kimsey, 1986). Other ELISA has been developed (Bon Durant, 1997; Gault et al., 1995). Molecular-based techniques that use PCR technology have been developed for the identification of *T. foetus* (Campero et al., 2003; Cobo et al., 2007; Parker et al., 2001). A PCR diagnostic test offers a number of potential advantages, including increased analytical sensitivity, faster diagnostic turn around time, and the fact that the organisms in the collected sample are not required being viable.

Artificial Insemination is an excellent way to prevent or control genital diseases in animals. The vaccine protects against various bovine infections may not only protect the elite herd, but it is useful to protect against human sexually transmitted diseases. The testing of bulls entering AI should be mandatory.

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Effect of Milk Fever on Reproductive Performance of the Dairy Cattle

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Abstract :
High productive dairy cattle are the most susceptible to milk fever during periparturient period. It may contribute for other conditions like immuno suppression, ketosis, fatty liver syndrome and other related reproductive disorders. Various method of managing and preventing hypocalcemia were discussed.

Key words: Dairy cattle, Milk Fever, Hypocalcemia, Ketosis.

Introduction

During transition from late gestation to early lactation, tremendous adaptations occur in the dairy cow because of the increased need for nutrients to support milk synthesis. The requirement for calcium increases approximately two- to three-fold than required before calving. Most cows have some degree of hypocalcaemia at calving because intestine and bone adapt to the Ca demands of lactation (Reinhardt *et al.*, 2010). However in certain cases, the mammary drain of Ca causes an acute drop in blood Ca concentrations to a level(<5mg/dl) that disturb neuromuscular function, termed as "Milk Fever". Milk fever is more prevalent in Jersey and Guernsey cattle. One of the reasons for this is that Jersey cattle have fewer vitamin D receptors than Holstein cattle. Incidence is higher in high producers and increases with subsequent lactations. First-calf heifers rarely develop clinical hypocalcemia as they produce less colostrum and milk and can more rapidly mobilize calcium from bone in their growing skeleton. Milk fever is a contributing factor for development of various uterine diseases in dairy cattle. By nutritional management of animal before calving and during early lactation these diseases could be prevented.

Hypocalcemia and Immunosuppression

Ionized Ca is regarded as an important second messenger in cellular signal transduction. Fluctuations in intracellular Ca2+ concentrations are critical to activate immune cells, primarily neutrophils that are responsible for clearing the uterus of infectious agents after parturition. The source of the calcium for this is primarily the endoplasmic reticulum and mitochondria of the cell. Due to hypocalcemia, there is a decline in calcium concentration in extracellular fluids as well as intracellular stores of calcium within endoplasmic reticulum. Because of which, the response of immune cells to activating stimuli is hindered in cows with hypocalcemia.

Cows with milk fever have a three- to four-fold increase in plasma cortisol at the initiation of parturition and the typical milk fever cow may

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exhibit plasma cortisol concentrations about ten- to fifteen-fold higher than pre-calving plasma cortisol concentration (Horst and Jorgensen, 1982). Cortisol is generally considered a powerful immune suppressive agent and likely exacerbates the immune suppression normally observed in the periparturient period .These changes collectively could probably contribute to the impaired immune system of the periparturient cow and its increased susceptibility to infectious diseases.

Ketosis and Fatty Liver Syndrome and Immunosuppression

Ketosis primarily occurs 2-7 weeks after calving and is the consequence of negative energy balance(NEB). Because of high demand of nutrients around parturition and simultaneous reduction in feed intake cows develop a NEB. This will promote fat mobilization in the form of triacyl glycerides (TGL). In the liver, TGL will be transformed into ketone bodies *viz*. acetoacetate (AcAc) and acetone (Ac) and β-hydroxybutyrate (BHB) to generate energy. The appearance of these ketone bodies in the blood, milk and urine is diagnostic of ketosis. Also, during the period of NEB, there is increased lipolysis and decrease lipogenesis, causing high levels of non-esterified fatty acids (NEFA). Liver is not able to esterify high levels of NEFA into triglycerides and fatty liver syndrome occurs.As fat accumulates in the liver, it reduces liver function and a major function of the liver in the dairy cow is to produce glucose a major fuel of the immune system.

Therefore, in many respects, cow in early lactation is in a physiological state comparable to prolonged protein-calorie restriction in humans and rodents. Glutamine amino acid is utilized at high rates by rapidly dividing cells, including leucocytes, to provide energy and optimal conditions for nucleotide biosynthesis, thus considered to be essential for proper immune function. In humans, plasma glutamine is known to fall in patients with untreated diabetes mellitus, in diet-induced metabolic acidosis and in the recovery period following high-intensity intermittent exercise. Plasma glutamine level does not depress in the dairy cow at the time of calving when body fat mobilization as well as protein catabolism are increasing rapidly. However, as the cow progresses into the early weeks of lactation, plasma glutamine level does decrease, indicative of immunosuppression.

Franklin *et al.* (1991) examined the effects of beta-hydroxybutyrate, acetoacetate, acetone, acetate, butyrate, and glucose on *in vitro* proliferation of lymphocytes stimulated with concanavalin A, phytohemagglutinin-P, or pokeweed mitogen. No effect was observed on bovine lymphocyte proliferation *in vitro* when the concentration of these compounds was same as *in vivo*. Only very high levels of beta-hydroxybutyrate inhibited proliferation. Nonnecke *et al.* (1992) examined the effect of ketones and glucose on *in vitro* lymphocyte immunoglobulin secretion. Results indicated that plasma glucose concentration associated with the ketotic state (1.66 mM, a plasma glucose concentration associated with the ketotic state), compared with normal plasma glucose concentration (3.33 mM), and did not affect total or antigen-specific IgM secretion. Adding a mixture of ketones to the culture media which approximated plasma levels of severely ketotic cows inhibited mitogen-induced IgM secretion in 11.1 mM glucose-supplemented cultures but not in cultures with 3.33 or 1.66 mM glucose. These data indicate that effects of ketones and acetate on IgM secretion are dependent on the concentration of glucose in culture and suggest that changes in plasma glucose, ketone, and acetate concentrations associated with bovine ketosis do not alter IgM secretion *in vivo*. However, high ketones and high plasma glucose would be more likely in the human ketoacidotic

state and could help explain the immune deficits observed in diabetics. Beta-hydroxybutyrate (but not other ketones) at concentrations seen in mild ketosis could decrease bactericidal activity in sheep.

A few studies have been done on the response of lymphocytes to levels of interferons alpha and gamma, isolated directly from normal cows and cows with clinical and subclinical ketosis and then placed into culture. Results indicated that leukocytes of cows with clinical symptoms and the highest concentration of ketones and free fatty acids in blood responded with the lowest levels of interferons alpha and gamma. There is relationship between liver triacylglycerol (TAG) content and immunophenotypical and functional properties of neutrophils of dairy cows in the peripartum period. Increased liver TAG content (>40mg/g) is associated with reduced expression of function of surface molecules on blood neutrophils. Moreover, in cows with high liver TAG levels, the antibody independent and dependent cellular cytotoxicity (AICC, ADCC) of blood neutrophil preparations is markedly reduced. Also, the increased serum free fatty acid concentrations inhibited calcium response of both CD4(+) and CD8(+) subsets. These studies suggest that NEFA could be associated with immunosuppression in periparturient cow.

Milk Fever and Reproductive Disorders

Hypocalcemia has been also related to dystocia and retained placenta. It has been reported that cows that developed milk fever were 3.0 to 4.2 times more likely to experience retained placenta (RP) than normal cows (Houe *et al*., 2001). Fetal placenta must be recognized as "foreign" tissue and rejected by the immune system after parturition to cause the expulsion of placenta. If the function of neutrophil is impaired at the time of calving, as in hypocalcemia, retention of placenta takes place. Further, cows developing RP were demonstrated to have reduced levels of antioxidants in their blood. A profound decrease in plasma content of vitamin E and beta-carotene,begins to occur about two weeks before calving and does not recover until several weeks after parturition, suggesting the periparturient period is a period of increased oxidative stress to the cow. It is interesting to note that deficiency of two nutrients, vitamin E and selenium, well known as anti-oxidants and known to be risk factors for RP, metritis and mastitis.

At the time of calving, cow's reproductive tract is most vulnerable to attack by bacteria. Cow survives because her immune system provides protection from infection. Neutrophils provide the first line of defense, moving out of the blood to clear the invading bacteria. They ingest the bacteria and release enzymes and free radicals onto the bacteria to kill them. Occasionally, the neutrophils don't succeed in killing the intruder. In this situation, second line of defense i.e. macrophages and lymphocytes, work together to produce antibodies and other antibacterial factors. These factors will eventually eliminate most infections that neutrophils cannot handle. However, situation is not same in immune-compromised animals. Bacteria are not kept in check in such animals and grow to large numbers in the uterus, causing a condition known as metritis. Around 20 to 30% of cows will develop metritis, which is characterized by a foul smelling, red-brown, watery discharge from uterus within 10 to 14 days after calving. It is often, but not always, accompanied by a fever. Additionally, the loss of muscle tone in the teat sphincter and in the uterus due to low Ca levels may increase the risk of uterine prolapse.

Endometritis is a uterine problem characterized by inflammation of the lining of the uterus lasting more than three to four weeks after calving. Studies suggest 40 to 50% of cows can

have endometritis at four weeks after calving. These cows are less likely to be successfully bred back. It is found that cows with metritis, or diagnosed with subclinical or clinical endometritis, had higher NEFA levels in their blood than did cows with a healthy uterus. Also endometritis is associated with a reduction in conception rates and extended intervals to pregnancy. High prepartum NEFA and postpartum BHBA have been found to be associated with losses in milk production during subsequent lactation (Ospina *et al.*, 2010).

Prevention of Hypocalcemia

Prevention of hypocalcemia generally involves modifications in the pre-calving diet. These changes allow for the physiological adaptations so that animal is prepared for the increased demand for calcium associated with the synthesis of colostrum and milk.

1. **Low calcium diets:** To be effective, diets must provide less than 20 g of available calcium. In some grazing situations (e.g., depending on forage species and pasture fertility), low calcium diets may be possible. Although this practice does reduce the incidence of hypocalcemia, it is difficult to implement on the farm.

2. **Low potassium forages/diets:** Incorporating low potassium forages (e.g., corn silage) into diets for pre-calving dairy cows may decrease the likelihood of clinical hypocalcemia but not the incidence of subclinical hypocalcemia. By excluding sodium and potassium (cations) and including more chloride and sulfate (anions), the DCAD becomes more negative. The DCAD influences the pH of the blood and the responsiveness of tissues to PTH and the cow's ability to reabsorb calcium from bone and absorb dietary calcium from the small intestine.

3. **Feeding anionic salts for 21 days pre-calving:** Feeding a negative DCAD diet 21 days pre-calving has been shown to prevent clinical (a five-fold reduction) and subclinical hypocalcemia. Diets should be formulated to result in a dietary DCAD of -10 to -15 mEq/100g dietary dry matter using the most palatable of anionic mineral supplements. Many commercially available anionic mineral or protein-based supplements are available for use in formulating these diets. Before formulating diets, the amount of potassium and sodium provided through forages and other feedstuffs should be kept as low as possible. Diets should be formulated with about 1.0% calcium and 0.35% magnesium to prevent hypocalcemia. Phosphorus concentration of close-up diets should be 0.25% to 0.3% because excess phosphorus (0.4% total diet) increases the risk for hypocalcemia.

4. **Oral sources of calcium:** Calcium supplemented orally (not part of the diet) after calving has shown a positive response for preventing a drop in concentration of blood calcium. Many oral supplements are absorbed within 30 minutes after administration and blood calcium concentration is increased for 4 to 6 hours. Oral supplementation of calcium often is in the form of calcium chloride in gel or paste forms. The calcium chloride in these forms can result in respiratory problems if aspirated, and as such, care must be taken when administering it. More recently, a solid bolus coated with fat containing calcium chloride and calcium sulfate was tested and found effective at increasing the concentration of blood calcium when two doses (one at calving, a second 12 hours post-calving) were given after calving. Coated boluses would help reduce the chances of cows aspirating the product. In another study, these boluses

were tested in combination with anionic salts fed pre-calving, but no differences were seen versus providing anionic salts alone.

Conclusion

Milk fever or hypocalcemia is one of the most common disorders occurring around parturition. Adequate calcium is important for colostrum and milk synthesis, muscle and nerve function, and immunity. Milk fever affects the reproductive health of dairy cattle and predisposes the animal to various diseases. The reproductive health of the animal is key to profitability of any livestock farm. Therefore, implementing proper nutritional and managemental programs that reduce the risk of metabolic disturbances are expected to improve cow health, but also improve economics of farm.

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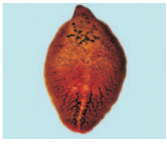
Top three diseases of Livestock in India are caused by parasites

As per the Annual report submitted by NIVEDI (National Institute of Veterinary epidemiology & disease informatics) on animal disease monitoring (2014-15), top three ranks in India were parasitic diseases namely Fascioliasis, Trypanosomiasis and Babesiosis. Parasitic diseases were reported from almost all the states irrespective of the seasonal changes. It is well known that the parasitic diseases are, many times the sole cause of economic loss to the farmers. A Nationally approved package is necessary for the control of these parasitic diseases.



(Source: 2014-15 annual report on animal disease monitoring, NIVEDI)

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Recent Uses of Prostaglandin ($PGF_2\alpha$) for Estrus Synchronization in Bovine

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Abstract

Prostaglandin is member of a group of lipid compounds that are derived enzymatically from fatty acids. The use of $PGF_2\alpha$ is based on its luteolytic effect causing regression of corpus luteum. The duration of the luteal phase of the estrous cycle can be modified by administering prostaglandins or using progesterone/progestagen implants. To perform timed artificial insemination, these treatments have to be used in combination with GnRH, hCG or eCG. Single or double treatment regimens of $PGF_2\alpha$ resulted in induced estrus and ovulation during the breeding season. Recently use of Ovsynch protocol for estrus synchronization and fixed time AI is effective in cattle and buffalo. The uses of heat synch, pre-synch and double Ovsynch protocols are highlighting the importance of targeting pre-synchronization protocols to stimulate cyclicity in anoestrus dairy animals.

Key words: Bovine, Corpus Luteum, Estrus synchronization, GnRH, $PGF_2\alpha$, Progestogen

Introduction

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) has been the most commonly used treatment for synchronization of estrus in cattle and buffaloes. The duration of the luteal phase of the estrous cycle can be modified by administering prostaglandins or using progesterone/progestagen implants. To perform timed artificial insemination, these treatments have to be used in combination with GnRH, hCG or eCG. The administration of $PGF_2\alpha$ from about day 5 of the estrous cycle causes regression of the corpus luteum (CL). Thereafter, progesterone declines rapidly to basal concentrations within 24 hour resulting in induction of estrus and ovulation (Chohan *et al.*, 1995). The intravaginal submucosal administration of low doses of $PGF_2\alpha$, ipsilateral to the side of the CL, is as effective at inducing luteolysis as the conventional intramuscular administration of the hormone (Rao, 1988).

Efficient estrus control treatments should have the ability to affect the wave pattern by preventing the development of persistent dominant follicles containing ageing oocytes and the recruitment of the future ovulatory follicle whatever the stage of the wave at the time of treatment which would allow synchronous ovulation of growing dominant follicles. (Driancourt, 2001).

1. $PGF_2\alpha$ (Single or Double injection)

Single or double treatment regimens of $PGF_2\alpha$ in buffalo induce estrus and ovulation in about 60

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to 80% of animals during the breeding season (Brito *et al*., 2002). The mean interval from $PGF_2\alpha$ treatment to estrus reported in these studies was 88 hr (range 48 to 144 hr; 78% from 72 to 96 hr) and from treatment to ovulation was 100 hr (range 60 to 156 hr; 81% from 84 to 108 hr). These intervals are shorter, however when $PGF_2\alpha$ is given during the early luteal phase of the estrous cycle in the presence of dominant follicles, as opposed to the late phase of the cycle (Baruselli, 2001). Pregnancy rates following prostaglandin treatment are 45 to 50 per cent on average (Neglia *et al*., 2003) and appear to be similar to those obtained after natural estrus. The efficacy of treatment is dramatically reduced during the non-breeding season, with conception rates falling to below 25 per cent (Chohan *et al*., 1995) even when a remarkably high percentage (88 %) of buffaloes display standing estrus following $PGF_2\alpha$ treatment.

The buffaloes during the breeding season were treated with two doses of $PGF_2\alpha$ separated by an interval of 13 days and ovarian activity was examined by ultrasound two days after the second $PGF^2\alpha$ administration. Animals with a dominant follicles measuring at least 10 mm were inseminated 16 to 22 hr later, while animals without a DF were inseminated two days later, depending on the stage of follicular development at the time of the ultrasound exams. To ensure ovulation, GnRH was given at the time of insemination. Pregnancy rates were similar to those of animals managed under the classic Ovsynch protocol (48% vs. 50%, respectively). Rosenberg et al. (1990) and Folman *et al*. (1990) compared estrus synchronization and pregnancy rates in groups of lactating cows given two injections of $PGF_2\alpha$ either 11 or 14 day apart and inseminated according to detected estrus following the second injection. Estrus occurred earlier after the second PGF injection when it was given 11 day after the first, but a similar percentage of animals were detected in estrus for each group (Rosenberg *et al*., 1990). Pregnancy rates were greater for cows receiving $PGF_2\alpha$ injections 14 day intervals (Folman *et al*., 1990).

Attempts have been made to develop management practices to identify cows that should respond to $PGF_2\alpha$ and avoid treating cows that will not respond, such as those without a functioning corpus luteum (CL). Stevenson and Pursley (1994) used milk progesterone tests to identify cows with elevated concentrations of progesterone that subsequently were treated with PGF. Reproductive performance of these cows was compared with cows submitted for AI based on twice daily estrus detection. The use of milk progesterone tests decreased days to first AI, calving intervals and cost per pregnancy, but the cost was greater than a blind weekly injection of PGF to all non-inseminated animals. Similar results were found when trans-rectal palpation was used to identify cows for $PGF_2\alpha$ treatment. Kristula, *et al*. (1992) found that weekly PGF injections followed by standard estrus detection decreased days to first insemination and increased pregnancy rate by 30 per cent over the first 90 day postpartum compared with trans-rectal palpation followed by $PGF_2\alpha$ administration to cows identified as having a palpable CL.

2. Progesterone plus $PGF_2\alpha$

This method consists of inserting a progestin implant (CIDR) with a plastic applicator into the vagina of the animal on day 1. On day 6, inject all animals with $PGF_2\alpha$. Remove all vaginal implants on day 7. Monitor for heat and bred standing heat for the next four days. Most females will exhibit estrus within 24 to 72 hours after the $PGF_2\alpha$ injection. The progestogen based estrus synchronization programs for cattle are

associated with a reduction in conception rate at the synchronized estrus (Ryan *et al*., 1995). The reduction in fertility after estrus synchronization with progestogen has been attributed to the development of persistent dominant follicles and subsequently the ovulation of aged oocytes that if fertilized, result in a poor quality embryo with reduced developmental capacity (Mihm *et al*., 1994).

Instead of administering an estrogen at the beginning of a progestogen treatment to reduce the length of progestogen exposure, a prostaglandin may be administered at or near the end of a progestogen treatment. This approach was first taken by Heersche *et al*. (1979). They combined a norgestomet implant with an injection of $PGF_2\alpha$ before or at implant removal. At the end of the implant period, cattle should either have a corpus luteum that is susceptible to regression by prostaglandin or have undergone natural corpus luteum regression already. Therefore, cattle should show estrus soon after implant removal and prostaglandin injection. When beef heifers were treated with a 7 day norgestomet implant and injected with $PGF_2\alpha$ on day 6 or 7 after implantation, 93 per cent showed estrus within 5 day and 62 per cent of these conceived, which was similar to a 60 per cent first service conception rate for controls. Combined with an injection of alfaprostol at implant removal, a 9 day norgestomet implant was as effective as Syncro-Mate B in postpartum beef cows (Brown *et al*., 1988). Whittier *et al*. (1986) reported that a 7 day norgestomet implant with alfaprostol on day 7 resulted in conception and pregnancy rates similar to those obtained with SyncroMate-B, however the degree of synchrony was better for heifers treated with SyncroMate-B.

A synchrony of estrus, which is important for timed insemination, can be attained by injecting prostaglandin 2 day prior to norgestomet implant removal. This concept also has been using applied a PRID for 7 day and injected $PGF_2\alpha$ on day 6. Synchronized pregnancy rate was greater for the PRID- $PGF_2\alpha$ group than for cattle treated with two injections of $PGF_2\alpha$ 11 day apart. Short term feeding of MGA was combined with an injection of $PGF_2\alpha$, at the end of MGA feeding. This system induced cycling successfully in some non-cycling cows. However, fertility of the estrus after treatment in cattle known to be cycling prior to this treatment was reduced relative to controls. This reduced fertility was present in cattle that were greater than day 13 of the estrous cycle at the progestogen treatment may lower fertility beginning of treatment. This day of cycle even though the progestogen treatment is 9 day effect was similar to that reported for SyncroMate-B (Brink and Kiracofe, 1988).

3. Progesterone, Estradiol, hCG or eCG plus $PGF_2\alpha$ Combinations

Estradiol, hCG and/or prostaglandin have been successfully used to improve the synchrony of estrus and conception rates in progestagen based protocols (Chohan *et al*., 1995). Barile *et al*. (2001) used eCG at the time of progesterone device withdrawal followed by two timed artificial inseminations 72 and 96 hr later. This protocol achieved a conception rate of 51 per cent. Treatment with progestagens in conjunction with eCG can induce the resumption of estrus in anestrous buffalo, yielding pregnancy rates close to 30 per cent (Neglia *et al*., 2003). The treatment of acyclic buffaloes displaying some ovarian follicular growth with Ovsynch or PRID plus PMSG during the months of increasing day length gave pregnancy rates of 40 per cent and 70 per cent, respectively (Presicce *et al*., 2005).

3.1 Ovsynch Protocol

In recent years, the impact of the novel Ovsynch

protocol for estrus synchronization and fixed time AI effectively carried out in cattle. The initial GnRH injection caused ovulation or luteinisation of the large follicle present at that time and synchronized the recruitment of a new follicular wave. At 7 days following GnRH injection, an injection of $PGF_2\alpha$ induced regression of the CL and allowed for final maturation of the synchronized DF. A second GnRH injection given 48 hr after $PGF_2\alpha$ injection, synchronized the time of ovulation of the DF. These systems promote ovulation of the DF by GnRH administration, CL regression by prostaglandin administration 7 days later, and thereafter the control of ovulation of the new DF by a second injection of GnRH. For its success, this protocol requires the presence of a DF at the time of the first GnRH treatment (De Rensis *et al*, 2005). In buffalo, the Ovsynch protocol has been observed to synchronize ovulation in 78 per cent (Baruselli, 2001) and 90 per cent of animals (Paul and Prakash, 2005) with conception rates during the breeding season average between 33, 50 and 60 per cent (Baruselli, 2001). Parity shows an effect on treatment efficacy with pluriparous animals responding better than primiparous animals (51 % vs. 35 %).

The degree of synchronization after Ovsynch in cyclic animals can be improved by initiating treatment in the presence of a DF, with the administration of progesterone between the first GnRH and $PGF_2\alpha$ administration (De Renis *et al.*, 2005) and when the second GnRH treatment is substituted by LH (Berber *et al*., 2002). However conception rates following the Ovsynch protocol are reduced during transition from the breeding to non-breeding season (Neglia *et al*., 2003), and dramatically decrease during non-breeding season to 7 per cent (Baruselli, 2001).

3.2 Heat Synch Protocol

The Ovsynch variation using second estradiol benzoate instead of second dose of GnRH called Heat Synch protocol. Cows treated (day 0) at random stages of the estrous cycle with GnRH and after seven days, all animals were treated with $PGF_2\alpha$. These cows were received one injection of estradiol benzoate on day 8. AI was performed at 30 to 34 hr after the estradiol benzoate injection. Pregnancy rate was found to be 43.30 per cent for timed AI compared to 47.7 per cent obtained after regular Ovsynch protocol.

An estrus synchronization protocol called Heat synch developed in cattle which makes use of a combination of GnRH - $PGF_2\alpha$ - Estradiol benzoate (EB) injection was adopted in buffaloes by Mohan and Prakash (2009). EB is less expensive hormone in place of second GnRH injection of Ovsynch protocol. EB is a commercially available form of the natural hormone, estrogen, and creates a surge type release of gonadotropin releasing hormone (GnRH) from the brain. GnRH in turn causes the release of LH which results in ovulation of the mature follicles and ovulations may occur 48 to 60 hours after EB injection. Estrus symptoms are also improved using this technique. The success rate in terms of induction of ovulation (80 %) in buffaloes (Mohan and Prakash, 2009) was higher than that reported in cows (70 %) by Barros *et al*. (2000) using Estradiol benzoate in Heat synch protocol. Using Estradiol cypionate in the Heat synch protocol on cattle, Pancarci *et al*. (2002) reported similar success rate (86 %) in cows. The timing of ovulation (50 hr) in relation to estradiol benzoate injection was similar to those in cows 55 hr (Pancarci *et al*., 2002) and 59 hr (Stevenson *et al*., 2004). Mean ovulation time in relation to LH surge of 29 hr was also similar to observations in cows using Heat synch protocol (Stevenson *et al*., 2004).

3.3 Pre-synchronization before Ovsynch/ Heatsynch protocol

Various experiments using lactating dairy cows

and dairy heifers found that the ideal phase to initiate the Ovsynch protocol is from Days 5 to 12 of the estrous cycle (Vasconcelos *et al*., 1999). Based on this idea, researchers have developed pre-synchronization systems that attempt to increase the proportion of cows in the ideal part of the estrous cycle on the day of the first GnRH of Ovsynch. For instance, Moreira *et al*. (2001) reported that two PGF treatments 14 day apart increased the percentage of cows in the early to mid luteal phase and improved fertility in cycling cows when Ovsynch was initiated 12 day later. However anovulatory cows did not benefit from this pre-synchronization protocol (Moreira *et al*., 2001). Other studies using similar Pre-synch protocols with two PGF treatments reported an improvement (Navanukraw *et al*., 2004) in fertility following an Ovsynch protocol, however a single treatment with PGF prior to Ovsynch was not effective (LeBlanc *et al*., 2003). Anovulatory cows have been found to be well synchronized by the Ovsynch protocol, but have greatly reduced fertility to the TAI protocol (Gumen *et al*., 2003). This reduced fertility may be due to the increased percentage of short cycles in anovulatory cows following Ovsynch. There are a substantial percentage of cows that are anovulatory (20 to 30 per cent) at the time of the first GnRH of Ovsynch (Moreira *et al*., 2001), highlighting the importance of targeting presynchronization protocols to stimulate cyclicity in anovulatory cows. Limitation of the standard PGF based Pre-synch protocol is that follicular and luteal stages are not precisely synchronized, due to the variability in time to estrus or ovulation following PGF treatments. Ovulation to the first GnRH of Ovsynch increased circulating progesterone (P4) at the time of PGF, reduced variation in the size of the ovulatory follicle and increased synchronization rates during Ovsynch.

3.4 Double Ovsynch Protocol

In spite of the high fertility to Presynch-Ovsynch, cows were presynchronized with double Ovsynch had even greater fertility than Presynch-Ovsynch cows, with TAI reaching close to 50 per cent for double Ovsynch (49.70 %). The improved fertility with double Ovsynch in primiparous cows appears to be improved treatment of anovulatory cows and increased synchronization of stage of the cycle at initiation of Ovsynch (Souza *et al*., 2008). In addition to treatment of anovulatory cows, it seems likely that double Ovsynch more tightly synchronized the stage of the estrous cycle at initiation of Ovsynch compared to Presynch. Ovulation to the first GnRH of the Ovsynch protocol and fertility during the Ovsynch protocol was dependent on stage of the estrous cycle at Ovsynch initiation (Vasconcelos *et al*., 1999). In designing the double Ovsynch protocol, it seemed likely that cows on Day 7 of the estrous cycle, the stage selected for initiation of the breeding Ovsynch, would be very likely to ovulate in response to the first GnRH of Ovsynch (Vasconcelos *et al*., 1999).

Perhaps the two PGF treatments of Presynch-Ovsynch had some positive effects on uterine health that were not observed with double Ovsynch, but these effects were offset by more dramatic positive effects of double Ovsynch on fertility (Souza *et al*., 2008). The improvement in fertility during double Ovsynch seemed to be mostly due to increases in fertility in primiparous rather than multiparous cows. It is generally accepted that primiparous cows are more likely to be anovulatory compared with multiparous cows (Chebel *et al*., 2006). Thus the parity differences in effects of double Ovsynch might be related to the percentage of anovulatory cows, and high percentage of anovulatory cows could result in reduced effectiveness of Presynch-Ovsynch in primiparous cows. In contrast, double Ovsynch may effectively treat anovulatory cows and the increased percentage of primiparous cows that are anovulatory may make this protocol more useful for primiparous cows.

Conclusion

The use of progesterone based protocols during the non-breeding season allows the insemination and induction of pregnancy in animals that would be non-productive. The novel Ovsynch protocol is most commonly used for estrus synchronization and fixed time AI effectively in cattle and buffaloes. Prostaglandin in combination with different hormonal regimens has proved to be efficient in synchronizing estrus along with induction of estrus and ovulation in postpartum dairy animals with improving reproductive efficiency.

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An Overview of Antibacterial Therapy in Small Ruminants

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Abstract :

Antibacterial have revolutionized Veterinarian approach to treatment, control and prevention of animal infection diseases, specially in small ruminants. A brief overview of commonly used antibacterials in small ruminant were discussed in this article.

Key words: Small ruminants, Antibacterials.

The primary goal of antibacterial drug use for treatment and prevention of bacterial infections should be to control bacterial growth to enable host responses to contain or eliminate bacteria responsible for disease. Although antibacterial drugs can help the host contain and eliminate infections, these drugs should not be considered solely responsible for eliminating infections in the host as both innate and developed immune responses are critical and optimizing immune status can minimize the effect of infections without use of antibacterial drugs. A brief overview on the role of the commonly used antibacterial classes in small ruminants is presented in this article.

Fluoroquinolones (FQ):

They are broad spectrum rapidly acting bactericidal agents, exhibiting concentration dependent killing and post antibiotic effect. Spectrum of activity is against gram positive, gram negative organisms; some of the agents showing extended spectrum against mycoplasma, Chlamydia and anaerobes.

- Better ocular tolerability with less toxicity to corneal epithelium makes FQs as good ocular anti infectives, with ofloxacin achieving the highest aqueous concentration. They are active in presence of abscess in spite of often unfavorable environmental conditions.
- Of the currently available fluoroquinolones, all have a similar spectrum of activity, but they may vary in potency.
- Against some gram-negative bacilli, especially *Pseudomonas aeruginosa*, ciprofloxacin is more active than veterinary quinolones.
- Levofloxacin possesses excellent activity against gram positive, gram-negative and anaerobes compared to ofloxacin and ciprofloxacin. Well distributed in to tissues : kidney, lung, prostate, genital tract, bones, phagocytes and inflammatory fluid.
- They are distributed into cytosol where they can reach intracellular pathogens *(Brucella, Mycoplasma, Mycobacterias)*. They are synergestic with beta-lactams, aminoglycosides. and antagonistic with macrolides, tetracyclines, chloramphenicol.

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- Arthropathy : (Noninflammatory erosive arthropathies) in young growing animals, retinal degeneration (in cats) CNS-excitability and seizure (Rapid IV administration). Phototoxicity are the adverse effects .

- Concurrent administration of drugs that contain divalent or trivalent cations, such as aluminum, calcium, iron, magnesium, or zinc cations (antacids, sucralfate, laxatives iron supplements, and molasses. multivitamins) will significantly inhibit oral absorption and NSAIDs may increase the risk of CNS stimulation and convulsions. The hepatic clearance of methylxanthines, like theophylline and caffeine gets reduced on concurrent administration with FQs , resulting in CNS related toxicity signs

- Doses: *sheep, goats* : enrofloxacin (2.5 to 5mg/kg, PO, IM, SC, q 24h), marbofloxacin (2 mg/kg, IM, IV, SC, q 24h), danofloxacin(6 mg/kg, Imq 48h).

Cephalosporins:

They are wide-spectrum β-lactum bactericidal antibiotics exhibiting time-dependent efficacy. They are classified into four generations, based primarily on their spectrum of antibacterial activity and resistance to β-lactamases.

- Generation wise, the spectrum of activity against gram negative organisms and the stability against β-lactamase increase from first to fourth, along with same or reduced spectrum of activity against gram positive organisms, except for 4th generation agents, which have enhanced activity aginst gram positive pathogens.

- Oral cephalexin, cefadroxil and parenteral cefazolin are the most commonly used first generation cephalosporins, primarily for skin and soft tissue infections such as pyoderma

- The oral absorption is poor and highly erratic in ruminants, thus used only in pre-ruminant young ones.

- Cefoxitin and cefotetan (cephamycins) are second generation ones, effective against anaerobic gram negative organisms, are used in the treatment of anaerobic infectious conditions like aspiration pneumonia, bite infections, ruptured intestine gangrene, peritonitis and pleuritis.

- **Ceftriaxone, ceftizoxime, cefotaxime and ceftazidime** are the third generation ones, have the ability to cross blood-brain barrier, effective in therapy for bacterial meningitis.

- **Ceftazidime** and **Cefoperazone,** are highly active against *Pseudomonas aeruginosa* among all cephalosporins, compared to Ceftriaxone and Ceftizoxime, which also have antipseudomonal activity to some extent.

- **Ceftiofur** has broader activity against gram-positive and beta-lactamase - producing strains as well as anaerobes. Ceftiofur presentation is in three formulations: ceftiofur crystalline freee acid, ceftiofur hydrochloride and ceftiofur sodium salt.; is indicated for treatment of bronchopneumonia in sheep, caused by *Pasteurella hemolytica or P. aeruginosa.*

- Ceftiofur sodium (50mg/ml powder vials for inj) and ceftiofur hydrochloride (50mg/ml sterile suspension) are the formulations approved for use in dogs, horses, catlle, sheep, goats and swines. Ceftiofur sodium has been used for treating coliform mastitis to reduce toxaemia. .Cefquinome, an extended spectrum beta-lactam, is used for treating bovine respiratory disease and mastitis.

Adverse Effects : *Gastrointestinal disturbances, Hypersensitivity reaction Bleeding disorders :* as all cephalosporins can inhibit vitamin K synthesis by suppressing gut flora. Prophylactic vitamin K therapy is recommended when any of these medications is used for prolonged periods in malnourished or seriously ill patients. *Hepatic dysfunction, Renal insufficiency,* Abscesses or other severe local tissue reactions (with IM injections) and thrombophlebitis (IV). Most of the cephalosporins are synergistic with aminoglycosides and nephrotoxic risk when used with other nephrotoxic medications: loop diuretics, NSAIDS.

Dosages (mg/kg) of approved cephalosporins in sheep and goats:

Ceftiofur sodium - 1.1-2.2 mg/kg, IM ,SC,q24h; Cefazolin-10 mg/kg ,IM, q8h; Cephapirin - 200mg TD, I/mammary, q12h; Cefoperazone-250 mg Total dose(TD), I/mammary,,q12h; Cefuroxime- 250 mg TD, I/mammary,q12h;Cephacetrile - 1.1-2.2 mg/kg, IM ,SC,q24h Ceftriaxone- 25-50 mg/kg, IM ,IV,q12h; Cefadroxil - 25 mg/kg,PO, q12h; Cephradine - 7, PO,q12h ; Cefetamet – 10 mg/kg,PO, q12h; Ceftazidime- 20-40 mg/kg, IM,,q24h

Macrolides:

Bacteriostatic antibiotics,(bactericidal at high concentrations.), with the spectrum similar to that of penicillins and are often used as penicillin substitutes. In addition,they are active against *Mycoplasmae, Chlamydiae, Legionellae,* gram positive anaerobes etc. The newer agents like clarithromycin (Not indicated in goats and horses), azithromycin, dirithromycin are found to be effective against opportunistic pathogens :toxoplasma, cryptosporidia, *H. pylori* etc. They should not be used with chloramphenicol or lincosamides.

- **Erythromycin:** The spectrum is narrow-mostly gram positive organisms and a few gram negative organisms. It is the drug of choice in corynebacterial infections , respiratory , neonatal ocular inflammation, or genital chlamydial infections . Erythromycin is not recommended in adult horses (oral and systemic) and ruminants (oral). Erythromycin estolate, can produce acute cholestatic hepatitis (fever, jaundice, impaired liver function), probably as a hypersensitivity reaction. Dose: cattle: 8-15mg/kg,IM,bid; Foals: 25mg/kg,PO or IM, tid; Sheep and swine: 2-6mg/kg, IM, sid

- **Tilmicosin** recommended for treatment of pneumonia in cattle, sheep and pigs, associated with *Pasteurella, Actinobacillus, mycoplasma* species. It is as effective or more effective than other established treatments like ceftiofur, oxytetracycline, or florfenicol for treating : bovine respiratory disease. Injections to horses, goats, swine, or nonhuman primates can be fatal; heart,the target of toxicity via depletion of cardiac intracellular calcium and thus contraindicated in goats,. *Dose :* Cattle, sheep: 10mg/kg, SC, q 72 h.

- **Tylosin** is used to treat : swine dysentery, pleuropneumonia due to *Haemophilus parahemolyticus,* colitis in dog, *Mycoplasma canis and Mycoplasma haemocanis* infection in dog & cattle. It is used against gram positive bacteria for shipping stress, pink eye, footrot in goats. Dosage : Kids: 1-2ml SC; Adults: 5ml per 100lb SC every 24 hours for 5 days. Use a larger (18ga or 20ga) needle for this thickish medication. Dose:Cattle: 10-20mg/kg,IM,bid; Pig: 10mg/kg, IM, bid

Lincosamides:

Bacteriostatic drug (particularly active against grampositive bacteria, and mycoplasma, and

good activity against anaerobic bacteria .

- **Lincomycin** has efficacy against *Erysipelothrix,, Leptospira pomona, Mycoplasma,* (activity similar to that of erythromycin, but less than that of other macrolides). Staphylococcus, and Streptococcus species.

- **Clindamycin** has a spectrum of activity- *Mycoplasama,* anaerobes such as *Actinomyces, Bacteroides sp, Clostridium perfringens.* They are widely distributed in to respiratory tissue, soft tissue and, bones; indicated for streptococci ,staphylococci and anaerobic infections. In horses, rabbits, chinchillas, guinea pigs, neonates and hamsters: lincosamides are contraindicated due to the risk of gastrointestinal adverse effects like serious fatal enterocolitis and diarrhoea. Oral lincomycin in ruminants results in : anorexia, ketosis, and severe diarrhoea. Antidiarrheals, adsorbent (kaolin/astringent)with oral lincomycin significantly decrease absorption. Dose: Lincomycin: 10 mg/kg, IM, bid- Cattle, sheep.

Tetracyclines:

Broad-spectrum with activity against gram-positive and gram-negative bacteria, including some anaerobes. They are also active against chlamydia, mycoplasma, some protozoa, and several rickettsiae, including Anaplasma, Ehrlichia, and Haemobartonella.

- **Oxytetracycline** for medicated feed and soluble powder and tetracycline soluble powder are indicated in the treatment of enteritis, pneumonia. Long-acting oxytetracycline injection is indicated in the treatment of pododermatitis ('foot rot') caused by susceptible *Fusobacterium necrophorum.* Oxytetracycline HCl injections indicated in uterine infections, skin, soft tissue infections & septic arthritis (joint ill).

- Antacids, Calcium supplements, such as calcium carbonate, Iron supplements, Magnesium-containing laxatives and sodium bicarbonate : Concurrent use of oral tetracyclines with these may result in formation of nonabsorbable complexes; Further,concurrent use within 1 to 3 hours of antacid or sodium bicarbonate administration may result in decreased absorption of oral tetracyclines because of increased intragastric pH.

Aminoglycosides:

Amikacin's spectrum of activity include coverage against many aerobic gram negative and some aerobic gram positive bacteria, including most species of *E. coli, Klebsiella, Proteus, Pseudomonas, Mycoplasma,* and *Staphylococcus.* Several strains of *Pseudomonas aeruginosa, Proteus,* and *Serratia* that are resistant to gentamicin will still be killed by Amikacin (10mg/kg. IM, q 24h).

- **Amikacin** is used clinically to treat serious gram negative infections in most species. Because Neomycin is more nephrotoxic and less effective against several bacterial species than either Gentamicin or Amikacin, its use is generally limited to the oral treatment of enteral infections -colibacillosis (bacterial enteritis) caused by *Escherichia coli* . (20mg/kg. PO. q 12h).

- **Gentamicin** (1-2mg/kg, IV, Im q 8h) is effective against gastro-intestinal and respiratory infections of cattle, calves, sheep, goats and swine, caused by bacteria such as E. coli, Klebsiella, Pasteurella and Salmonella spp.

- These agents can cause : nephrotoxicity, ototoxicity, neuromuscular blockade, facial

edema, pain/inflammation at injection site, peripheral neuropathy and hypersensitivity reactions

Penems (carbapenems):

Carbepenems are a new class of beta-lactam antibiotics that include imipenem-cilastatin sodium, meropenem, faropenem and ertapenem; the first two currently approved for use in dogs only.

- Imipenem is a broad-spectrum antibiotic with excellent activity against a variety of gram positive and gram negative organism (both aerobic and anaerobic), by comparison to third and fourth generation cepahalosporins. It is resistant to most forms of beta-lactamase including that produced by staphylococcus. The carbapenems are more rapidly bactericidal than the cephalosporins and less likely to induce release of endotoxin in an animal from gram-negative sepsis. Resistance to carbapenems has been extremely rare in veterinary medicine.

- The disadvantages of carbapenems include induction of resistance, inconvenient administration, and high cost. *Side effects:* Allergy, gastrointestinal disturbances: nausea and vomiting, seizures, hypersalivation and vocalization , indicating pain after IM amd SC administration in dogs was noticed. The empirical dose in dogs and cats is 5-10mg/kg, IV or deep IM, every 8 hours ,and in horse : 10-20mg/kg, IV, every 6 hours .

- **Meropenem,** has antibacterial activity greater than imipenem, with advantage over imipenem is that it is more soluble and can be administered in less fluid volume and more rapidly. Dose in dog is 2-5mg/kg, slow IV (with IV fluids) , every 6 hours; 5 -10mg/kg, deep IM every 8 hours or 8-12 mg/kg SC, every 8 -12 hours. Carbapenems are synergistic with aminoglycosides against *P. aeruginosa.*

Metronidazole:

Nitroimidazoles with activity against trichomonads and amebae include metronidazole, tinidazole, nimorazole, flunidazole, and ronidazole. Metronidazole and nimorazole are effective in the treatment of giardiasis, while dimetridazole, ipronidazole, and ronidazole control histomoniasis in poultry. Several nitroimidazoles have activity against trypanosomes.

- The principal clinical indications for metronidazole include the treatment of specific protozoal infections (amebiasis, trichomoniasis, giardiasis, and balantidiasis) and anaerobic bacterial infections such as those that may be seen in abdominal abscesses, peritonitis, empyema, genital tract infections, periodontitis, otitis media, osteitis, arthritis, and meningitis, and in necrotic tissue; primarily used with other antibiotics to treat mixed bacterial infections in which anaerobic bacteria are present. It is also used prophylactically after colic or other abdominal surgery when mixed bacterial infections are a risk. Metronidazole is generally given orally although it is also absorbed rectally.

- Rectal administration is occasionally used in the very sick patient when anorexia and weight loss are a problem.

- Dose : 15-25mg/kg, q 6h; IV, IM, PO, IR; 20-25mg/kg,q12h; IV, IM,PO,IR (in horses, dogs)

Suggested Empirical Antibacterials in Small Ruminants

Infection site	First choice drugs	Alternate choice drugs
Skin	Amoxicillin-clavulanate Cephalosporins	Trimethoprim-sulfonamides Fluoroquinolones
Urinary (Genital) tract	Cephalosporins Amoxicillin / Ampicillin Amoxicillin-clavulanate	Trimethoprim-sulfonamides Fluoroquinolone Tetracycline
Respiratory tract	Amoxicillin-clavulanate Fluoroquinolones Cephalosporins	Macrolides Aminoglycosides (amikacin, gentamicin) Chloramphenicol Extended-spectrum cephalosporin (2^{nd}/3^{rd} generation)
Septicemia	Amoxicillin-clavulanate Cephalosporin Fluroroquinolones	Aminoglycoside Extended-spectrum cephalosporins
Bone and joint	Cephalosporins Amoxicillin-clavulanate	Trimethoprim-sulfonamides Extended spectrum cephalosporins Fluoroquinolones, Clindamycin
CNS	Penicillin G, Trimethoprim-sulfamethoxazole, Ampicillin, (plus gentamicin)	Ceftriaxone, cefotaxime, ceftazidime, Cefuroxime,Ceftizoxime fluoroquinolones

Spectrum of activity of various Antibacterial classes

Class	Activity against Type of microorganism							
	Bacteria				Myco plasma	Rickettsia	Chlamydia	Protozoa
	Aerobic		Anaerobic					
	Gram + ve	Gram - ve	Gram + ve	Gram -ve				
Aminoglycosides	+	+	--	--	+	--	--	--
Penicillins	+	+	--	--	--	--	--	--
Potentiated penicillins	+	+	+	+	–	–	–	–
Cephalosporins	+	+	+	+	--	--	--	--
Chloramphenicol	+	+	+	+	+	+	+	--
Lincosamides	+	+	+	+	+	--	–	+

Class	Activity against Type of microorganism							
	Bacteria				Myco plasma	Rickettsia	Chlamydia	Protozoa
	Aerobic		Anaerobic					
	Gram + ve	Gram - ve	Gram + ve	Gram -ve				
Macrolides	+	--	+	+	+	--	+	--
Pleuromutilins	+	--	+	+	+	--	+	--
Tetracyclines	+	+	+	+	+	+	+	--
Fluorouinolones	+	+	+	+	+	+	+	--
Sulfonamides	+	+	--	--	+	--	+	+
Trimethoprim	+	+	--	--	--	--	--	+
Nitroimidazoles	--	--	+	+	--	--	--	+
Polymixin	--	+	--	--	--	--	--	--
Bacitracin	+	–	+	–	–	–	–	–

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Immunoneutralization of Hormones in farm animals

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Abstract :
Immunization against circulating hormones has various benefits in the farm animals. By controlling the secretion of endogenous hormone can control the body weight, reproductive performance and some clinical conditions in the farm animals.

Key words: Immunoneutralization, Hormones, benefits.

Immunoneutralization provides a simple, non-invasive approach to endogenous hormone deprivation. It includes both active and passive immunization procedures that can be used to neutralize circulating hormones (antigens). The passive immunity provides specificity and quickness to hormonal neutralization owing to the nature of the immune response and heterogeneity of antibody production among individuals. Comparatively, only small amounts of antigen are required to actively immunize a group of individuals and once established, biologically active antibody titres can persist throughout the course of an investigation. The impact of hormonal immunoneutralization depends on the hormone against which antibodies are produced and the relative physiological dependence of the individual.

Immunization against growth hormone (GH)

Pituitary GH is associated with stimulation of postnatal growth and is regulated not only by hypothalamic releasing factor(GRF), but also by somatostatin, a GH release inhibiting factor. Immunoneutralization of somatostatin to growth promotion has been evaluated in preliminary trials with young lambs. Somatostatin immunization stimulates average daily gain of young lambs by approximately 21%. Further, increased long bone growth in immunized lambs resulted in longer carcasses with no effect on body composition. However, studies are required on the potential success of somatostatin immunoneutralization as an aid to improve the efficiency of red meat production.

Immunization against luteinising hormone releasing hormone (LHRH)

LHRH can be considered as the trigger for the reproductive hormone cascade. The immunological neutralisation of LHRH would be expected to block pituitary secretion of LH and FSH and result in gonadal quiescence. Animals immunised against LHRH before puberty would remain pre-pubertal, while sexually mature animals would revert back to a pre-pubertal condition. In laboratory animals, the LHRH immunization suppresses the reproductive function. Immunization against LHRH in farm animals particularly cattle can be used to control sexual and aggressive behaviour, as well as

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fertility under different management systems. Immuno-neutralization of LHRH delayed the onset of puberty and hence fertility in heifers in a tropical environment. Young bulls immunised against LHRH also show a delay in the onset of puberty while sexually mature bulls undergo atrophy of the testes and a decline in circulating testosterone to castrate levels.

Immunization against gonadotrophic hormones

The gonadotrophins LH, FSH and human chorionic gonadotrophic (hCG) share a common α subunit but differ in the β subunit. Immunizations can be carried out using the purified native hormones comprising α and β subunits, however, antibodies of increased specificity are obtained if only the β subunits of the respective hormones are used. Selective immunization against either LH or FSH determines specific hormonal requirements for game to genesis and steroid ogenesis. Immunization against FSH in males may disrupt spermatogenesis without affecting normal production of steroid hormones. This could provide a contraceptive vaccine in species where the maintenance of sexual function is important. However, it is possible that strategic FSH immunization before puberty may induce a permanent lesion in the spermatogenic cycle regardless of species. In females, FSH stimulates early development of follicles and is therefore required on a continuing basis. Accordingly, FSH immunization may have potential as a contraceptive vaccine in females. However, females immunized against FSH may not maintain sexual characteristics because of a lack of sufficient oestrogen normally secreted by developing follicles. Young bulls immunised against LH showed reduced testis growth and had serum testosterone concentrations. Immunization of heifers and ewes using a bovine LH-ovalbumin conjugate induces an anovulatory condition. Similarly, active immunization of cows against pregnant mare serum gonadotrophin induced an anovular condition.

Immunization against gonadal steroids

Gonadal steroids serve important roles in sexual behaviour, accessory sex gland function and in the establishment and maintenance of pregnancy. These include androgens, oestrogens and progestagens. Immunization against testosterone in males has been shown to reduce feedback on gonadotropin secretion and promote increased testis size with increased testosterone secretion in rams and bulls. Although testosterone-immunised males have higher circulating testosterone levels, the anti-testosterone antibodies neutralise the actions of testosterone on sexual behaviour and somatic tissues. Testosterone immunization may be associated with pro-fertility rather than anti-fertility responses. In ewes and heifers, testosterone immunization can stimulate ovarian activity and cause abnormal ovarian cycles, cystic follicles and a reduced incidence of oestrus. A second androgen that has received attention in females is androstenedione. Immunization against androstenedione induces aberrations in ovarian function including varying degrees of superovulation and follicular cysts and reduced incidence of oestrus. Immunization protocols which induce infertility by causing abnormal ovarian function would be acceptable as contraceptive vaccines. Oestradiol-17β stimulates oestrous behaviour in females and also triggers the preovulatory LH surge. It could therefore be regarded as a good target for an anti-fertility vaccine. Ewes immunised against oestradiol-17β become anovular and anoestrus. Moreover, immunization against oestradiol-17β could also precipitate abnormal ovarian activity including cystic follicles. Oestradiol-17β, like androgens, is therefore an unlikely candidate for a contraceptive vaccine. Progesterone plays a

critical role in the establishment and maintenance of pregnancy and is perhaps the most likely gonadal steroid to be targeted by a contraceptive vaccine in females. Immunization against progesterone block the actions of this steroid on the uterus and prevent implantation. It could also influence embryonic development directly. Immunization against progesterone might therefore be acceptable as a contraceptive vaccine for livestock.

Immunization against oxytocin

Oxytocin secretion from the ovary commences with preovulatory follicles and proceeds throughout the luteal phase. Secretion of oxytocin in the late luteal phase acts on the uterus to stimulate prostaglandin F2α (PGF2) production, which in turn initiates luteolysis. Therefore, immunization of ewes and goats against oxytocin extends the oestrous cycle. More recently, it was found that ewes actively immunised against oxytocin exhibited significantly reduced conception rates.

Immunization against prostaglandin F2α (PGF2α)

PGF2α is secreted by the uterus and induces lysis of the corpus luteum. Accordingly, neutralisation of endogenous PGF2α results in maintenance of the corpus luteum. In heifers, active immunization against PGF2α, extended the length of oestrous cycle and conception was blocked for upto 4 months. Immunization against PGF2α results in continued secretion of progesterone that may provide an endogenous anabolic effect in meat producing heifers. However, pregnant animals should not be immunised against PGF2α, owing to interfere with parturition.

Immunization against inhibin

Inhibin is secreted by the gonads of mammals and it plays an important role in the control of FSH release. It is a non-steroidal glycoprotein hormone of gonadal origin with major action as negative feedback control of the production of follicle stimulating hormone (FSH) by anterior pituitary gland which in turn modulates male and female reproductive functions. Its physiological role has led to the development of inhibin based immunogens for fertility enhancement in farm animals. It is envisaged that a reduction of endogenous inhibin secretion would increase FSH concentrations and thus offers a potential for increasing the number of ovulatory follicles in the ovary. Immunization against inhibin has been reported to be a useful method for inducing multiple ovulations in farm animals.

Immunization against gamete antigens

The interaction between eggs and sperm at fertilization involves specific surface antigens present on gametes of both sexes. The particular attraction of gamete antigens for immunological contraception is that other reproductive functions should remain normal. In immunized males, the antibodies bind to sperm both during maturation and/or ejaculation and in immunized females, the antibodies bind to ova during development during immunoneutralization against surface antigens of gametes. The antibody binding to sperm or ova blocks gamete interactions required for fertilization.

Immunization against sperm antigens

The main sperm antigens are PH-20, SP-10, lactate dehydrogenase-C4, fertility antigen-1 (FA-1) and germ cell antigen-1 (GA-1). The sperm surface antigen, PH-20, was originally isolated from guinea pig sperm and appeared to be essential for fertilization in this species. Active immunization against PH-20 resulted in total infertility in male and female rabbits. The sperm antigen, SP-10, present on the sperm surface has received similar attention as the basis for a

contraceptive vaccine in man. Another antigen of interest in male gamete contraception is the sperm specificisozyme lactate dehydrogenase-C4. Reduced fertility after immunization of females with LDH-C4 has been observed in rabbits and mice.

Immunization against zonapellucida antigens

The zonapellucida (ZP) forms a coating around mammalian eggs and is comprised primarily of three glycoproteins, ZP1, ZP2 and ZP3. The ZP glycoproteins form a coating around the ovum and are involved in sperm binding during penetration of the zona. The glycoprotein ZP3 has been identified as the major sperm receptor and targeted as the most likely female gamete antigen for a contraceptive vaccine. Immunization of females against whole ZP and ZP antigens has shown to reduce fertility in a number of species. Further, immunization against ZP antigens has also been associated with abnormal ovarian function and altered reproductive hormone profiles.

Applications of immunoneutralization of hormones

1. Anti-self immunity (auto immune diseases, anti-tumour vaccines)
2. Better understanding of endocrine regulation.
3. Control fertility by providing new contraceptives for humans and animals.
4. Increase meat quality.
5. Combat diseases and boost productivity.
6. Practical way to control fertility of stray animals.

Conclusions

In conclusion, the immune system of animals plays an important role in reproduction. For inducing or controlling fertility, immunological interventions are an important aspect of management in domestic animals. Immunization against reproductive hormones such as luteinising hormone releasing hormone, luteinising hormone, follicle stimulating hormone gonadotrophic hormones, gonadal steroids, oxytocin, PF2 and inhibin has provided a significant effect on reproduction of animals. Further, immunoneutralization against gamete antigens such as against sperm antigens and zonapellucida antigens has shown also a profound effect on reproduction of farm animals. Therefore carrying out different types of reproductive immunization based on their scientific perspectives and procedures with a great precaution helps to induce or suppress of fertility in animal reproduction.

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Alternative Parasite Control in Grazing Livestock of India

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Abstract :

This paper deal with General alternative approaches to control parasites in the graizing livestocks. Use of anthelmentic drugs may not give full protection because of resistance to these drugs and use of these drugs with alternative approaches can provide good effect on eradication of parasites.

Key words: Alternative parasite control, FAMACHA, Vaccines.

Introduction

Parasitic worm infections can cause serious clinical disease, welfare problems and loss in production in farm animal species. As animal production has become more intensive the threat of parasitic disease has increased. Farmers are now very reliant on strategic, and in many cases "blanket", use of anthelmintic drugs to maintain health and production levels. Over the last forty years a number of very effective anthelmintic drug treatments have been discovered and made available to farmers to successfully control parasite disease. However, resistance to these drugs is becoming increasing prevalent, particularly in ruminants, and there is little prospect of new drugs coming onto the market in the near future. Therefore there is an urgent need to re-evaluate parasite control measures to ensure animal production is sustainable by using alternative methods. The present theme explains the alternative controls to overcome resistance and minimize the morbidity to farmers in terms of production and economy.

The parasite control program is to minimize the risk of parasitic disease, to control parasite egg shedding and to maintain efficacious drugs and avoid further development of anthelmintic resistance as much as possible.

Alternative parasite control includes:

- Housing management
- Nutritional management
- Pasture management -
- Genetics of animals
- Biological control
- Alternative dewormers-
- Targeted Selective Treatment- FAMACHA© system
- Vaccines

Housing Management

Animal shed must be well ventilated and lighted to maintain required humidity and air circulation (Madke et al., 2010).

- In high humidity and low light there will be accelerated growth of parasites population.

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- Water should be clean and free from faecal matter and watering areas should be situated in well drained places with gravel or even cemented floors.
- Facilities of proper drainage in the animal shed reduce the chances of survival of the parasites.
- Always keep the manure by making heap so that eggs, larvae, cyst or other stages of parasites are killed due to heat generated during composting (Williams and Warren, 2004).
- Application of nitrogen fertilizers like urea (1:25) to the surface of manure also eliminate the parasites (Howell *et al.,* 1999).

Nutritional Management

- Vitamin A, D and B complexes are essential in developing the immunity against parasites.
- Minerals like zinc, iron, cobalt, sodium, potassium, phosphorus, etc. are very essential for proper functioning of immune system against the parasites (Hughes and Kelly, 2006).
- Vitamin A is essential to improve the intestinal epithelial integrity (Villamor and Fawzi, 2005).
- Following deficiency in animals the intestinal immune system is disrupted that leads weakening the host defense against intestinal parasites (Coop and Kyriazakis, 1999).
- The zinc deficient animals have impaired cell mediated cytotoxicity and T helper cell function.
- Scott and Koski (2000) using a zinc deficient nematode infected mouse model noted that parasites are better able to survive in the zinc deficient hosts than in well nourished hosts.
- Cook-Mills *et al.,* (1990) while studying Trypanosomiasis recorded that NK cell function and phagocytosis by macrophages are impaired in zinc deficient animal, and this may be a consequence of reduced oxidative burst capacity.
- The deficiency reduces thymulin levels in blood, and thus reduces the CD4/CD8 ratio.
- Iron supplements are also very important, where animals are affected by blood sucking worms, *like Haemonchus spp., Bunostomum spp.* etc.
- Animals on low protein diets are more susceptible to infection because they produce less immunoglobulin IgA.

Alternative forages

- The pasture plants containing condensed tannins have anthelmintic properties (Min *et al.,* 2004).
- Forage plant species (Marley *et al.,* 2003) which contain high levels of condensed tannins include Sericea lespedeza (warm season legume), Birds foot trefoil (perennial legume) and Chicory (leafy perennial).
- Tropical legumes contain more condensed tannins than temperate legumes.
- Normally trees and shrubs contain higher levels of tannins than pasture grasses (Niezen *et al.,* 1998).

Pasture Management

- The scientific management of pasture is an effective way to control internal parasites in grazing livestock (Stuedemann *et al.,* 2004).
- Regular burning of old or grazed pasture should always be practiced to obtain parasites free pasture land.

- Overstocking of animals in a small piece of land increases the concentration of parasites. So allow optimum number of animals to graze in a given piece of land.

1. Pasture rotation and rest

- Pasture rotation is optimum use of grass by distributing the pastures into parcels of land of varying sizes called paddocks and frequently moving the livestock from one paddock to another (Wells, 1999; Johns *et al.,* 2004).
- The main objective of pasture rotation is not to put the animals back into the same field until the risk of infection has diminished.
- A rest of 3–6 months is required for an infected pasture to return to a low level of infectivity.

2. Grazing by age group

- As susceptibility of animals against parasites varies with age, it is reasonable to graze different age group animals in different fields.

3. Multispecies grazing

- Sheep parasites cannot infect the cattle.
- Cattle parasites cannot infect the horse.
- Horse parasites cannot infect the goat (Christensen, 2005).
- Each species have different grazing behavior.

4. Zero grazing

- It means keeping the animal in captivity to reduce the parasitic load.
- During confinement the animal should be fed off the ground in feeders and watering containers should be kept free from faecal matter.

Genetics of animals

- Genetics is probably the best long term weapon against internal parasites in animals.
- Some animal breeds are more resistant and resilient to internal parasites.
- Zebu cattle is resistant to ticks.
- N Dama breed of cattle is resistant to trypanosomes.
- Plymouth rock and Rhode island red breeds of chicken is genetically resistant to Ascaridia galli infection.
- Merino sheep is more resistant to trichostrongylus
- Red masai sheep is more resistant to *Haemonchus contortus*

Biological control

- Biological control of parasites means controlling them with other **natural organisms** that are their **natural enemies**.
- **Predators:** Other animals that just feed on the parasites, e.g. birds, ants, beetles, etc. that feed on ticks, flies, maggots, etc.
- **Parasitoids:** Mainly insects (mostly wasps and flies) that lay their eggs on the parasites. The larvae that hatch out of these eggs feed on the tissues of the parasite that is ultimately killed. They can be considered as "parasites of the parasites".
- **Pathogens:** Microorganisms such as bacteria, fungi, viruses, worms, etc. that infect and kill the parasites. They can be considered as "diseases" of the parasites.

Biological control of arthropods

Use of larvivorus fishes

- Gambusia: Most widely used biological control agent for Mosquitoes.

- *Oreocromis spilurus spilurus* (Tilapia)

Birds

- Many birds are very effective natural predators of flies, fly larvae, and many other insects, e.g. chicken, turkey, guinea fowls and many other domestic and wild birds.

Predatory insects

- such as Toxorhynchites larva feeds on *Culex* larva

Ants as predators of ticks

- Ant species such as *Pheidole megacephala, Solenopsis* and *Camponotus predate* on eggs and larvae of ticks and other arthropods.

Mites as predator of flies

- *Macrocheles muscaedomestica* can eat up to 10 housefly eggs a day.
- *Fuscuropoda vegetans* is another mite species that prefers deeper layers in the manure heap. It feeds on young fly larvae.

Mites as predators of ticks

- *Anystis baccarum* predates on tick larvae, particularly those that climb onto shrubs or grass blades questing for potential hosts.

Beetles:

- *Carcinops pumilio* is a tiny beetle that feeds on both larvae and eggs of flies that breed in animal dung.

Black dumpfly or Black garbage fly

- *Ophyra (Hydrotaea) aenescens*
- Occur in manure pits
- Larvae preys upon housefly larvae.

Soldier flies (Hermetia illucens)

- Larvae repel ovipositing female houseflies.
- Death of houseflies by competitive inhibition.

Parasitoids of flies

- Hymenopteran wasps are natural enemies of fly pupae, including houseflies. Wasp deposits egg inside pupa, developing wasp slowly kills pupa, and adult emerges. Hymenopteran wasps: *Muscidifurax* - houseflies. *Spalangia* - horn flies and stable flies

Parasitoids of ticks

- All parasitoid species of ticks are small hymenopteran wasps of the genus *Ixodiphagus*, particularly *Ixodiphagus hookeri.*

Pathogenic bacteria

- *Bacillus thuringiensis (Bt)* is the best-known bacterial pathogen of insects. It produces **thuringiensin**, a protein that is lethal to many insects.
- Mainly used for mosquito control, but also for controlling other insects that develop in water (e.g. black flies, midges, etc).
- *Brevibacillus laterosporus*-houseflies

Entomopathogenic fungi

- The best-investigatedspecies- *Entomophthora muscae, Beauveria bassiana* and *Metarhizium anisopliae, Coelomomyces, Lagenidium, Leptolegnia, Pythium* and *Conidiobolus.*

Pathogenic nematodes:

- *Steinermena, Heterorhabditis, Romanomermis, Culicimermis, Octomyomermis, Hydromermis*
- **Protozoans:** *Nosema, Amblyospora, Thelohanaia* and *Vavria*

- **Viruses:** Baculoviruses and Irridescent viruses.

Sterile Insect Technique:

- It is an environmentally friendly method for biological control of pests.
- It is successfully used for control of Callitroga hominivorax in USA

Biological Control of Helminths: Nematophagus fungi:

- Fungi that exhibit anti-nematode properties have been known for a long time.
- They consist of a great variety of species characterized by their ability to capture and exploit nematodes either as the main source of nutrients or supplementary to a saprophytic existence.
- They are divided into three major groups based on their morphology and types of nematode destroying apparatus (Barron,1997; NordbringHertz, 1988).

Nematode trapping fungi:

- They produce specialized nematode trapping structures (adhesive knods, networks, rings)on the mycelium. *eg. Arthobotrys oligospora* - reduce the no. of developing Cooperia oncophora larva (Gronvald *et al.,* 1985). Duddingtonia flagrans-control of strongyles in horse (Fernandez *et al.,* 1997)

Endo parasitic fungi:

- These invade either by penetration of cuticle from sticky spores adhering to the cuticle or following ingestion of spores which lodged in the gut.
- Eg. *Drechmeria coniospora & Harposporium anguillulae*

Egg parasitic fungi:

- These have the ability to attack the egg stage by degrade the egg shell enzymatically. eg. *Verticillium chlamydosporium-Ascaris lumbricoides* and *Ascaridia galli* & Parascaris equorum, *Paecilomyces lilacinus-Toxocara canis*

Alternative dewormers:

1.Herbal Dewormers

Plant name	Active Principle	Effect
Acacia auriculaeformis	Saponins	Tapeworms
Annona squamosa	Powdered seeds and immature fruits	Fly larvae (myiases), lice and other insects.
Artemisia vulgaris	Thujone	Haemonchus, Bunostomum, and Protostrongylus.
Azadirachta indica	Azadirachtin	Ticks

Butea monosperma	Palasonine	Roundworms, tapeworms and flukes
Calotropis procera	Aglycans	Haemonchus contortus
Chrysanthemum cinerariaefolium	Pyrethrins	Natural insecticides
Derris elliptica	Rotenone	Natural insecticide
Dryopterisfilix-mas Fern)	Phloroglucinol	Tapeworms
Eucalyptus globulus	Eucalyptol	Housefly larvae and pupae

Diatomaceous earth (DE)

- It is the fossilized remains of long-dead sea creatures, and it is mined from ancient sea beds and ground to a fine, powder-like consistency.
- It is believed that the microscopic sharp edges of the DE particles scrape off the adult worms have attached to the sheep's intestinal walls, so they can pass out with the feces.
- The sheep get their DE in a ratio of about 1/4 DE and 3/4 salt-mineral mix.

Copper Sulphate

- Important for immune function in livestock.
- Effective against certain internal parasites, notably barber's pole worm *(Haemonchus contortus)*.

Copper oxidewireparticles (COWP)

- Boluses of copper oxide wire particles (COWP) given orally are a much safer form of copper supplementation.
- Copasure© is a Cu supplement (12.5 & 20 g) for cattle that can be repacked into smaller doses for sheep and goats(0.5 & 4 g).
- Copasure capsules are now available in 2g & 4 g doses for sheep and goat (as Cu supplement).
- The boluses lodge within the forestomachs and release needle-like particles of copper oxide that move with the ingesta to the abomasum. The low pH here facilitates release of soluble copper (Bang, 1990).
- Note that COWP's have been found to be effective against barber's pole worm but not intestinal worms (Burke *et al.,* 2004) .

Targeted Selective Treatment - FAMACHA© System

The name derived from its originator **Dr. Francois FAffa MAlanCHArt-South African Parasitologist**

- It is a method of identifying individual sheep and goats that are heavily parasitized, based on physical evidence of anemia caused by *Haemonchus contortus* and subsequently tested and adopted in several other countries, including Brazil, Morocco, Kenya, Italy, Switzerland and India (Singh and Swarnkar, 2012).
- Store in dark place when not in use
- Replace card after 12 months use
- Keep a spare card in a light protected place

FAMACHA© System "rules":

- Score using the chart
- Evaluate in bright light (sunlight)
- Be quick
- Score both eyes (Kaplan, 2004).

Vaccines for Trematodes

Vaccine/antigen	Host	Parasite
FABP and G-S-T	Sheep & Cattle	*Fasciola hepatica*
Sm - 28	Rats and Mice	*Schistosoma mansoni*
Sj - 26	Mice	*Schistosoma japonicum*

Vaccines for Cestodes

Vaccine/antigen	Host	Parasite
TSOL - 18	Pig	*Taenia solium*
45W	Sheep	*Taenia ovis*
Eg95	Dog	*Echinococcus granulosus*

Vaccines for Cestodes

Vaccine/antigen	Host	Parasite
H 11	Sheep	*Haemonchus contortus*
Tropomyosin 41	Sheep	*Trichostrongylus colubriformis*
ES 31	Sheep	*Ostertagia circumcincta*
Dictol	Cattle	*Dictyocaulus viviparus*
Difil	Sheep	*Dictyocaulus filaria*
Irradiated L_3	Dog	*Ancylostoma caninum*

Vaccines for Cestodes

Vaccine/antigen	Host	Parasite
Live virulent vaccines - coccivac, immucox Precocious - paracox	Chicken	Eimeria spp.
Liva cox (egg adoptedline)	Chicken	*Eimeria tenella*

Vaccine/antigen	Host	Parasite
Vac M	Chicken	*Eimeria maxima*
Pirodog	Dog	*Babesia canis*
Rakshavac-T	Cattle	*Theileria annulata*
P-67	Cattle	*Theileria parva*
Toxovac	Sheep	*Toxoplasma gondi*
T-263	Cat	*Toxoplasma gondi*
Anaplaz (killed vaccine) Amvac (attenuated)	Chicken	*Anaplasma marginale*
Trichgard	Cattle	*Trichomonas foetus*
Giardiavax	Dog	*Giardia canis*

Vaccines for Ectoparasites

Vaccine/antigen	Host	Parasite
BM 86 (TICKGARD & GAVAC)	Cattle	*Boophilus microplus*
PM 44	Sheep	*Lucilia cuprina*

Conclusion

- Previous control methods are no longer viable, so new techniques must be used. Techniques such as increased pasture management, FAMACHA© and selecting parasite-resistant animals can help to manage internal parasites.
- These techniques reduce dependence on dewormers and lead to a more sustainable parasite management program.
- None of the single control measures will give long term solution.
- Integration of more than one measure like good farming practices, best breeding strategies, appropriate biological control measures and scientific utilization of biotechnological tools are essential to achieve the sustainable control on the parasites.

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Estrus Synchronization in cross-bred heifers maintained at Gaushala under group feeding & management practices

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Abstract

The present study was undertaken on sixteen Sahiwal cross-bred heifers of age ≥ 22 months and body weight ≥ 240 kg at Panchayati Gaushala, Hapur. These animals were individually tied in shed and managed under similar feeding practices. All the animals were given intramuscular injections of GnRH analogue (Receptal - 5ml) and $PGF_2\alpha$ (Lutalyse - 5ml) 7 days later. Heat detection was carried out twice daily during the cooler hours (6 AM and 5 PM) of the day by visual observations. The animals detected in estrus were inseminated with frozen thawed semen. Pregnancy diagnosis was carried out 3 months post-insemination by per-rectal examination. In this study, out of 16 heifers, 14 were observed in estrus within 3-5 days after the end of the $PGF_2\alpha$ injection and were inseminated using frozen thawed semen from Frieswal bulls. Twelve heifers were confirmed pregnant by per-rectal examination. In the present study, 87% of the heifers were detected in estrus using the synchronization protocol mentioned above. The overall conception rate observed was 75% with an average 1.16 services per conception. Our findings suggest that this protocol can be used as an economic and efficient tool for estrus synchronization in heifers maintained at Gaushala or field conditions for optimum fertility and milk production during the peak or desired season.

Keywords: estrus, synchronization, cross-bred, heifers

Introduction

Synchronization of estrus is a technique by which most of the females in a herd can be brought into estrus at a pre-determined time using hormonal treatment (Odde, 1990). Estrus synchronization offers major advantages like reduced time required for the heat detection and allows planned breeding of more number of females at a time and ultimately helpful scheduling the parturition as well as milk production at the most favourable or required season and when the newborns can be reared in suitable environment with ample food for enhancing their survivability (Islam, 2011). The precise synchronization of bovine estrous cycle requires control of follicular waves and life span of corpus luteum (Patterson et al., 2000). A protocol which combines the administration of gonadotropin-releasing hormone (GnRH), followed by prostaglandin F2 α (PGF2α) 7 days later, was termed as GnRH-PGF2α protocol (Thatcher et al., 1989) or Select Synch (Downing et al., 1998). Administration of GnRH induces ovulation or luteinization of dominant follicles in both normally cycling cows and those that are anestrous which results in the resumption of ovarian cycle (Troxel et al., 1993; Thompson et al., 1999). Administration of PGF2 synchronizes luteal regression, resulting in the

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synchronization of estrus (Twagiramungu et al., 1992). The present experiment was planned to observe the estrus synchronization response using Select Synch protocol in animals reared under Gaushala or field conditions.

Material & methods

The present study was undertaken on sixteen Sahiwal cross-bred heifers of age ≥ 22 months and body weight ≥ 240 kg at Panchayati Gaushala, Hapur. These animals were individually tied in shed and managed under similar feeding practices with green fodders@ 7-8kg and concentrate mixture containing mineral supplements @ 1-2% of the ration @ 1.5kg per animal along with ad-libitum feeding of wheat straw.

All the animals were given intramuscular injections of GnRH analogue (Receptal-5ml) on day 1 (start of treatment) and PGF2α (Lutalyse - 5ml) 7 days later, that is a Select Synch protocol. Heat detection was carried out twice daily during the cooler hours (6 AM and 5 PM) of the day by visual observations for 20 minutes each time. The animals detected in estrus were inseminated as per AM/PM schedule artificially using Frieswal bull semen and pregnancy diagnosis was carried out 3 months post-insemination by per-rectal examination.

Result and discussion

In this study, out of 16 heifers, 14 (87%) were exhibited estrus within 3-5 days after the end of the PGF2α injection, which was higher as compared to report of El-Zarkouny (2010), who observed 68% estrus response in heifers synchronized using Select Synch protocol. Under present experiment, out of 14 heifers detected in estrus, 12 (85%) confirmed pregnant with 75% conception rate and an average 1.16 services per conception. Perusal of available literature reveals that pregnancy rates were lower in heifers synchronized with Ovsynch (35.1%) than the Select Synch (74.7%) protocol (Pursley, et al., 1997). However, El-Zarkouny (2010) reported significantly ($P < 0.05$) higher pregnancy rates (97.0%) in heifers with Select Synch protocol as compared to control as well as other protocols, our findings from present study are similar to their observations. Therefore, it can be concluded that Select synch protocol followed in the present study is economic and efficient for synchronizing estrus in heifers in group management.

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Studies on the incidence of Yersinia enterocolitica in Swine

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Abstract:

Yersinia enterocolitica is an important food and water borne gastrointestinal agent and regarded as an emerging pathogen, due to its ability to grow in many refrigerated foods. Present study was envisaged on the incidence of *Y. enterocolitica* in swine and environmental samples. Out 30 samples, each of pork, pork tonsils, pork tongue, carcass swabs, faecal samples and farm water, 10 (33.33%), 20 (66.67%), 21 (70.00%), 08 (26.67%), 07 (23.33%) and 16 (53.33%) respectively were positive by cultural method, whereas PCR assay revealed 12 (40.00%), 23 (76.67%), 24 (80.00%), 10 (33.33%), 08 (26.67%) and 18 (60.00%) respectively. Heat stable enterotoxin targeting Yst-B gene was carried mong the PCR positives which revealed 06 (50.00%), 11 (47.82%), 10 (41.67%), 05 (50%), 03 (37.5%) and 08 (44.44%) respectively for pork, pork tonsils, pork tongue, carcass swabs, faecal samples and farm water.

Key words: Yersinia enterocolitica, Swine, PCR

Introduction:

The genus *Yersinia* spp. belongs to Enterobacteriaceae family and are gram negative, coccobacillus, pleomorphic, non-lactose fermenting, urease positive microorganism (Bottone, 1977). *Y. enterocolitica* is wide spread in the environment and animal populations, posing a potential source of infection to humans. The main reservoir of *Y. enterocolitica* for humans are pigs (Huovinen *et al.,* 2010).They found in water, soil, vegetables, milk, vacuum packed meats, pork, beef, lamb, tofu, fish, chicken and drinking water. It is an important food and water borne gastrointestinal agent and regarded as an emerging pathogen worldwide (Ostroff, 1995). Among the food borne diseases infection with *Y. enterocolitica* is frequently reported zoonotic gastrointestinal disease after Campylobacteriosis and salmonellosis in many developed countries, especially in temperate zones (EFSA journal, 2007). *Y. enterocolitica* is psychotropic and able to grow in many refrigerated foods. Contamination is possible at the manufacturing site or home, refrigerated foods are potential vehicles. Yersiniosis in children less than 5 years occurs as enterocolitis and shows diarrhoea which is often bloody, fever, abdominal pain and vomiting. In older than 5 years and young adults it presents as a pseudo-appendicular syndrome including symptoms such as fever, abdominal pain and tenderness of the right lower quadrant (Robins-Browne, 1997). In adults it causes acute gastroenteritis and mesenteric adenitis as well as variety of extra intestinal disorders. In older patients sore throat is a frequent accompaniment.

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A large variety of immunological complications may follow the acute infections including reactive arthritis, erythema nodosum, iridocyclitis, glomerulonephritis, carditis and thyroiditis (Cover and Aber, 1989). Bacteraemia is a rare complication except in immune compromised patients and in patients with iron over load.

Although *Y. enterocolitica* is a ubiquitous microorganism, the majority of isolates recovered from asymptomatic carriers, infected animals, contaminated food, untreated water and contaminated environmental samples are non-pathogenic having no clinical importance (Fredriksson- Ahomaa and Korkeala, 2003). Contaminated pork and pork products are believed to be the most important source of infection (Bucher *et al.,* 2008). At the same time, the epidemiology of *Y. enterocolitica* infection is complex and remains poorly understood because most sporadically occurred cases of Yersiniosis are reported without an apparent source (Bottone, 1999). However, most pathogenic *Y. enterocolitica* strains associated with human Yersiniosis belong to bio-serotypes 13/0:8, 2/0:5,27, 2/0:9, 3/0:3 and 4/0:3. (Portnoy and Martinez, 1985). Very meagre information is available on the incidence of Yersinia enterocolitica. As swine products are the major source of this organism, an attempt was made to study the incidence of Yersinia enterocolitica in swine and farm environmental samples.

Materials And Methods

Isolation and Identification:

All the samples were collected aseptically in sterile polythene bags and transferred on ice to the laboratory at the earliest possible for the further analysis.

About 10 g of each sample(pork, pork tonsils and pork tongue) was homogenized in mortar and pestle and enriched into 90 ml ITC (Irgasan-Ticarcillin- potassium Cholate) broth in individual sterile polythene bags and incubated at 37^{0}C for 24hrs.10ml of farm water samples were inoculated into 90 ml ITC broth and incubated at 37^{0}C for 24hrs. Faecal and swab samples were incubated into 50 ml ITC broth and incubated at 37^{0}C for 24hrs. The enriched inoculum from broth was streaked onto Mac Conkey agar plates and incubated at 37^{0}C for 24hrs and pale pink colour colonies were noticed. The presumptive colonies of *Y. enterocolitica* were picked up and confirmed by standard biochemical tests.

DNA isolation:

The genomic DNA isolation was isolated carried out by phenol: chloroform: I & O amyl alcohol method from the bacterial strain *Y. enterocolitica to* standardize PCR assay for detection. DNA templates were prepared from samples by boil and snap chilling method. All the enriched samples were subjected to PCR analysis for the presence of *Y. enterocolitica* using primers specific to Ail gene. These positive samples were further examined for the presence of *Y. enterocolitica* heat stable enterotoxin using primers specific to *yst Bgene.* (Table: 1)

In this method about 1000 µl of the 24h inoculums from the selective enrichment was centrifuged at 6000 rpm for 5 min and resuspended in 50 µl of molecular grade water. The suspension was then kept in a boiling water bath for 10 min and immediately transferred onto ice, later it was centrifuged at 13000 rpm for 5 min. For PCR technique, five µl of supernatant used as template.

PCR was done in 25 µl reaction mixture containing 5 µl of template, 0.5 µl of dNTP's, 2.5 µl of forward primers, 2.5 µl of reverse primers,2.5 µl of buffer with MgCl2, 0.5 µl of 10x Taq polymerase and 11.5 µl of nuclease free water. Amplification was done in thermal cycler

Table 1: Primers used for Ail and Yst-B gene

Target gene	Primer	Length	Primer sequence	Amplification product (bp)	Reference
Ail	Ail-F	21	5' TTA ATG TGT ACG CTG CGA GTG 3'	425	Bowman et al, 2006
	Ail-R	21	5' GGA GTA TTC ATA TGA AGC GTC 3'	425	
Yst B	Yst B-F	20	5' GTA CAT TAG GCC AAG AGA CG 3'	146	Thoerner et al, 2002
	Yst B-R	20	5' GCA ACA TAC CTC ACA ACA CC 3'	146	

following standardized conditions.The amplified DNA fragments were resolved by agarose gel electrophoresis stained with ethidium bromide (0.5 µg/ml) and detected the band in the Gel doc instrument. PCR conditions are showed in table 2.

Table 2: PCR conditions

S.No.	Step	Ail gene	Yst B gene
1.	Initial denaturation	94°C / 4 min.	94°C / 15 min.
2.	Final denaturation	94°C / 1 min.	94°C / 30 sec.
3.	Annealing	62°C / 2 min.	62°C / 1 min. 30 sec.
4.	Initial extension	72°C / 2 min.	72°C / 1 min. 30 sec.
5.	Cycles	35	40
6.	Final extension	72°C / 5 min.	72°C / 10 min.
7.	Hold	4°C	4°C

Results and Discussion:

The results of the samples by cultural and PCR assay (both for Ail- gene and YST- B genes) are presented in table: 3, fig 1 & 2

The incidence of *Y. enterocolitica* in the pork was 33.33% and 40.00% by cultural and PCR assay in the present study, out of 12 positives by PCR assay 6(50%) were positive for Yst-B gene. Low incidence (0%, 2% and 7%) than the present study was reported by Boyapalle *et al.* (2001), Johannessen *et al.* (2000) and Lambertz and Danielson-Tham. (2005) respectively by cultural methods. The incidence in the present study was almost similar to the incidence (35%) reported by Thisted Lambertz *et al.* (2006) and lower than the incidence (66.6%) reported by Vishnubhatla *et al.* (2000). Lower incidence of 10% and 17% by PCR was reported by Boyapalle *et al.* (2001) and Lambertz and Danielson-Tham. (2005) and

Table-3: Incidence of Y.enterocolitica in swine and environmental samples by cultural and PCR assay

Sample	Sample culture	Positive result by culture method	Positive result by PCR for Ail gene	Positive for Yst B gene from positive of Ail- gene
Pork	30	10 (33.33%)	12 (40.00%)	06 (50.00%)
Pork tonsils	30	20 (66.67%)	23 (76.67%)	11 (47.82%)
Pork tongue	30	21 (70.00%)	24 (80.00%)	10 (41.67%)
Carcass Swab samples	30	08 (26.67%)	10 (33.33%)	05 (50%)
Swine Faecal samples	30	07 (23.33%)	08 (26.67%)	03 (37.5%)
Swine Farm Water	30	16 (53.33%)	18 (60.00%)	08 (44.44%)
Total	180	82 (45.56%)	95 (52.78%)	43 (45.26%)

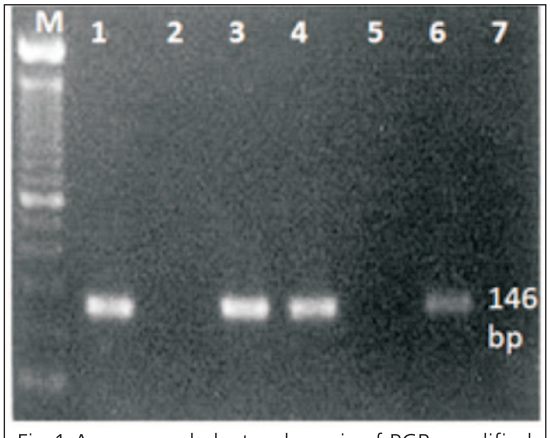

Fig 1:Agarose gel electrophoresis of PCR amplified product yst B-gene of Y. enterocolitica toxin in swine and environmental samples (lane M: 100bp DNA ladder; Lane 1, 2, 3, 4 and 6: showing positive results)

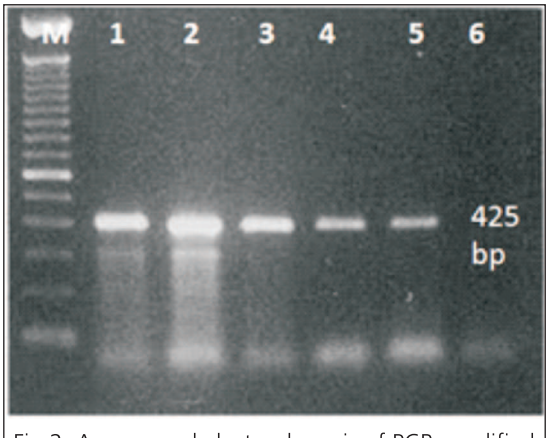

Fig 2: Agarose gel electrophoresis of PCR amplified product Ail gene of Y. enterocolitica toxin in swine and environmental samples (lane M: 100bp DNA ladder; Lane 1, 2, 3, 4 and 5: showing positive results)

Johannessen *et al.* (2000) respectively than the incidence (40%) in the present study. Higher incidence by PCR was also reported by Theotner *et al.* (2002) and Vazlerova and Stienhauserova (2006).

The incidence in the pork tonsils by cultural methods was 66.67%. Lower incidence of 0%, 8%, 10%, 22%, 26%, 34%, 44% and 50% was reported by Boyapalle *et al.* (2001), Weber and Knapp.(1981), De boer *et al.* (1986), Thibodeau

et al. (1999), Friedrickson-Ahomaa *et al.* (2000), Friedrickson-Ahomaa *et al.* (2007), Martinez *et al.* (2010) and Martinez *et al.* (2011) respectively, whereas almost similar results were reported by Nesbakken *et al.* (2003). The incidence by PCR assay was 76.67% in the present study. Higher incidence (88%)by PCR was reported by Friedrickson-Ahomaa *et al.* (2007), whereas low incidence (0%, 31% and 36%) was reported by Martinez *et al.* (2010 & 2011), Friedricksson-Ahomaa *et al.* (2000) and Boyapalle *et al.* (2001) respectively, but almost the similar results were reported by Nesbakken *et al.* (2003).Out of 23 positives by PCR assay 11(47.82%) were positive for Yst-B gene.

In the present study, the incidence of *Y. enterocolitica* in the pork tongue was 70% and 80% by cultural method and PCR assay respectively, out of 24 PCR positives 10 (41.67%) were positive for Yst-B gene. Higher incidence of 80% by cultural method was reported by Friedrickson-Ahomaa and karkeala, (2003) whereas low incidence (47%) was reported by Vishnubhatla *et al.* (2000). Low incidence (67%) by PCR assay was reported by Vishnubhatla *et al.* (2000) whereas almost similar incidence (83%) was reported by Friedrickson-Ahomaa and karkeala, (2003).

In the present study, the incidence of *Y. enterocolitica* in the carcass swabs was 26.67% and 33.33% by cultural and PCR assay respectively, out of 10 positives by PCR 5(50%) were positive for Yst-B gene. Lower incidence of 17% by cultural method and 28.2% by PCR than the present study was reported by Novoslauskij et al. (2010) and Funk *et al.* (1997) respectively.

The incidence of *Y. enterocolitica* in the faecal samples in the present study by cultural methods and PCR assay was 23.33% and 26.67% respectively, out of 8 positives by PCR 3(37.5%)were positive for Yst-B gene. By cultural method, lower incidence (0%, 2%, 4% and 13%) than the present study was reported by Boyapalle *et al.* (2001), Okwari *et al.* (2009), Bhaduri *et al.* (2005) and Nesbakken *et al.* (2003) respectively. The high incidence (31%) by PCR assay was reported by Boyapalle *et al.* (2001) whereas lower incidence of 0%, 0.2%, 2.3%, 4%, 5%, 12% and 13% reported by Okwari *et al*. (2009), Hunter and Hughes (1983), Khorramizadeh et al. (2007), Fukushima *et al.* (1989), Gurgui Ferrer *et al.* (1987), Bhaduri *et al.* (2005) and Nesbakken *et al.* (2003) respectively than the present study.

The incidence in swine farm water was 53.33% and 60% by cultural methods and PCR assay respectively, out of 18 PCR positives 8(44.44%) were positive for Yst-B gene in the present study. Sandery *et al.* (1996) reported lower incidence (4.3%) than the present study by PCR assay.

Out of total 180 samples (30 each of pork, pork tonsils, pork tongue, carcass swabs, swine faecal samples and farm water) were screened for Y.enterocolitica and it was found that 45.56% and 52.78% were positive by cultural methods and PCR assay respectively, out of 95 (52.78%) PCR positives for Y.enterocolitica 43 (45.26%) were positive for Yst-B gene controlling enterotoxin production, which accounts for 23.89% of all samples. Lower incidence of 11.84% for Yst-B gene was reported by Ramamurthy *et al.* (1997) than the present study, whereas higher incidence of 80, 81.3 and 96.3% were reported by Thoerner *et al.* (2003), Grant *et al.* (1998) and Singh & Virdi (2004) respectively.

Conclusion:

The incidence of *Yersinia enterocolitica* in swine and farm environmental samples is quite high and alarming and so necessary precautionary methods should be followed while handling and consuming swine products.

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Lymphocytosis in Malassezia Dermatitis of Companion Dogs: Clinical Significance and Response to Composite Remedial Therapies

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Abstract

Study was conducted on client-owned dog patients brought for treatment in the Out Patient Department (Medicine), Teaching Veterinary Clinical Complex, Veterinary College, Jabalpur. Of the total 240 dogs with clinical signs of dermatitis, Malassezia dermatitis was confirmed in 60 cases on microscopic examination of the modified Wright-stained impression smears, corroborated by mycotic culture on Sabouraud's dextrose agar plates. Synthetic antifungal, ketoconazole and flucoconazole were offered in 2 different doses (pulse therapy, 4 cycles). Herbal Neem (*Azadarichta indica*, Linn.) leaf extract (powder) at 2 different dose levels was offered with food daily for 4 weeks. The broad spectrum anti-bacterial cephalexin daily for 5 days and hepato-protective sylimarin herbal, b.i.d. 4 weeks were offered orally in all combination regimens. Herbal Neem seed oil (NSO) was applied topically on the skin lesions daily, and shampoo (2% miconazole plus 2% chlorhexidine), followed by body wash once a week was also recommended. In all treatment groups, statistically significant ($P < 0.05$) changes in the lymphocyte % appeared to be clinically meaningful.

Key words: Malassezia dermatitis, Dogs, lymphocytosis, fluconazole, Neem, composite therapy

Introduction

Malassezia dermatitis, non-contagious skin disease of yeast aetiology in dogs, generally represents a flare up of the underlying canine atopic dermatitis, CAD (Carlotti, 2006). However, each case being different, a holistic management protocol and effective symptomatic therapy is recommended (Scott et al., 2001). While coccoid bacteria participate in the pathogenesis, *M. pachydermatis* is the recognized primary agent. Part of the normal skin flora, especially of the wild and domestic carnivores (Cook, 1958; Brito *et al.,* 2009), some strains of the yeast may elicit highly exaggerated immune responses (Morris *et al.,* 1998) contributing to chronic inflammation in the stratum corneum and dermatitis (Aspre and Anderson, 2004). Dogs of certain breeds including White Highland terrier, Poodle, German shepherd and Daschund exhibit increased predisposition (Mircean *et al.,* 2010). Flea infestation or mild pyoderma may lower the pruritic threshold leading to marked discomfort to the patient. Clinical presentation includes persistent itching, alopecia, excessive greasiness, scaly skin, erythema, typical foul rancid odour, hyperpigmentation, and epidermal thickening in the chronic cases. Mechanical cleansing action of

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the medicated shampoos removes deleterious scales, crusts, exudates and the invading microbial pathogens. Penetration of the active ingredients inside the stratum corneum facilitates the therapeutic action. Moisturizers enhance the state of cell hydration and promote effective epidermal barrier against pathogenic ingress. Topicals synergize with the systemic treatment and elicit a faster and stronger therapeutic response, preventing relapses. Local action minimizes the risk of adverse general reactions. Further, improved skin and coat texture fosters owner satisfaction.

Materials and Methods

Dogs: Total 60 client-owned, referred for treatment with recurring bouts of dermatitis with foul musty odour to the Teaching Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry (NDVSU), Jabalpur, M.P. were subjected to initial screening. Following routine clinical examination, 42 dogs, irrespective of breed, sex, and the reproductive status (in females) were admitted to the study (December 2013-January 2014).

Skin cytology: The impression smears from the skin lesions on the tape were stained with modified Wright's stain (Diff-Quick) and applied on a clean grease-free glass microslide with the adhesive side down. The preparation was examined under OIF (1000X) to identify oval or elongated cells of *Malassezia spp.* (3-5 µm in diameter) with typical unipolar budding imparting a peanut or boot shaped appearance. More than 2 organisms/OLF was considered to be significant (Borkar *et al.,* 2015).

Leucocyte picture: Total leucocyte count was done with auto blood cell counter, and uniformly thin blood films were stained with modified Wright's stain for differential leucocyte count, manually.

Treatment:

Table I: Combination therapeutic regimens

Groups	Dogs (n)	Drugs	Dose level
T_1	6	Ketoconazole	@ 5 mg/kg o.d., PO, two successive days/week x 4 weeks
		+ Cephalexin	20 mg/kg PO, b.i.d. x 5 days
		+ Neem oil	Topical, b.i.d. x 4 weeks
T_2	6	Ketoconazole	@ 10 mg/kg o.d., PO two successive days/week x 4 weeks
		+ Cephalexin	20 mg/kg PO b.i.d. x 5 days
		+ Neem oil	Topical, b.i.d. x 4 weeks
T_3	6	Fluconazole	@ 5 mg/kg o.d., PO two successive days/week x 4 weeks
		+ Cephalexin	20 mg/kg PO b.i.d. x 5 days
		+ Neem oil	Topical, b.i.d. x 4 weeks
T_4	6	Fluconazole	@ 10 mg/kg o.d., PO two successive days/week x 4 weeks

Groups	Dogs (n)	Drugs	Dose level
		+ Cephalexin	20 mg/kg PO b.i.d. x 5 days
		+ Neem oil	Topical, b.i.d. x 4 weeks
T_5	6	Neem* Tablet	@ 25 mg/kg PO b.i.d. x 4 weeks
		+ Cephalexin	20 mg/kg PO b.i.d. x 5 days
		+ Neem oil	Topical, b.i.d. x 4 weeks
T_6	6	Neem* Tablet	@ 50 mg/kg PO b.i.d. x 4 weeks
		+ Cephalexin	20 mg/kg PO b.i.d. x 5 days
		+ Neem oil	Topical, b.i.d. x 4 weeks
T_0	6	Healthy Control	

* 1 tab of neem contains 250 mg of neem extract; hepatoprotective Silymarin @ 5 ml PO twice daily for 4 weeks in all treatments (T_1-T_6).

Results and Discussion

The itchy dog is subjected to persistent stress (Srikala *et al.,* 2010), and bringing succor to the listless patient is the primary concern of the caring pet physician. Abatement of fungal/ bacterial dermatitis was evidenced by progressive resolution of pruritis and perceptible improvement in the clinical status (Fig. 1, 2). Fluconazole @ 5 mg/ kg (T_3) and Neem leaf extract @ 50 mg/kg body weight (T_6) were found to be the most effective (Borkar *et al.,* 2014). Evaluation of the blood picture pre-treatment and at regular post-treatment intervals helps in objective assessment of restoration of homeostasis (Roy *et al.,* 2013). The response to secondary *Malassezia pachydermatis* yeast infection in the atopic dogs by mononuclear cells

Fig. 1 Prominent skin lesions pre-treatment

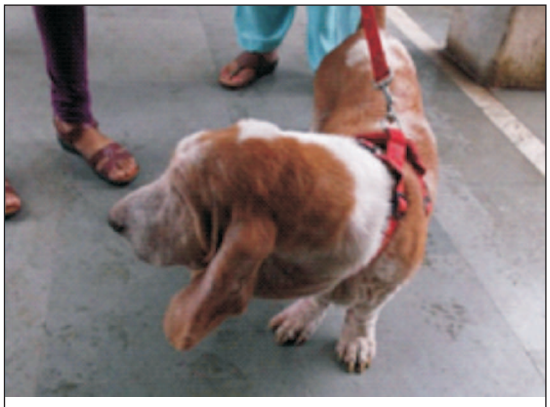

Fig. 2. Same patient on day 14 (T3 regimen)

in circulation, suggestive of phagocytosis is on record (Morris *et al.,* 1998). In this study, the leucocyte profile (DLC, Table 1) revealed perceptible lymphocytosis, consistent with an earlier report (Saranya *et al.,* 2011). In this context, it is pertinent to recall that monocyte-derived dendritic cells MDDC, as representatives of antigen presenting cells, can efficiently bind, and rapidly engulph/disintegrate *Malassezia sympodialis* and allergenic components from degenerating pathogenic yeast cells. This process is linked to the maturation of MDDC, induction of lymphocyte proliferation, and of a Th2-like immune response in the humans (Saunders *et al.,* 2012). Thus, lymphocytosis in *Malassezia spp.* induced dermatitis in the dog patients (present study) is explained. However, in all holistic treatments, the lymphocyte % had reverted to the normal range, which is a noteworthy first report.

Table 1. Leucocyte profile of dogs in Malassezia dermatitis before and after treatment.

Parameters / Groups	Intervals		
	Pre-treatment	Post-treatment	
	Day 0	Day 14	Day 28
TLC (x 10^3/ µl)			
T_0	5.57 ± 0.79	16.06 ± 0.79	16.54 ± 0.54
T_1	15.78 ± 0.64	16.53 ± 0.48	16.01 ± 0.71
T_2	15.94 ± 0.70	16.33 ± 0.62	15.29 ± 0.69
T_3	16.40 ± 0.70	14.90 ± 0.47	14.54 ± 0.49
T_4	15.77 ± 0.79	15.86 ± 0.42	14.77 ± 0.27
T_5	16.36 ± 0.55	14.23 ± 0.45	13.83 ± 0.37
T_6	16.91 ± 0.42	14.90 ± 0.44	14.25 ± 0.54
DLC-N (%)			
T_0	67.0 ± 0.43	65.3 ± 0.21	66.3 ± 0.88
T_1	58.8 ± 0.65	59.7 ± 1.15	66.6 ± 0.49
T_2	57.8 ± 0.70	62.7 ± 0.68	66.3 ± 0.56
T_3	58.3 ± 0.42	59.6 ± 0.67	66.2 ± 0.70
T_4	57.5 ± 0.56	58.3 ± 0.61	66.0 ± 0.58
T_5	57.8 ± 0.48	59.0 ± 0.61	65.8 ± 0.70
T_6	57.7 ± 0.56	58.2 ± 0.48	64.8 ± 1.00

Parameters / Groups	Intervals		
	Pre-treatment	Post-treatment	
	Day 0	Day 14	Day 28
DLC-L (%)			
T0	27.3 ± 0.72	26.8 ± 0.63	27.8 ± 0.60
T1	36.5 ± 0.43	34.7 ± 0.76	28.0 ± 0.58
T2	36.8 ± 0.48	31.2 ± 1.08	28.8 ± 0.60
T3	37.5 ± 0.43	34.8 ± 0.70	27.8 ± 0.60
T4	37.7 ± 0.42	36.8 ± 0.48	27.8 ± 0.60
T5	37.8 ± 0.48	36.5 ± 0.48	27.5 ± 0.76
T6	37.5 ± 0.48	36.3 ± 0.42	28.3 ± 0.88
DLC-E (%)			
T0	2.8 ± 0.31	2.5 ± 0.22	2.7 ± 0.21
T1	2.0 ± 0.26	2.3 ± 0.21	2.3 ± 0.21
T2	2.5 ± 0.22	2.5 ± 0.22	2.3 ± 0.17
T3	2.0 ± 0.22	2.0 ± 0.26	2.5 ± 0.22
T4	2.2 ± 0.17	2.3 ± 0.21	2.5 ± 0.22
T5	2.0 ± 0.26	2.0 ± 0.26	2.7 ± 0.21
T6	2.2 ± 0.17	2.7 ± 0.21	2.8 ± 0.17
DLC-M (%)			
T0	3.5 ± 0.43	4.5 ± 0.50	4.3 ± 0.49
T1	2.7 ± 0.33	3.3 ± 0.42	3.0 ± 0.37
T2	2.8 ± 0.31	4.5 ± 0.67	2.7 ± 0.33
T3	2.2 ± 0.17	3.5 ± 0.43	3.5 ± 0.43
T4	2.7 ± 0.33	2.5 ± 0.34	3.7 ± 0.33
T5	2.3 ± 0.21	2.5 ± 0.34	4.0 ± 0.45
T6	2.7 ± 0.33	2.8 ± 0.31	4.0 ± 0.37
T0 = Normal control; T1-T6, different combination treatments			

Conclusions

Lymphocytois is the hallmark of pathoclinical response to Malassezia dermatitis in dogs, and remission is one of the dependable parameters that attest to successful outcome of effective holistic remedial therapies.

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Antimicrobial sensitivity pattern of Staphylococcus aureus isolated from pus and mastitic milk samples collected from Veterinary College Hospital,Rajendranagar, Hyderabad.

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Abstract

The incidence of *Staphylococcus aureus* infections are being common in veterinary field. This study was an attempt to know the antibiotic sensitivity pattern of the *S. aureus* in the samples collected from the Veterinary College Hospital Rajendranagar, Hyderabad. Samples comprising were nine pus samples and sixteen mastitic milk samples, from which the colonies were isolated and grown in *S. aureus* selective mannitol salt agar plates. The antimicrobial sensitivity pattern of seven antibiotics was evaluated in this study. The antibiotics selected for the study were ampicillin(30 µg), neomycin (30 µg), erythromycin (15 µg), cefoperazone and sulbactam – CFS (75/30 µg), gentamicin (10µg), and tetracycline (10 µg). The maximum inhibitory zone was shown by ampicillin, CFS and erythromycin, and the resistance was the maximum towards neomycin and tetracycline. Hence it could be concluded that ampicillin is the best cost effective drug with maximum inhibitory zone against S. aureus infections among the seven antibiotics studied.

Key words:

Introduction

S. aureus is Gram positive cocci which is a facultative anaerobe. *Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. It is commonly present on skin flora and in nasal passage. It is one of the most common causes of nosocomial infections and can also cause post-operative infections (Shorr *et al.,* 2005). *S. aureus* is coagulase-positive; having golden yellow colonies. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, post influenza pneumonia, sepsis. Toxins produced by *S. aureus* cause food poisoning, dermatitis exfoliativa and toxic shock syndrome.

S. aureus has often been encountered and thought to be a Nosocomial infection (hospital acquired) and community acquired infection. In 1881, Alexander Ogston recognized that it is responsible for a number of infections in man and animals especially when there is a breakage of the skin and epithelial linings. It's now known that the organism is a medical hazard. It takes advantage when the inert and active immunity is suppressed (Taussing, 1984).

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The problem with *S. aureus* became more complicated when it was found that it quickly developed resistance and was capable of producing many antibiotic resistant strains. This is very common in hospitals where drug resistant "hospital strains" have caused *S. aureus* infection outbreaks resulting in deaths in surgical units and newborn nurseries (Meyers *et al.,* 1970). These are the Beta-lactam resistant strains such as MRSA that cause high mortality and morbidity.

Antibiotic resistance leads to prolonged hospital stay and increased costs in terms of treatment. In addition to these, it causes life threatening infections such as in cases of pyomyositis and chronic osteomyelitis. The majority of the MRSA strains worldwide have become resistant to multiple antibiotics including beta-lactams; tetracyclines, macrolides and more recently fluoroquinolones. Excessive use of penicillin antibiotics over the years has led to the development of resistant strains of bacteria that are no longer killed by other beta lactam antibiotics.

Antimicrobial agents are among the most commonly used and misused of all drugs. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, fueling an ever increasing need for new drugs. However, the pace of antimicrobial drug development has slowed dramatically, with only a handful of new agents, few of which are novel, been introduced into clinical practice each year. Reducing the inappropriate antibiotic use is thought to be the best way to control resistance (Cookson and Phillips, 1998).

The microbiology laboratory plays a central role in the decision to choose a particular antimicrobial agent over others. First, identification and isolation of the causative organism should be taken place in the microbiology laboratory. Once the microbial species causing the disease have been identified, a rational choice of the class of antibiotics likely to work in on the patient can be made (Henry, 2010).

Materials and Methods

Isolation and Identification

First step done was the collection of pus samples from the wound using sterile swabs and mastitic milk samples in sterile vials from the university hospital. The samples were immediately brought to the laboratory after collection. In the laboratory the swabs were inoculated in 7% sodium chloride solution and incubated at 37°C overnight. Later the prepared samples were subcultured in mannitol salt agar plates. The milk samples were directly inoculated into mannitol salt agar plates and incubated for 24 hours at 37°C. The presumptive colonies were picked up and were subjected to biochemical tests. The isolates were identified with standard tests used to identify *S. aureus* such as Gram stain, catalase and coagulase tests. After this, sample was inoculated on plates by four flame method and incubated at 37°C for 24 hours (Forbes, 2007)

Antimicrobial susceptibility tests

Antibiotic sensitivity was performed by Disc Diffusion Method of Bauer *et al.* (1966). MH broth was inoculated with five colonies from plates and tubes were incubated at 37°C for 2-8hrs until achieving a turbidity equivalent to 0.5 on the Macfarland scale. After turbidity adjustment a sterile swab was introduced, pressed against the tube well in order to remove any excess liquid; and then seeded on the surface of a petri dish containing MH agar, rotating atleast twice. After the liquid was placed the disc was left at rest for five minutes to absorb any

excessive humidity (Kumar and Kalpana, 2013) Then same commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. Filter paper discs containing designated amount of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. Antimicrobial susceptibility isolates was established by the disc diffusion assay with Muller- Hinton agar in accordance with French National antibiogram committee guidelines.

The antibiotic sensitivity of *S. aureus* was tested for antibiotics such as ampicillin (30 µg), amikacin (30 µg), neomycin(30 µg), erythromycin (15 µg), cefoperazone and sulbactam – CFS (75/30 µg), gentamicin (10 µg) and tetracycline (10 µg). The antimicrobial activity was present on the plates, was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter after 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

Results

From the nine pus samples and sixteen milk samples tested, organisms isolated were identified as *Staphylococcus aureus*. The colonies were gram positive cocci, non motile, non capsular and non sporulating organisms. They were tend to occur in irregular clusters resembling bunches of grapes. The coagulase test was positive and with golden yellow colonies.

Percentage of antibiotic sensitivity ranged between 52-72 % (as shown in Figure 1 and Table-1), for ampicillin, CFS and erythromycin. Among them, ampicillin was with highest inhibitory zone, followed by CFS and erythromycin. The intermediate sensitivity was exhibited to amikacin and gentamicin discs. The inhibitory zone was least for neomycin and tetracycline.

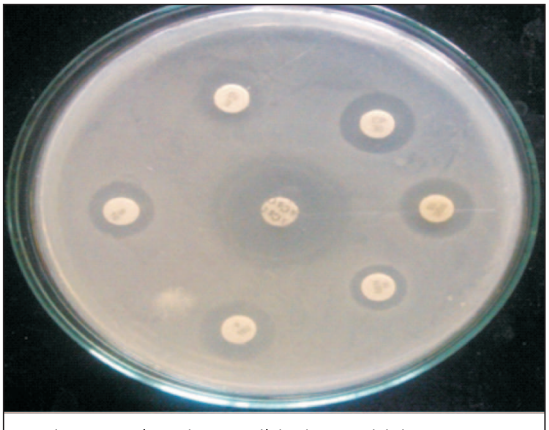

Figure 1 showing antibiotic sensitivity pattern in S. aureus culture

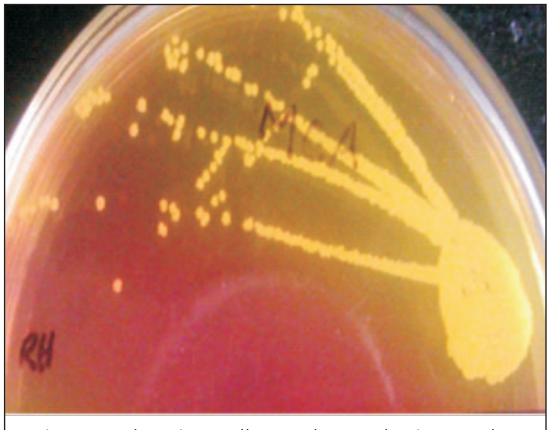

Figure 2 showing yellow colour colonies on the Mannitol Salt agar plates

Table1: Antibiotic sensitivity of S. aureus isolated from pus and mastitic milk samples

Antibiotics	Concentration (μg)	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	30	72	20	8
CFS	75/30	68	16	16
Erythromycin	15	52	28	20
Amikacin	30	28	60	12
Gentamicin	10	32	48	20
11Neomycin	30	32	16	52
Tetracycline	10	36	20	44

Discussion

Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make their cell walls. It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis; therefore ampicillin is usually bacteriocidal (AHFS Drug Information 2006). S. aureus isolates were highly sensitive to ampicillin (72%) in the present study which was almost similar to the findings (74.6%)of Kumar (2013). Lower sensitivity of 24% and 6.8% were reported by Brinda *et al.* (2010) and Rajaduraipandi et al. (2006) respectively. On contrary higher resistance of 93.2%, 68% and 25.5% were reported by Rajaduraipandi *et al.* (2006), Brinda *et al.* (2010) and Kumar (2013) respectively.

Erythromycin belongs to macrolide group of antibiotic which slow down the growth or kill the sensitive organism by reducing the production of important proteins needed by the bacteria to survive (Kibwage *et al,* 1985). A sensitivity of 52%, 28% intermediate and 20% resistance to Erythromycin were observed in the present study. High sensitivity (71.43%) was reported by Adamu *et al.* (2010), whereas lower sensitivity of 45.2%, 20% and 14.8% were reported by Kumar (2013), Brinda *et al.* (2010) and Rajaduraipandi *et al.* (2006) respectively. On contrary higher resistance of 60%, 54.8%, 52% and 28.57% than the present study were reported by Rajaduraipandi *et al.* (2006), Kumar (2013),brindaet al. (2010) and Adamu *et al.* (2010) respectively whereas low resistance (11.6%) was reported by Gentilini *et al.* (2000).

Amikacin and Gentamicin belong to an aminoglycoside family, work by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth (Edson and Terrell, 1999). For Amikacin, sensitivity (28%), resistance (12%) and intermediate (60%) was observed in this study. High sensitivity (40.8%) and higher resistance of 40.8% than the present study was reported by Rajaduraipandi *et al.* (2006). A sensitivity of 32%, 48% intermediate and 20% resistance to Gentamicin were observed in the present study. Similar sensitivity (32%) was reported by Brinda *et al.* (2010) whereas higher sensitivity of 86.7%, 51.79% and 35.6% were reported by Kumar (2013), Adamu *et al.* (2010) and Rajaduraipandi

et al. (2006) respectively. Higher resistance of 63.2% and 48.21% were reported by Rajaduraipandi *et al.* (2006) and Adamu *et al.* (2010) respectively. Lower resistance of 16%, 13.3% and 3.4% than the present study were reported by Brinda *et al.* (2010), Kumar (2013) and Gentilini *et al.* (2000) respectively.

Neomycin is an aminoglycoside antibiotic found in many topical medications such as creams, ointments and eye drops, which bind to duplex RNA with high affinity. For Neomycin, sensitivity (32%), intermediate (16%) and resistance (52%) was observed in the present study. High sensitivity (54.1%) and lower resistance of 45.9% than the present study was reported by Kumar (2013).

Tetracyclines broad spectrum antibiotic inhibiting cell growth by inhibiting translation. It binds to the 16S part of the 30S ribosomal subunit, prevent the amino-acyl tRNA from binding to the A site of the ribosome and interferes with protein synthesis (Chopra *et al.,* 2001).Sensitivity of S. aureus was 36%, resistance 44% and intermediate 20% to Tetracycline was observed in this study. Low sensitivity (28%) was reported by Brinda *et al.* (2010), whereas high sensitivity (60.2%) than the present study was reported by Kumar (2013). Higher resistance of 60% was reported by Brinda *et al.* (2010), whereas low resistance (39.8%) was reported by Kumar (2013).

Cefoperazone is the third generation cephalosporin antibacterial component of Cefoperazone Sodium and Sulbactam Sodium, which inhibit the biosynthesis of cell wall mucopeptide. Sulbactam is an irreversible inhibitor of β-lactamase; it binds to the enzyme and does not allow it to degrade the antibiotic. Cefoperazone Sodium + Sulbactam Sodium combination is indicated for the treatment of infections of respiratory tract, urinary tract, skin soft tissues, bones and joints. (Zhanel *et al.,* 2014).

Conclusion:

From the present study it could be concluded that ampicillin is the best antibiotic of choice, among the seven antibiotics studied against *S. aureus* infection, with the highest inhibitory zone and the cost effective of therapy.

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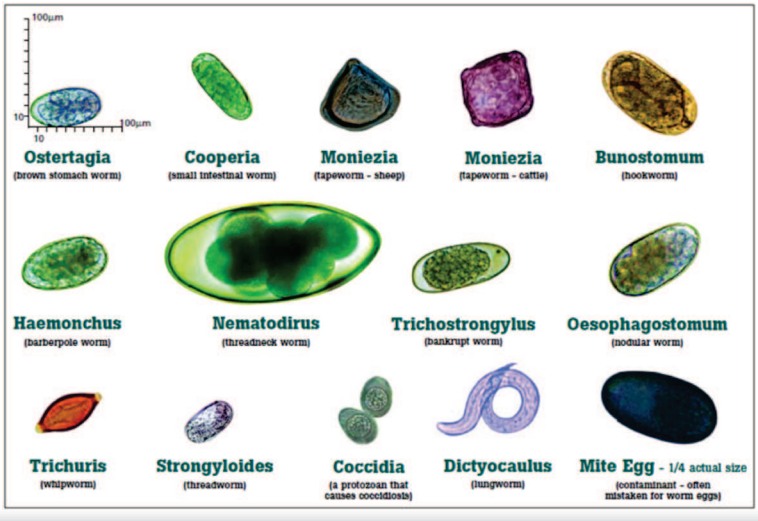
Eggs of different parasites affecting Ruminants



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Studies on the Incidence of Shigella spp in Livestock Products

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Abstract

Shigella spp is the main cause of dysentery and diarrohea in India. In the present study 50 samples each beef, mutton, chicken, egg, fish and milk were investigated for incidence of Shigella spp. The Incidence of *Shigella* spp in beef, mutton, chicken, egg, fish and milk were 12, 16, 14, 8, 18 and 8 percent respectively. The isolated *Shigella* spp were differentiated according to their biochemical characteristics. The incidence of *S. flexneri* was, in beef (8 percent), mutton (10 percent), chicken (6 percent), egg (4 percent), fish (10 percent) and in milk (6 percent) respectively. The incidence of *S. sonnei* was, in beef (4 percent), mutton (4 percent), chicken (6 percent), egg (2 percent), fish (6 percent) and in milk (2 percent) respectively. The incidence of *S. dysenteriae* was, in beef (0 percent), mutton (2 percent), chicken (2 percent), egg (2 percent), fish (2 percent) and in milk (0 percent) respectively. Incidence of *Shigella* spp in livestock products indicates serious public health hazard.

Key Words : *Shigella* spp, beef, mutton, chicken, egg, fish, milk

Introduction

Shigella is one of the most important pathogen causing diarrheal disease in both developing and developed countries (WHO, 2001). Shigellosis or Bacillary dysentery was first discovered over 100 years ago by Japanese scientist Kiyoshi Shiga (Anonymous, 2002). *Shigella* are gram negative non spore forming facultative anaerobic bacilli belonging to the family Enterobacteriaceae and are genetically nearly identical to *E. coli* and closely related to *Salmonella* and Citrobacter spp. (APHA, 2001).

Shigella species are transmitted by ingestion of contaminated food, water and/or through person to person contact (WHO, 2001). Even though it is classically known as water borne disease, is also significant cause of food borne diseases (June *et al.*, 1993). Epidemic and endemic Shigellosis are most frequently caused by *S. dysentriae* and *S. flexneri* in developing countries, where as *S. sonnei* causes epidemic Shigellosis in industrialized countries (Chun, 1964). It is also travel associated disease (Mintz et al., 2002). *S. flexneri* is found more often in outbreaks at mental institutions or nursing homes, where as S. sonnei is seen more often in schools or communities (Chiu et al., 1994). The hallmark of clinical shigellosis is an acute rectocolitis associated with nausea, fever, anorexia, dehydration, mucopurulent and bloody diarrohea, tenesmus. HIV associated immunodeficiency leads to more severe clinical manifestations of *Shigella* infection including recurrent intestinal disease and bacterimia (Angulo and Swerdlow, 1995).

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The incidence of Shigellosis ranges 3-13% among certain Asian countries (Von Seidlein *et al.,* 2006) and it is major G.I tract disease in India. Identification of *Shigella* in environment and food samples, where the number of organisms are few and some may be injured/dormat, becoming difficult for isolation and identification (AOAC, 1998). Hence the present study was undertaken to study the incidence of *Shigella* spp in livestock products

Material & Methods

A total of 50 samples of each beef, mutton, chicken, eggs, fish and milk were collected. An amount of 50 grams of beef samples from Greater municipal slaughter house, Chengicherla, mutton as well as chicken samples from local markets, fish samples from local markets and ponds, milk sample from dairy experimental station, C.V.Sc, Rajendranagar, local vendors and local dairy farms and eggs from local poultry farms and markets. All samples were packed in icebox and transported to the laboratory.

Isolation of *Shigella* spp. were done as per method BAM (1998). The 25 grams of sample was added to 225 ml of *Shigella* broth containing 0.5µg/ml novobiocin supplement which after homogenization for 2 min transferred to 500 ml flask and kept in incubation for 16-22 hr at 37^{0}C. The loop of enriched culture sample was streaked over MAC, DCA, XLD and SS agar and incubated at 37^{0}C for 24 hr. The colony characteristics on MAC agar pale pink or colorless *(S. flexneri)*, colorless *(S. dysentriae)*, red *(S. sonnei)*, on DCA agar plate colorless *(S. flexneri* and *S. dysentriae)* and red *(S. sonnei)* and on XLD agar, red *(S. flexneri* and *S. dysentriae)* and yellow *(S. sonnei)* were identified. On SS agar plates colorless *(S. flexneri* and *S. dysentriae)* and red *(S. sonnei)* will be identified. Typical colony characteristics features of different *Shigella* spp. on selective agar plates were further confirmed by grams staining and biochemical tests such as indole test, MRVP, citrate utilization test, mannitol fermentation test, TSI, motility and catalase tests were done in the laboratory for confirmation of Shigella spp.

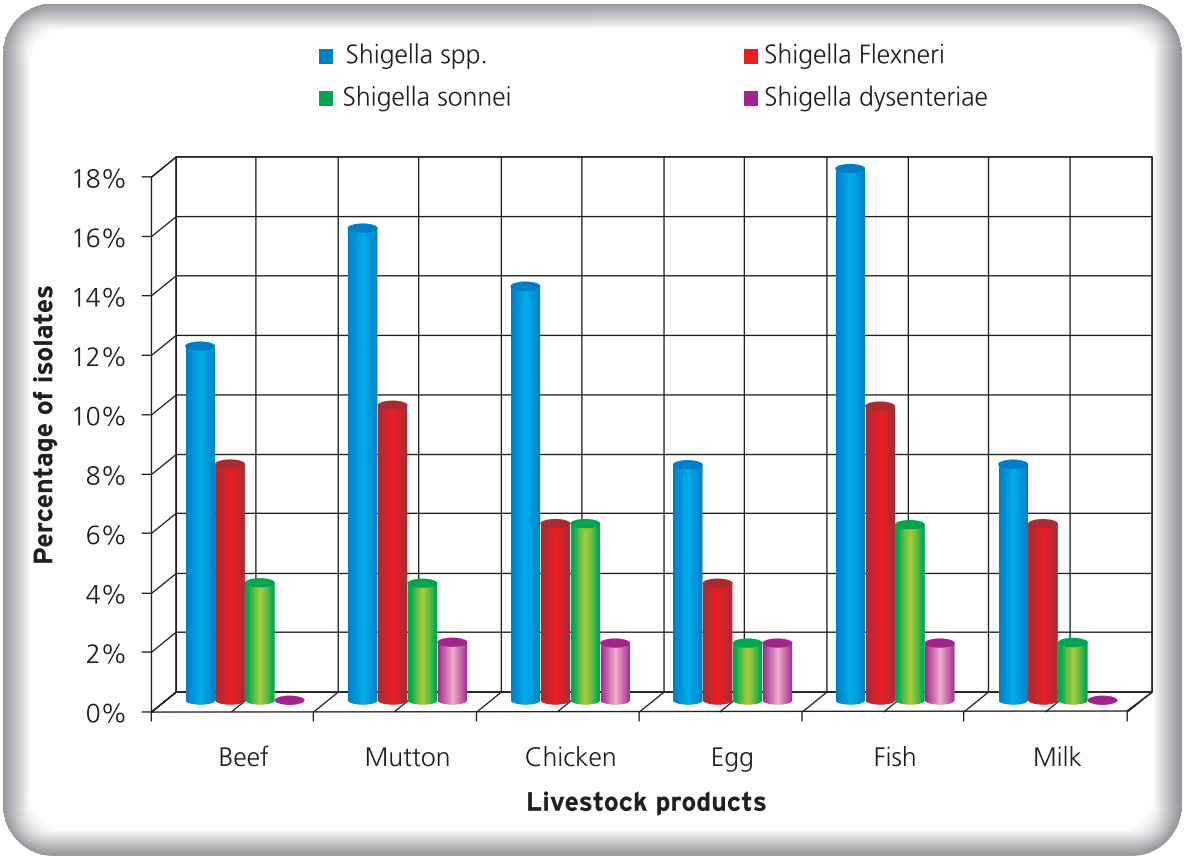
Results and Discussion

The incidence of Shigella spp in different samples are presented in Tab-1 and Fig-1. Out of 50 beef samples, six were positive for *Shigella* spp. accounting for 12 percent. Out of which four (66.66 percent) were positive for *S. flexneri* and two (33.33 percent) were positive for *S. sonnei* and no samples were found positive for *S. dysenteriae*. On the basis of 50 samples incidence of *S. flexneri, S. sonnei* and *S. dysenteriae* were 8 percent, 4 percent and 0 percent respectively. Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs (Fratamico *et al.* 2005). Earlier Garedew *et al.* (2015) examined the raw cattle meat samples in Gondar town Ethiopia and reported 11.11 percent incidence of *Shigella* spp. which was almost similar to the present study. No incidence of *Shigella* spp in beef samples collected from Seoul, South Korea was reported (Kim *et al.* 2008). A lack of sanitary conditions is the most common cause of contamination of meat from different sources (Ukuwuru *et al.,* 2012).

Out of 50 mutton samples, eight samples were positive for *Shigella* spp. accounting 16 percent. Out of eight samples positive for Shigella spp five (62.5 percent) were positive for *S. flexneri*, two (25 percent) were positive for *S. sonnei* and only one (12.5 percent) was positive for *S. dysenteriae.* On the basis of total 50 samples *S. flexneri, S. sonnei* and *S. dysenteriae* were 10, 4 and 2 percents respectively. Lower incidence of 2 % and 0.6 % were reported by Ashenafi (1994) and Tassew *et al.* (2010) respectively. Personal hygiene are the primary factors for the

Table-1: Showing the incidence of various Shigella spp. in livestock products

Livestock Products	Sample size	Shigella spp.		S. Flexneri			S.sonnei			S. Dysenteriae		
		No. of positive	%	No. of positive	% of Shigella positive	% over total samples	No. of positive	% of Shigella positive	% over total samples	No. of positive	% of Shigella positive	% over total samples
Beef	50	6	12	4	66.66	8	2	33.33	4	0	0	0
Mutton	50	8	16	5	62.5	10	2	25.0	4	1	12.5	2
Chicken	50	7	14	3	42.8	6	3	42.18	6	1	14.28	2
Egg	50	4	08	2	50.0	4	1	25.0	2	1	25.0	2
Fish	50	9	18	5	55.55	10	3	33.33	6	1	11.11	2
Milk	50	4	08	3	75	6	1	25.0	2	0	0	0



incidence of *Shigella* spp in meat (Asghar *et al.* 2002). Understanding the prevalence and distribution of *Shigella* species in food animals and determining management strategies associated with lower prevalence is key to decreasing the risk of high pathogen loads at harvest (Foley and Lynne, 2008).

Out of 50 chicken samples, seven positive for *Shigella* spp. accounting 14 percent of total samples. Out of seven positive for *Shigella* spp., three sample each (42.8 percent) positive for *S. flexneri* and *S. sonnei* and one sample (14.28 percent) was positive for *S. dysentriae*. On the basis of 50 samples tested the incidence of *S. flexneri, S. sonnei* and *S. dysentriae* were 6 percent, 6 percent and 2 percent respectively. Thanigaivel et al. (2015) reported an incidence 2.5 percent of *S. flexneri* in raw chicken meat from market places in and around Chennai which was lower than the present study (6 percent). Cardoso *et al.* (2006) also reported the incidence 14.3 percent in freshly dressed chicken which is similar to the present study. Higher incidence of *Shigella* spp. was also reported by Bhatia and Patak (1978) in poultry which was 80 percent.

Out of 50 egg samples, four were positive for *Shigella* spp accounting 8 percent. Out of eight positive for *Shigella* spp., two (50 percent) positive for *S. flexneri* and one each (25 percent) positive for *S. sonnei* and *S. dysenteriae*. On the basis of total 50 samples the incidence of *S. flexneri, S. sonnei* and *S. dysenteriae* were 4 percent, 2 percent and 2 percent respectively. Higher incidence of *S. flexneri* in eggs and egg products (66.7 percent, 70 percent and 79 percent) was reported by Weil and Gall (1941). Sahilu *et al.* 2015 reported 6.25 percent *Shigella* spp incidence in table eggs of retail outlets, Sokoto metropolish, Nigeria, which is lower than the present study. *Shigella* isolation from eggs and egg surface is due to contamination with feaces (WHO/FAO, 2002).

Out of 50 fish samples, nine (18 percent) were positive for *Shigella* spp. Out of eight positive for *Shigella* spp, five (55.55 percent) were *S. flexneri,* three (33.33 percent) positive for *S. sonnei* and only one sample(11.11 percent) was positive for *S. dysenteriae.* The incidence of *S. flexneri, S. sonnei* and *S. dysenteriae* were 10 percent, 2 percent and 2 percent respectively, when expressed for total 50 samples. Higher incidence (39.5%) of Shigella spp was reported by Onyango *et al.* (2009) in fish sample collected from Winam Gulf of lake Victoria. Yagoub (2009) reported 2.2 percent incidence of *Shigella* spp from raw fish which was lower than the present study. Sources of contamination like the utensils used in the preparation, water used in the washing of the fishes, working tables, showcases and trays used in processing and carrying of the fishes and also unhygienic practices of the food handlers or the consumers may resulted in incidence of *Shigella* spp (Chukwu *et al.,* 2013).

Out of 50 milk samples four (8 percent) were positive for *Shigella* spp. Out of four positive *Shigella* spp, three (75 percent) were positive for *S. flexneri,* one sample (25 percent) was positive for *S. sonnei* and no sample was positive for *S. dysenteriae.* Out of total sample tested *S. flexneri* and *S. sonnei* were 6 percent, 2 percent respectively. Jeyakumar and Lawrence (2014) reported *Shigella flexneri* 14% from raw milk samples of Allahabad district of Uttar Pradesh, India which was higher than the present study. Unhygienic environment as well as handlers are the main cause of *Shigella* spp. incidence in raw milk (Girma, 2015).

Shigella infections remain a global public health concern, causing diarrhea in both the developing and developed regions (Guerrant *et al.,* 1990). The organism is readily transmitted through the fecal-oral route, with the majority of illnesses

arising through the consumption of contaminated food and water. Poor personal hygiene and sanitation are the common sources of such food and water contaminations (Sapsford *et al.,* 2004).

Conclusion

The incidence of Shigella spp in livestock products is increasing day by day due to poor sanitary conditions. So strict sanitary condition compiled with high personal hygiene will decrease the incidence.

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Faecal Egg Counts in Young Animals

Parasite	Degree of infection (eggs per gram of faeces)		
	Light	Moderate	Heavy
CATTLE			
Mixed infection	50-200	200-800	800+
Pure *Haemonchus* infection	200	200-600	600+
Pure *Trichostrongylus* infection	50-100	100-400	400+
Pure *Cooperia* infection	200-300	300-2500	2500+
SHEEP AND GOAT			
Mixed infection	50-800	800-1200	1200+
Mixed infection with *Haemonchus* absent	300-800	800-1000	1000+
Pure *Haemonchus*	100-2000	2000-7000	7000+
Pure *Trichostrongylus*	100-500	500-2000	2000+
Pure *Nematodirus*	50-100	100-600	600+
Pure *Oesophagostomum*	100-800	800-1600	1600+

Source : FAO

(Note: Provides guidelines to aid in interpreting fecal egg counts in young animals but it may vary for different area/country/region)

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Antibiogram of Shigella spp. isolated from Chicken, Milk and Fish samples

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Abstarct

Twenty isolates of *Shigella* species obtained from chicken, milk and fish samples were tested for the presence of sensitivity/resistance against nine selected antibiotics. *Shigella* species was highly sensitive to kanamycin (85%), followed by nalidixic acid (35%), chloromphenicol (25%), ciprofloxacin (20%), very low sensitivity was reported for the antibiotics like ampicillin, erythromycin (10%), tetracycline, cotrimoxazole (5%) and gentamycin (0%). The resistance was high to gentamycin (80%), followed by ampicillin, erythromycin (60%), cotrimaxazole, tetracycline(50%), nalidixic acid (45%), chloromphenicol (30%) and ciprofloxacin (10%).

Keywords: Antibiogram, Antibiotic resistance, Antibiotic sensitivity, *Shigella spp.*

Introduction

Shigella is a genus of Gram-negative, facultative anaerobic, non-spore-forming, non-motile, rod-shaped bacteria closely related to *Salmonella.* The genus is named after Kiyoshi Shiga, who first discovered it in 1897 *(Yabuuchi, 2002)*. These organisms are members of the family Enterobacteriaceae and tribe Escherichieae; they are grouped into 4 species: *Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei,* also known as groups A, B, C, and D, respectively (Gomez and Clearly, 1997), Group A has 13 serotypes, group B has 6 serotypes, group C has 18 serotypes, and group D has 1 serotype. *Shigella* infection is the third most common cause of bacterial gastroenteritis throughout the world, after *Salmonella* infection and *Campylobacter* infection and ahead of *E. coli* O157 infection (Gupta et al, 2004).

The disease caused by the ingestion of *Shigella* bacteria is referred to as shigellosis, which is most typically associated with diarrhea and other gastrointestinal symptoms (CDC, 2009). *Shigella* is easily spread person-to-person because of its relatively tiny (compared to other bacteria) infectious dose (Kotloff, 1999 and DuPont, 2000). Infection can occur after ingestion of fewer than 100 bacteria (APHA, 2008). Another reason *Shigella* so easily cause infection is because the bacteria thrive the low pH of gastric juice in the human intestine and are commonly spread both by person-to-person contact and through the contamination of food. (Keusch and Acheson, 1996 and Reller *et al,* 2006). Most people who are infected with *Shigella* develop diarrhea, fever, and stomach cramps after being exposed to the bacteria (CDC, 2009). Symptoms may start 12 to 96 hours after exposure, usually within 1 to 3 days (APHA, 2008). Diarrhea may range from mild to severe, and it usually contains mucus (DuPont, 2000). When more severe, the

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diarrhea is bloody 25% to 50% of the time. Rectal spasms, which are technically referred to as "tenesmus," are common (Keush and Acheson, 2000).

The progressive increase in antibiotic resistance because of overuse and misuse of antibiotics in the treatment of diarrhea in developing countries is becoming a critical area of concern (Ecker *et al,* 2011 and Reda *et al,* 2011). Antibiotic therapy reduces the duration of Shigella dysenteriae infection and, therefore, is recommended, for the treatment of moderate to severe dysentery (David *et al,* 2010). Appropriate antibiotic treatment of shigellosis depends on identifying resistance patterns (Ashkenazi *et al,* 2003). Rapid emergence of resistance warrants the need for continuous monitoring of sensitivity patterns (Zafar *et al,* 2009) and the choice of antibiotic should be governed by periodically updated local antibiotic sensitivity patterns of *Shigella* isolates (David *et al*, 2010).

Hence this investigation was carried out to test the antibiotic susceptibility of *Shigella* organisms isolated from chicken, milk and fish samples collected from local markets of Hyderabad city, Telangana.

Materials and Methods

A total of 60 samples of chicken (20), milk (20), fish samples (20), 10 g each were collected aseptically from local markets and brought to the laboratory in an icepack. All the samples were inoculated into nutrient broth and incubated at 37^{0}C for 24 hrs. One ml of pre enrichment broth inoculums was transferred to selective enrichment broths like *shigella* broth and selenite F broth and incubated at 37^{0}C for 24 hrs. The enriched inoculums from the selective broths were streaked onto selective medias like XLD agar, DCA agar plates and incubated at 37 0C for 40 hrs. The presumptive colonies of *Shigella* species were picked up and subjected to Biochemical tests. The confirmed twenty colonies of different species of *Shigella* were subjected for antibiotic sensitivity test, using disk diffusion assay with Muller-Hinton agar in accordance with French National Antibiogram committee guide lines. The antibiotics tested were - Ampicillin (30 µg), Chloramphenicol (10 µg), Ciprofloxacin (30 µg), Cotrimoxazole (30 µg), Erythromycin (10 µg), Gentamycin (30 µg), Kanamycin (10 µg), Nalidixic Acid (30 µg), Tetracycline (30 µg).

MH broth was inoculated with five colonies from the sample and tubes were incubated at 37^{0}C for 2-8hr until achieving a turbidity equivalent to 0.5 on the Mac Farland scale. After turbidity adjustment, a sterile swab was introduced, pressed against the tube wall in order to remove any excess liquid, and then seeded on the surface of a petridish containing MH agar, rotating atleast twice. After the liquid was placed, the disc was left at rest for five minutes to absorb any excessive humidity. Using sterile forceps, ten discs (sensifar) impregnated with antimicrobials were placed at equal distances from each other on the surface of each dish. Subsequently the plates were inverted and incubated at 37^{0}C for 24 hr. Disc readings were performed 24hr after incubation. The zones showing complete inhibition were measure. The diameters of the zones to the nearest millimeter were measured. The interpretation was made as per the zone size interpretation chart provided by manufacture of discs.

Results and Disussion

In the present study *Shigella* species was highly sensitive to Kanamycin (85%), followed by nalidixic acid (35%), chloromphenicol (25%), ciprofloxacin (20%), very low sensitivity was reported for the antibiotics like ampicillin, erythromycin (10%), tetracycline, cotrimoxazole (5%) and gentamycin (0%).

Antimicrobial sensitivity and resistance of Shigella spp. to different antibiotics

S. No	Antibiotic	No	Sensitive No. (%)	Intermediate No. (%)	Resistance No. (%)
1	Ampicillin	20	2(10)	6(30)	12(60)
2	Chloromphenicol	20	5(25)	9(45)	6(30)
3	Ciprofloxacin	20	4(20)	14(70)	2(10)
4	Cotrimoxazole	20	1(5)	9(45)	10(50)
5	Erythromycin	20	2(10)	6(30)	12(60)
6	Gentamycin	20	–	4(20)	16(80)
7	Kanamycin	20	17(85)	3(15)	–
8	Nalidixic acid	20	7(35)	4(20)	9(45)
9	Tetracyclin	20	1(5)	9(45)	10(50)

(Numericals in parenthesis indicates percentage)

The sensitivity of *Shigella* isolates to ampicillin was 10% in the present study, whereas the resistance was 60%. Lower resistance of 5% by *S. sonei* and 1% by *S. flexeneri* than the present study were reported by Hirose *et al*, (2005) and Manomando *et al,* (2009) respectively, whereas higher percentage of resistance 72.7%, 73.6% *(S. flexeneri)*, 90.6% and 100% *(S. dyssenteriae)* were reported by Bhattacharya *et al* (2012), Jomezadeh *et al* (2014), Gardew *et al* (2015) and Temu *et al* (2007) repectively.

In the present study the sensitivity of *Shigella* isolates to chloromphenicol was 25% and resistance was 30%. Almost similar percentage of resistance (27.3% and 31.5%) was reported by Battacharya *et al* (2012) in Andaman islands, India and Jomezadeh *et al* (2014) in Abandan, Iran for Shigella species isolated from stool samples of paediatric diarrhea patients. Lower percentage of resistance than the present study were reported by Hirose *et al* (2005) and Mandomando *et al* (2009) as 0% and 1% respectively. Higher percentage of resistance was reported by Temu *et al* (2007) for *S. dyssenteriae* as 71% and for *S. flexeneri* as 94% in case of stool samples of diarrheic patients of Mwanza, Tanzania. The sensitivity of *Shigella* isolates to ciprofloxacin in the present study was 20% and 10% resistance. No resistance of *Shigella* isolates to ciprofloxacin was reported by several studies. (Lin, 2000, Temu *et al* 2007, Bhattacharya *et al* 2012, and Jomezadeh *et al* 2014).

The sensitivity and resistance of *Shigella* isolates to cotrimaxazole was 5% and 50% respectively in the present study. In contrast to the present study 100% of sensitivity was reported by Garadew *et al* (2015) for *Shigella* isolates, isolated from meat and swab samples. Almost similar percentage of resistance was reported by Battacharya *et al* (2012) (57.6%). Higher percentage of resistance (100%) than the present study was reported by Tassew *et al* (2010), whereas Bhattachraya *et al* (2012) reported little higher resistance (57.6%). The sensitivity of Shigella isolates to Erythromycin was 10% and resistance of 60% in the present

study. Lower percentage of resistance than the present study was reported by Temu *et al* (2007) as 14% for *S. dyssenteriae* and 19% for *S. flexeneri* in stool samples of diarrheic patients of Mwanza, Tanzania.

A resistance 0% and sensitivity of 80% was observed in the present study. Almost similar type of sensitivity percentage to gentamycin was reported by Battacharya *et al* (2012), Hirose et al (2005), Jomezadeh *et al* (2014) for S.dyssenteriae, Garedew *et al* (2015), Temu et al (2007) both for *S. flexeneri* and *S. dyssenteriae*. Lower percentage (26.3%) of resistance to gentamycin than the present study was reported by Jomezadeh *et al* (2014) for *S. flexeneri* isolated from stool samples. The sensitivity of Shigella isolates to Kanamycin was 85% and zero resistance was observed in the present study. Almost similar type of resistance (0%) to kanamycin was reported by Hirose *et al* (2005) for *S. sonei* and slightly higher resistance (8%) was observed by Lin, 2000.

The sensitivity of *Shigella* isolates to Nalidixic acid was 35% and resistance of 45% in the present study. Higher percentage of sensitivity was reported by Garedew *et al* (2015) (81.2%) for Shigella isolates of meat and swab samples. Lower percentage of resistance of 0, 21, 26, 27.3 was reported by Temu *et al* (2007), Jomezadeh *et al* (2014) for *S. flexeneri*, Hirose *et al*, (2005) for *S. sonei* and Bhattacharya *et al* (2012) respectively. The sensitivity of Shigella isolates to Tetracyclin was 5% and resistance of 50% was observed in the present study. Higher percentage of sensitivity was reported by Garedew *et al* (2015) as 90.6% in meat and swab samples. Higher percentage of resistance 86%, 78.9%, 100% was reported by Temu *et al* (2007), Jomezadeh et al (2014) and Tassew *et al* (2010) respectively. Lower percentage of resistance 1%, and 9.1% were reported by Mandomando *et al* (2009) and Battacharya *et al* (2012) respectively.

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Effect of Ashwagandha (Withania somnifera) Herb on *In Vitro* Fermentation Characteristics of Wheat Straw and Wheat Straw-based Complete Feed

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Abstract

An experiment was conducted to assess the effect of Ashwagandha *(Withania somnifera)* herb supplementation to wheat straw based complete feed (60:40 ratio).@ 0, 1, 2, 3, 4 and 5% kg -1 dry matter substrate on degradability of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and total gas production (TGP) using goat rumen liquor. Significant improvement in nutrient degradability of DM, OM and NDF in wheat straw based complete feed and TGP production was observed due to Ashwagandha supplementation with maximum values at 3% kg -1 DM.

Keywords: wheat straw, complete feed, Ashwagandha, gas production, in vitro degradability.

Introduction

The ever increasing number of consumers demanding healthy and natural foods have pushed organic livestock farming in the present era. This system of livestock farming is environmental friendly, sustains animals in good health with high welfare standards and prohibits routine use of growth promoters, animal offals and antibiotics or any other chemicals and additives in the livestock ration. It is, therefore, imperative to explore alternative natural feed additives to improve animal productivity.

The use of herbs in livestock ration as feed additives is receiving increasing attention as it is considered safe without any side effect as well as enhances nutrient utilization and productivity in animals. The beneficial effects of herbs in farm animals may arise from activation of feed intake and secretion of digestive enzymes, improved feed efficiency, reduced incidence of digestive disturbance, stimulation of digestion, methane inhibition, improved reproductive parameters and productive performance, immuno-stimulation, antibacterial, coccidiostatic, anthelmentic, antiviral or antioxidative characteristics (Uegaki *et al.,* 2001). Herbs and/or herbal preparations stimulate the endocrine system and enhance the intermediate nutrient metabolism, thereby enhances the nutritional status of animal by maintaining the blood biochemical constituents and enzyme profile concerned with metabolic activities. The extent to which level the herbs can be incorporated in livestock ration to increase nutrient intake and utilization as well as animal performance has not been well established. Therefore, the present investigation was undertaken to study the effect of herb Ashwagandha *(Withania somnifera)* at different

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levels on *in vitro* nutrient degradability characteristics and total gas production of wheat straw and fermentation pattern in wheat straw-based complete feed using rumen liquor of goats.

Material and Methods

The samples of wheat straw, complete feed and Ashwagandha herb were oven-dried, ground and passed through 1 mm sieve in a Willy mill and stored in plastic bottles for further use. The proximate constituents of wheat straw, Ashwagandha and complete feed was done as per AOAC (1999) and fibre fractions as per VanSoest *et al.* (1991). The modified *in vitro* procedure of Tilley and Terry (1963) was used to assess *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD) and *in vitro* nutrient detergent fibre degradability (IVNDFD) at 48 and 72 hr post-incubation and total gas production at 24 hr post-incubation. Three adult bucks were given 20 days adoption time to complete feed containing wheat straw at 60% level to meet nutrient requirements as per ICAR (1998). Rumen fluid was collected from these bucks before morning feeding (Solvia and Hess, 2007) by perforated tubing device under negative pressure and squeezed through four layers of muslin cloth to get inoculums (SRL), then transferred to pre-warmed flask (39°C) and flushed with Co_2. SRL (10 ml) was dispensed into pre-warmed (39°C) fermentation vessels (Jakhmola et al., 2010) containing 500 mg finely ground wheat straw based complete feed (1 mm) without or with 1, 2, 3,4 and 5% herb kg -1 substrate DM + 40 ml buffer and flushed with oxygen free CO_2. The experiment was conducted in completely randomized block design and each treatment run in triplicate with negative controls (SRL + buffer alone). The controls were used to correct for fermentation residues resulting directly from SRL. Each vessel cork fitted with control value was kept in incubator at 39°C for 48 hr. *In vitro* gas production was measured periodically upto 24 hr incubation by Doctor's syringe (Jakhmola *et al.,* 2010). At the end of 48 hr incubation two drops of saturated HgCl2 was added in each vessel to stop microbial activity. Contents of each vessel were transferred to 1 L spout-less beakers. The vessels were thoroughly washed with neutral detergent solution and final volume made to 150 ml. The contents were refluxed for 1 hr at 100°C, filtered and washed through pre-weighed Gooch crucible (Grade 1, 50 ml capacity). This undigested residue (NDF) was oven-dried at 100°C for 24 hr, cooled in desiccator and weighed. Loss in DM and NDF was digested dry matter and digested NDF. The crucibles containing residue were ignited in muffle furnace at 500°C and the ash left in crucible after ignition was subtracted from residual dry matter to get the organic matter content. For *in vitro* studies at 72 hr, incubation of samples was done in same way as for 48 hr incubation. The reaction was stopped by adding 2 ml of 6 N HCl and 0.1 g pepsin powder (1:3000) to each vessel at the end of 48 hr incubation. Then the vessels were incubated for another 24 hr and procedure repeated as for 48 hr incubation, except the addition of $HgCl_2$ at 48 hr. The optimum level of herb, chosen from experiment I, was mixed with finely ground (1 mm) complete feed containing wheat straw and concentrate mixture in 60:40 ratio. The concentrate mixture contained barley 06, deoiled rice bran 05, groundnut cake 13, gaur korma 13, mineral mixture 02 and salt 01 parts. The experimental procedures for degradability of nutrients in complete feed were similar to experiment I. The pH in rumen liquor was determined immediately after the termination of incubation at 48 hr using portable digital pH meter. Total volatile fatty acids was determined as per method of Barnett and Reid (1957) using Markham still distillation apparatus, total rumen nitrogen as per AOAC (1999) and ammonia

nitrogen by spectrophotometer method (Chaney and Marbach, 1962). The data were analyzed statistically as per Snedecor and Cochran (1994) to draw the inference.

Results and Discussion

The herb *Withania somnifera* contained 4.13% crude protein, 0.56% ether extract, 15.02% crude fibre, 74.94% N- free extract, 42.35% neutral detergent fibre, 21.87% acid detergent fibre and 20.48% hemicellulose (Table 1). The chemical composition of Ashwagandha was comparable with Samal *et al*. (2009). The chemical composition of wheat straw is comparable with Manju *et al*. (2010).

Table 1: Chemical composition wheat straw, complete feed and Ashwagandha

Chemical components (% DM)	Wheat straw	Complete feed	Ashwagandha (Withania somnifera)
Dry matter	92.75	94.20	96.00
Organic matter	88.91	87.68	94.65
Crude protein	3.35	14.72	4.13
Ether extract	1.04	2.44	0.56
Crude fibre	38.65	26.48	15.02
Nitrogen free extract	45.87	44.04	74.94
Neutral detergent fibre	74.20	57.16	42.35
Acid detergent fibre	50.58	36.33	21.87
Hemicellulose	23.62	20.83	20.48

The IVDMD and IVOMD of complete feed supplemented with Ashwagandha was significantly higher as compared to control at 1, 2, 3, 4 and 5% level at both 48 and 72 hr incubation (Table 2). Maximum IVDMD and IVOMD were noticed at 3% supplementation though the differences between 3 and 4% and 4 and 5% were non-significant. Ashwagandha supplementation improved DM degradability at 48 and 72 hr incubation by 3.93 and 3.20%, 7.32 and 5.29%, 12.20 and 9.15%, 11.22 and 6.86%, 9.82 and 6.04% over control at 1, 2, 3 4 and 5% supplementation level, respectively. A trend similar to IVDMD was observed in IVOMD. The respective improvement in OM degradability for 48 and 72 hr incubation was 3.68 and 2.39, 7.09 and 4.4, 11.35 and 8.30, 10.40 and 4.96, 9.16 and 3.85% over control at 1, 2, 3,4 and 5% Ashwagandha supplementation, respectively. Addition of Ashwagandha affect degradability of IVNDFD to significant level at 48 and 72 hr incubation. The respective improvement over control in IVNDFD for 48 and 72 hr incubation was 1.95 and 1.94, 3.71 and 5.31, 5.44 and 7.50, 4.18 and 6.61 and 3.29 and 5.44% at 1, 2, 3, 4 and 5% Ashwagandha supplementation,

respectively. A linear increase in IVDMD, IVOMD and IVNDFD at 48 and 72 h incubation with increase in the level of supplementation of Ashwagandha upto 3% DM was observed and thereafter inclusion of Ashwagandha did not show any additional improvement on IVDMD, IVOMD and IVNDFD. The results suggest that the addition of herb might have increased the population of fibre degrading bacteria and/or their activity (Das, 1992; Manjunatha, 1998). These findings corroborate with those of Bakshi *et al.* (2004) who reported that in vitro digestibility of nutrients and availability of metabolizable energy improved with herbal feed additives using berseem and wheat straw as substrate. The findings are in line with Gupta *et al.* (2005) and Kumar *et al.* (2006) who reported that supplementation of herbs at 5% level increased IVDMD of paddy straw. Sardar et al. (1998) observed that a mixture of herb supplementation (50 mg g -1) improved IVDMD of oat fodder, concentrate mixture and oat fodder + concentrate mixture.

The improvement in IVTGP in wheat straw based complete feed supplemented with 1, 2, 3, 4 and 5% of herb was 20.50, 24.80, 29.70, 24.60 and 22.87% over control at 24 hr incubation. The results fall in line with the observations of Bakshi *et al.* (2004), who reported increased net gas production in berseem and wheat straw on supplementation of different herbs. The increase in total gas production appeared to be associated higher DM, OM and NDF degradability in substrate due to increase in bacterial population, activity and/or rate of fermentation.

Table 2: In vitro DM, OM and NDF degradability, and total gas production in wheat straw based complete feed

	Incubation (hr)	Ashwagandha supplementation (%)						
		0	1	2	3	4	5	SEM
IVDMD (%DM)	48	48.00^{a}	51.93^{b}	55.32^{c}	60.20^{e}	59.22^{de}	57.82^{d}	0.59
	72	58.05^{a}	61.25^{b}	63.34^{c}	67.20^{d}	64.91^{c}	64.09^{c}	0.54
IVOMD (%DM)	48	51.05^{a}	54.73^{b}	58.14^{c}	62.40^{e}	61.45^{de}	60.21^{d}	0.45
	72	60.35^{a}	62.74^{b}	64.75^{c}	68.65^{d}	65.31^{c}	64.20^{c}	0.31
IVNDFD (%DM)	48	28.84^{a}	30.79^{b}	32.55^{bc}	34.28^{d}	33.02^{cd}	32.13^{c}	0.44
	72	31.72^{a}	33.66^{b}	37.03^{c}	39.22^{d}	38.33^{cd}	37.16^{b}	0.42
IVTGP **(ml/200mg)**	24hr	19.30^{a}	20.50^{a}	24.80^{c}	29.70^{d}	24.60^{bc}	22.87^{b}	0.44

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

*IVDMD - *in vitro* dry matter degradability

*IVOMD - *in vitro* organic matter degradability

*IVNDFD - *in vitro* nutrient detergent fibre degradability

*IVTGP - *in vitro* total gas production

Ishtiyak *et al.* (2010) also observed increase in IVDMD and IVOMD on supplementation of different herbs in complete feed at 2 and 3% level in rumen fluid of goats. Similar findings have been reported by Sardar *et al.* (1998) and Wadhwa and Bakshi (2006). Ashwagandha supplementation did not alter pH of in vitro rumen fluid of goats which were within normal range of rumen pH (6.5-7.0) indicating no adverse effect of herb addition on rumen environmental conditions. The results are in agreement with Ishtiyak *et al.* (2010). Total nitrogen and total volatile fatty acid concentration was significantly higher (93.99 mg dl -1 and 84.91 m Eq L -1) in herb supplemented complete feed as compared to control (92.16 mg dl -1 and 81.22 m Eq L -1), which indicated stimulatory effects of herb on fibre degrading microorganisms. It appears that more energy may be available due to herb supplementation. Contrarily, there was significant decrease in rumen ammonia-nitrogen in herb supplemented complete feed as compared to unsupplemented one, indicating higher incorporation of ammonia-nitrogen into microbial protein (Sardar *et al.,* 1998; Wanapat *et al.,* 2008). The results recorded are authenticated by Manju (2010) and Wanapat *et al.* (2008) in *in-vivo* experiments with sheep and cattle, respectively.

Conclusion

The study revealed a significant improvement in digestibility of nutrients and rumen fermentation in *in-vitro* rumen fluid of goats as an effect of supplementation of Ashwagandha (Withania somnifera) at 3 % level of DM. However, in vivo trial in goats is required before advocating these findings to goat raisers.

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Seroprevalence of Brucellosis in Cattle related to Dairies in and around Pillibanga

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Abstract

Brucellosis is an emerging anthropozoonosis having serious public health implications in developing nations. Surveys were conducted on dairies. In the present study, 200 cattle serum samples that were found to be suspected for the brucellosis (still birth, premature birth, retention of placenta belonging to different localities of Pillibanga, Hanumangarh) were screened using RBPT and STAT. In cattle, an overall prevalence of 4.50 per cent and 5.50 was found using RBPT and STAT.

Key words : Cattle, brucellosis, RBPT, STAT

Introduction

Brucellosis is an anthropozoonosis of both public health and economic significance in most developing countries (WHO, 2006). According to OIE (Office International Des Epizooties), it is the second most important zoonotic disease in the world after rabies, causing extensive economic losses (Neta *et al.,* 2010). Brucellosis causes heavy economic losses to the animal based industries due to abortion, premature birth, total loss or decrease of milk production and infertility in the male as well as in female animals. The disease in animals is characterized by abortion in late pregnancy, followed by retained placenta and metritis. The organism may get localized in the udder (upper mammary lymph node) and there after appears in milk from infected cows. In bulls, orchitis and epididymitis may occur. There are about 204.3 million cattle and 85.3 million buffaloes in India (Renukaradhya et al., 2002), constituting about 16 per cent of the global livestock population. According to livestock census 2007, the cattle population in Rajasthan is 12.4 million, so bovine species are valuable component of rural household and this disease has also been detected in cattle and buffalo of western Rajasthan (Wadhwa, 2007). It has been found that the programmes for the control and eradication of bovine brucellosis markedly reduce the prevalence of the disease in humans (Renukaradhya *et al.,* 2002).

Materials and Methods

In the present study, the seroprevalence of brucellosis in cattle related to dairies in Bikaner were studied. The methodology/techniques approved by FAO (1971) were adopted.

Preparation of serum samples for serological test

Sterilized test tubes containing blood samples were incubated at 37^{0}C and subsequently transferred to refrigerator for two hours (for shrinkage of clot) and it was centrifuged at 2000

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rpm for 10 minutes. Afterwards, the serum thus separated was transferred to sterilized vials with the help of sterilized pipette. The Rose Bengal plate test (RBPT) and standard tube agglutination test (STAT) were performed on the same day and results were recorded. The RBPT was carried out by employing the technique directed by I.V.R.I., Izatnagar using Brucella Rose Bengal antigen. The technique approved by FAO (1971) was used for standard tube agglutination test.

Results and Discussion

Seroprevalence of bovine brucellosis:

Serum samples of 200 cattle screened by using RBPT and STAT. In Rose Bengal Plate Agglutination test (RBPT) an overall seroprevalence were 4.50 % (9 cattle were found positive) (Table 1, Fig. 1).

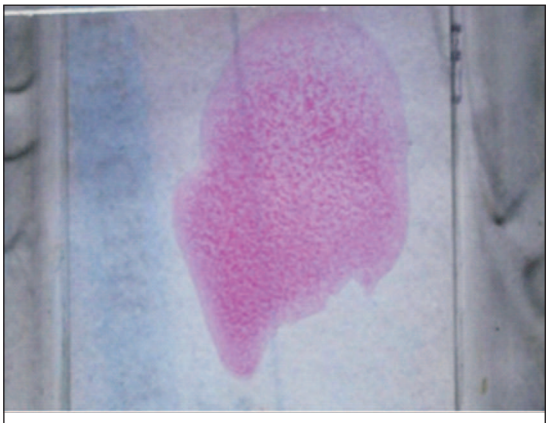

Fig. 1: RBPT showing positive result

Table 1: Overall seroprevalence of Brucella antibodies in bovine from different sources determined using RBPT

Serum samples	No. tested	No. positive	Percentage
Cattle	200	9	4.50

Standard Tube Agglutination Test (STAT) revealed overall seroprevalence of bovine brucellosis to be 5.50 per cent (Table 2). Out of 11 positive samples of cattle, one had a titer of 1:40, 8 had a titer of 1:80 and two samples were having a titer of 1:160 (Table 2, Fig 2). The present study found 6.79 per cent seroprevalence of bovine brucellosis which falls in line with the results of Vinod et al. (2006) who found 6.65 per cent of bovine brucellosis using STAT. However, a lower prevalence of 0.3, 4.44 and 0.8 per cent was reported by Renukaradhya *et al.* (2002); Kataria and Verma (1969) and Paloma *et al.* (1995), respectively. On comparing the current results with those of above workers, it can be concluded that the prevalence of brucellosis in this part of Rajasthan is higher compared to other parts of country.

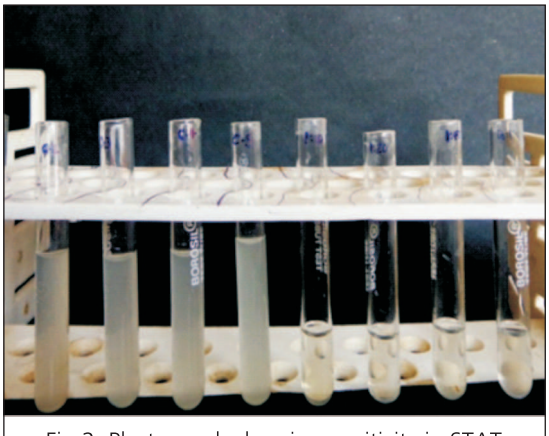

Fig 2: Photograph showing positivity in STAT

Table 2: Overall seroprevalence of Brucella antibodies is bovine from different sources determined using STAT.

Serum samples	No. tested	No. positive	Percentage	STAT titer				
				1:20	1:40	1:80	1:160	1:320
Cattle	200	11	5.50	-	1	8	2	-

Conclusion

On comparing the current results, it can be concluded that the prevalence of brucellosis of bovine in this part of Rajasthan is higher compared to other parts of country.

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Parasitic Zoonoses

Parasitic zoonotic diseases are prevalent throughout India at varying rates. The prevalence of zoonotic parasites is likely to be an underestimate, owing to the lack of proper surveillance and the shortage of information about the existence of asymptomatic animal carriers. Emergence of diseases such as human echinococcosis/hydatidosis, neurocysticercosis, cryptosporidiosis and toxoplasmosis in those with acquired immune deficiency syndrome, together with the reemergence of cutaneous leishmaniosis, poses a serious threat in India and the prevention and control of these parasitic zoonoses, and others, is a great challenge.

(Source: Parasitic zoonoses in India: an overview by BB Singh et al, Rev. sci. tech. Off. int. Epiz., 2010, 29 (3), 629-637)

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Dicephalic Monster in Buffalo

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Abstract

Monstrosities are malformed fetuses, which are rare in buffaloes. Duplication of the cranial portion of the fetus is more common than the caudal portion. Dystocia is common sequelae of fetal monstrosities. Two cases of dicephalic monsters in buffaloes were reported and the dystocia was relieved by cesarean and obstetrical operations in the respective cases.

Key words: - dicephalic monster, buffalo, caesarean section, carboxy methyl cellulose.

Introduction

Monstrosities are malformed fetuses, which are rare in buffaloes (Chauhan and Verma, 1995 and Bugalia et al., 2001). Incidence among all calves range from 0.2 to 3.0 percent with 40 to 50 percent born dead and only a small fraction of reported defects not being externally visible. Monstrosities are associated with either congenital defects or infectious disease (Arthur et al., 2001) and may or may not interfere with birth. Abnormal duplication of the germinal area during embryogenesis of a monozygotic fetus will give rise to partial duplication of body structures (Sharma et al., 2010). Duplication of the cranial portion of the fetus is more common than the caudal portion (Roberts, 2004). Dystocia is common sequelae of fetal monstrosities. Fetotomy offers a good alternative to the caesarean for relieving a fetal monster causing dystocia (Vermunt, 2009). In the present study, two cases of dicephalic monsters in buffaloes were relieved by caesarean and obstetrical operations respectively.

Clinical Examination and Treatment

Case 1:- An eight-year-old second parity Murrah buffalo was presented with the complaint of failure in delivering the fetus after initiation of labour pain. The clinical examination under epidural anesthesia revealed rupture of water bag, dilated cervix and a live fetus in anterior - longitudinal presentation, dorso-sacral position with both the forelegs upto the carpal joint outside the vulval lips. Repulsion and deeper exploration through the cervical canal into the gravid uterus revealed presence of two conjoined fetal heads in dorso ventral position. The per vaginal delivery could not be facilitated due to large size of the conjoined heads of the fetus. Hence, caesarean section was performed after restraint in lateral recumbancy under local infiltration with 2% Lignocaine hydrochloride . A 40 cm long oblique low flank (Young's approach) incision was taken and a live male monster calf was delivered through laparohysterotomy. The fetal membranes were carefully removed by separating cotyledons from caruncles of the uterus. The uterine incision was closed with

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cushing followed by Lambert suture pattern using chromic catgut No.1. The laparotomy incision was closed by standard technique. Supportive treatment was carried out with inj Calcium borogluconate @ 450 i/v, inj DNS @ 1000 ml i/v, and inj melonex (Meloxicam, Intas animal health, Gujarat) @ 0.5mg/ kg b.wt. Postoperative antibiotic therapy with inj Amoxicillin + Cloxacillin was followed for 5 days. Intrauterine therapy using C-flox TZ IU® (ciprofloxacin + tinidazole, Intas animal health, Gujarat) for three days. Daily antiseptic dressing was followed and the sutures were removed on day 15 after wound healing.

The fetus was a male and had two normal heads fused with each other at mandible and had one neck and four eyes, four ears, one trunk, one thorax, one pair of forelimbs, one pair of hind limbs, one sacrum and one tail (Fig 1). The calf was able to drink the colostrum through both the oral cavities on hand feeding. The calf was unable to independently bare weight. The calf died after five days. As per Roberts (1971), the condition could be classified as dicephalic conjoined twins are non inherited teratological defect (Noden and Lahunta, 1984). Conjoined twins are monozygotic and monstrosities arising due to incomplete division of embryo usually at the primitive streak development stage. Duplication of the cranial parts of fetus is more common than the caudal part. However, duplication can occur at both cranial and caudal end with the middle area of monster remaining single (Roberts, 1971). Similar to present report, Sloss and Dufty (1980) were also of the opinion that, fetotomy in such cases is difficult, hence caesarean section operation should be performed.

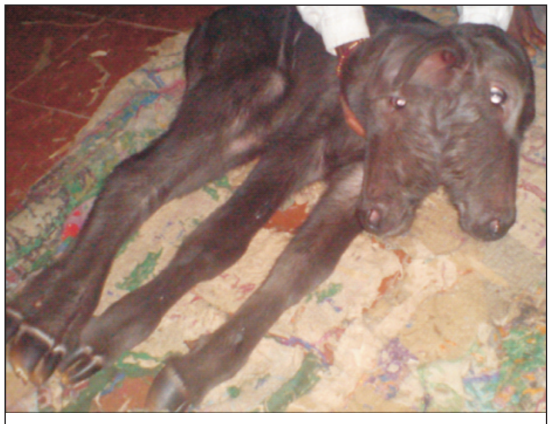

Fig:- 1 Dicephalus monster in a buffalo (Case 1)

Case 2:- A five year old she buffalo with full term gestation was presented to the outpatient ward of Teaching Veterinary Clinical Complex, Veterinary College, Bidar with a complaint of unsuccessful straining since ten hours. The clinical examination revealed an increase in respiration and heart rate with normal rectal temperature. Obstetrical examination under epidural anesthesia revealed a dead dicephalic fetus in anterio-longitudinal presentation, dorso-sacral position with bilaterally flexed knee joint and head deviated laterally. The buffalo was restrained in sternal recumbency and obstetrical maneuvers were carried out to deliver the calf per vaginally. The birth canal was lubricated with five liters of Carboxy methyl cellulose (CMC). The flexed knee joints were extended by repulsion and traction of the fetus. The position of the head was corrected using obstretical hook and the fetal head was brought near to the birth canal. The obstetrical chains were tied to both the forelimbs separately and the fetus was extracted after proper positioning by simultaneous three-point traction on both extended fore limbs and head with the help of obstetrical chains.

The delivered fetus was dead, male with two heads attached to single neck with fusion at the level of the cranium and ear; both heads had separate nostrils, eyes and ears. The neck, thorax, abdomen and limbs were grossly normal (Fig 2). These observations are in consonance with the

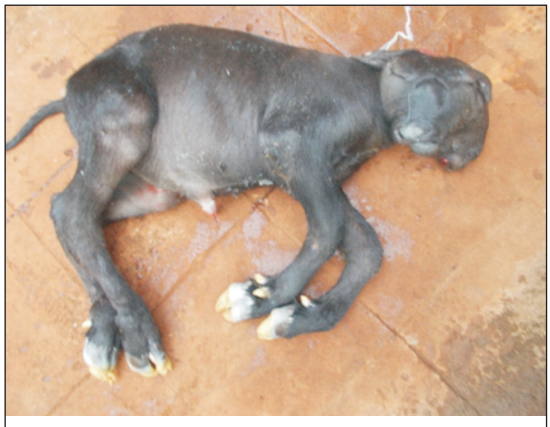

Fig 2 :- Dicephalus monster in a buffalo (Case 2)

earlier findings (Fisher *et al*., 1986 and Sharma *et al*., 2010). The report describes relieving dystocia due to dicephalic monster in a buffalo manually by proper positioning, correction and thorough lubricating with CMC. The technique was safer and less time consuming. It could be used successfully used as an alternative to the fetotomy and Caesarean section when the sizes of head is not too big and facilitate passage through the birth canal.

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Dystocia due to Complete Uterine Inertia in German Shepherd bitch

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(received 22/12/2015 - accepted 21/02/2016)

Abstract

A full term pregnant German Shepherd bitch of approximately 5 years of age with history of dystocia was presented in TVCC, Akola. The bitch has completed normal gestation period and presented with two hind legs of fetus in vaginal passage with no signs of straining and anorexia. The dystocia was diagnosed as maternal dystocia due to uterine inertia and treated successfully.

Keywords: Dystocia, Uterine Inertia, Bitch.

Introduction:

Dystocia is defined as inability to expel foetuses through the birth canal without assistance and occurs in about 5 % canine parturition (Eneroth et al., 2000). Dystocia is a common emergency in canine patients. The condition can be life threatening to both mother and fetus and it is of great emotional stress to owner (Davol, 2000). Dystotcia due to uterine inertia is observed most frequently in dog occasionally in cow and sow but rare in other species (Benesch, 2001). It is produced by a lack of tone or failure of uterine muscles to contract. The failure of uterine muscles to contract normally at parturition may be due to primary failure of muscle to respond hormonal stimuli (Roberts, 1971). The most common form in bitches is primary inertia, which can be classified as complete, or incomplete (Van den Weijden and Taverne, 1994). Complete inertia indicates that no puppies delivers because of apparent uterine muscle fatigue while incomplete inertia occurs when there is normal delivery of a portion of litter but uterus fatigues before parturition is complete (Jones and Joshua, 1982). Primary complete uterine inertia accounted for nearly 70% of maternal causes of dystocia (Shambu et al., 2001).

History and clinical observations:

A primiparus German shepherd bitch of approximately 5 years of age which has completed gestation period was presented with the history of dystocia. The history reveals that the two hind legs of pup were in the vaginal passage. Per vaginal examination reveals that there was no lubrication. The bitch was not exhibiting the signs of straining. The temperature was 100.9˚ F. While the heart beat was in normal range. The abdominal palpation was carried out which revealed there was no another fetus. Based on the history and clinical observations the present case was diagnosed as dystocia due to complete uterine inertia.

Treatment:

The bitch was treated with chorpheniramine maleate (Zeet, 20 mg, I/M), DNS 25% (200 ml I/V) and Calcium Gluconate 10% (10 ml I/V). After

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the treatment there was no progress and the pup was not expelled up to one hour so further injection of Oxytocin 10 IU (I/V) and Methyl Ergonetirne 0.2 mg (Methergin, I/M) was administered. The vaginal passage was lubricated with Gelly with the finger. Then after the treatment the bitch shows the straining and by slight traction the pup was removed (plate No. 1). The bitch was treated with antibiotic, analgesic and liver tonic for next 3 days.

Discussion:

Uterine inertia has been associated with inadequate uterine stimulation in one or two pup litters with systemic diseases such as hypocalcaemia or infection and with inadequate nutrition, uterine torsion and trauma (Johnston et al, 2001). The calcium gluconate increases the strength of myometrial contractions and Oxytocin increases the frequency of contractions. (Feldmon and Nelson, 2004). In the present article, the successful treatment of complete uterine inertia in the German Shepherd bitch reported.

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Feline Infectious Peritonitis -A clinical Case report

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(received 12/12/2015 - accepted 11/02/2016)

Abstract

A case of feline infectious peritonitis in a kitten, diagnosed on the basis of history, clinical picture, high serum proteins and globulins, high proteins in yellow coloured peritoneal fluid positive to Rivalta's test, is reported and discussed.

Key words : Feline infectious peritonitis, kitten, proteins.

Introduction

Feline infectious peritonitis is an immune mediated fatal disease of felines. Its diagnosis is usually achieved by assessing signalment factors, clinical signs are abnormalities in common diagnostic procedures, postmortem examination (when possible) and histopathology (Sharif et al.,2010). Classical indirect tests for feline infectious peritonitis include complete blood count , total serum proteins, albumin and globulin levels, A:G ratio and basic blood chemistries (Addie et al., 2009, Pedersen, 2009 and Drechsler et al.,2011).Scarcity of reports on feline infectious peritonitis in India prompted to report a case of feline infectious peritonitis in a kitten.

History and Investigations

A three month old male Persian kitten was referred at the hospital with the complaint of abdominal distension for a month and erratic fever (103.9 to 105^{0}F). The kitten has been refractory to treatment with frusemide, clindamycin, meloxicam and vitamin complex therapy.

Detailed clinical examination revealed antibiotic unresponsive fever (103.9 to 105.0^{0}F), erratic appetite, abdominal distension with fluid thrill on palpation (Fig.1), pale pinna and mucus membranes, dyspnoea, almost normal heart rate (180 bpm) with sinus rhythm. Abdominal X-rays, showed ground glass appearance. Sonography did not suggest any specific finding except hypoechoic fluid in the abdomen. Peripheral blood smears on two consecutive days were negative for Babesia and Ehrlichia spp. Biochemical investigations revealed almost normal values of serum creatinine (1.2 mg/dl), blood urea nitrogen (21.3 mg/dl), SGPT (19.4 IU/L), SGOT(70.1 IU/L), total bilirubin (0.43 mg/dl), direct bilirubin (0.22 mg/dl), indirect bilirubin (0.21 mg/dl); high values of total serum proteins (9.6 g/dl), and serum globulin (5.9 g/dl); and lower values of A:G ratio (0.627). Haemogram indicated severe anaemia (haemoglobin 2.9 g/dl, total erythrocytes 1.79 million/mm^3 and packed cell volume 7.7%) and almost normal value of total leucocytes (14.4 thousand/mm^3) .Electrocardiogram was normal with heart rate as 180 bpm and sinus rhythm(Fig.2). Peritoneal fluid was yellowish in colour

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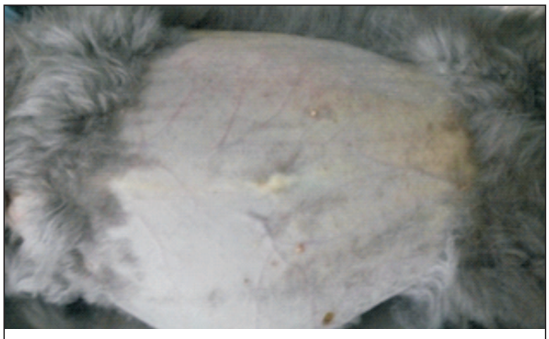

Fig. 1. Kitten with distended abdomen

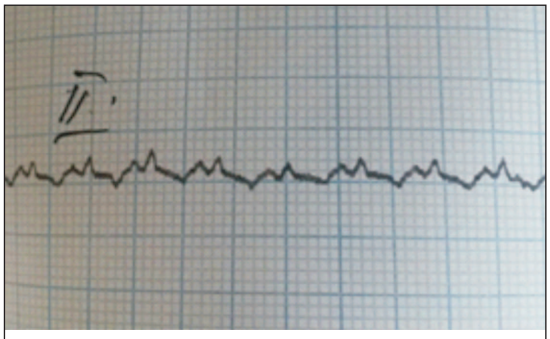

Fig.2.ECG of the kitten with normal complex

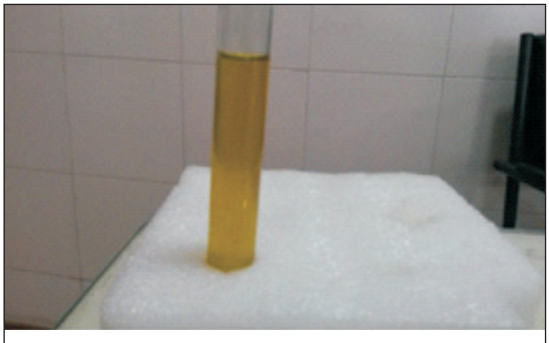

Fig.3.Turbid and yellowish peritoneal fluid of the kitten

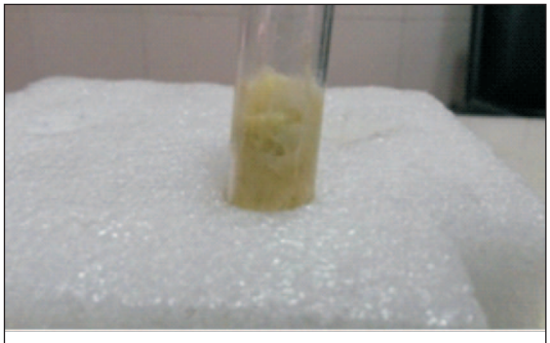

Fig.4. Peritoneal fluid showing positive Rivalta's test

and viscus in consistency(Fig.3). Its microscopic examination showed abundant erythrocytes and neutrophils. Peritoneal fluid had high protein (10.0 g/dl) and fibrinogen(500 mg/dl); and was positive to Rivalta' stest (Fig.4) suggesting feline infectious peritonitis(Hartmann et al.,2003).

Diagnosis and Treatment

Presumptive diagnosis of feline infectious peritonitis was made on the basis of history and clinical picture; high values of total serum proteins and globulin; high values of total proteins and fibrinogen in peritoneal fluid; and positive Rivalta's test.

Treatment was adopted with Ribavirin (10 mg/kg orally once daily), prednesolone (2.0 mg/kg orally twice daily), cefadroxil (10 mg/kg orally twice daily) and stanozolol (0.5 mg orally twice daily).

Discussion

Feline infectious peritonitis is a Corona viral disease affecting cats of all ages. The present case was initially misdiagnosed as ascites and treatment with furesemide, clindamycin and vitamin tonic for 10 days remained futile. Undulating antibiotic unresponsive fever with distended abdomen, marked anaemia and icterus at the time of referral aroused suspicious of feline infectious peritonitis. Severe anaemia, higher values of serum proteins and globulins; viscous yellowish Rivalta's positive peritoneal fluid with high proteins and fibrinogen reasonably confirmed feline infectious peritonitis (Addie et al.,2009; Pederson,2009 and Drechsler et al.,2011) as positive Rivalta's test with

peritoneal fluid has been considered highly specific for feline infectious peritonitis in Europe (Pederson,2014). Though there is no cure for feline infectious peritonitis, symptomatic and palliative therapy with antiviral ribavirin, immune-suppressive prednisolone, an antibiotic cefadroxil and bone marrow stimulant stanozolol was attempted without any success as the kitten collapsed on 10th day of therapy. Prognosis of wet feline infectious peritonitis has been reported as poor.

Acknowledgements :

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Important parasitic zoonoses reported in India

Disease name	Parasites name	Animals involved
Toxoplasmosis	*Toxoplasma gondii*	Cat, Sheep, Goats and Pig
Leishmaniosis	*Leishmania tropica*	Dogs and rodents
Taeniosis and Cysticercosis	*Cysticercus cellulosae and Taenia saginata*	Pigs and Cattle
Echinococcosis/ Hydatidosis	*Echinococcus granulosus*	Sheep, Cattle, Buffalo and dog
Larva migrans (Cutaneous and visceral larva migrans)	*Toxocara canis, Toxocara catis and Ancylostoma caninum*	Dogs and Cats
Sarcocystosis	*Sarcocystis suihominis and Sarcocystis hominis*	Cattle, Pig and Dogs
Trichinellosis	*T. spiralis*	Pigs
Crimean-Congo haemorragic fever	*Hyalomma anatolicum*	Cattle, Buffaloes, Sheep and Goats

(Source: Parasitic zoonoses in India: an overview by BB Singh et al, Rev. sci. tech. Off. int. Epiz., 2010, 29 (3), 629-637)

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A Rare Case of Mastitis with Haemogalactia in Goat

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Abstract

A 5 year old goat was brought to the veterinary hospital with the history of swelling of quarter and bloody milk. Milk was curdled and red in colour. On the basis of history and clinical observations it was diagnosed as a case of mastitis with haemogalactia and it is successfully treated with Ceftriaxone with Tazobactum, Meloxicam, Chlorpheniramine maleate, Vit. C, Vit. B complex and Haemostatic. Animal recovered completely after 5 days of treatment.

Keywords: Goat, haemostatic and mastitis.

Introduction

Mastitis is considered one of the most important diseases of domestic animals, caused by several etiologic agents. Transmission of the microorganisms primarily occurs by ascending teats canal, usually involving agents from animals and environmental origin and from milking process (Anderson *et al*., 2005). *Staphylococcus aureus* is recognized as the most common causal agent of goat mastitis. *E. coli* and *C. perfringens* are considered as the minor occurrences in mammary infections of small ruminants, although they have been associated to serious mastitis cases (Radostits *et al.,* 2007, Ribeiro *et al.,* 2007). An unusual case of mastitis in goat as presented.

History and clinical observations

A 5 year old goat was brought to the veterinary hospital with the history of swelling of quarter and bloody milk. Clinical examination revealed that the left quarter was abnormally swollen. Milk was curdled and red in colour. Temperature,

pulse and respirations were within the normal range. No prior treatment was given. On the basis of history and clinical observations it was diagnosed as a case of mastitis with haemogalactia.

Treatment

The animal was administered Inj. Intacef Tazo 500 mg i/m for 5 days, Inj. Vetalgin 2 ml i/m for 5 days, Inj. Pail C 1 ml i/m for 5 days, Inj. Botropase 1 ml i/m for 3 days, Inj. Tribivet 2 ml i/m for 5 days

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and Inj. Avilin vet 1 ml i/m for 5 days. The affected quarter was flushed with diluted Povidone Iodine 20 ml (1:10 dilution) in normal saline. The procedure was repeated for 3 days. Improvement was observed on second day. Swelling reduced slightly and the milk took the normal colour. Inj. Botropase was discontinued from third day and other treatment were repeated. Animal recovered completely after 5 days of treatment. No relapse was observed to the next 30 days. The treatment given aimed to diminished bacterial load in udder, reduce inflammatory changes and increase blood clotting mechanisms.

Discussion

Many studies have been conducted to determine the prevalence of mastitis in sheep and goats. Despite different breeds, management conditions and definitions of what constitutes a case of mastitis, these studies have had similar results in terms of the overall incidence of mastitis and the individual pathogens identified (Eugene White, 2007). Cases of mastitis can be divided into clinical and subclinical categories. Clinical mastitis can be determined by a change in milk secreted, a change in the udder and or a systemic change in the affected animal. Subclinical mastitis cannot be diagnosed by gross examination of the animal, its udder, or the milk secreted and instead requires a determination of the somatic cell count of the milk and/or a milk culture to determine the presence or absence of an intramammary pathogen. Clinical mastitis can be further divided into three categories: per-acute, acute, and chronic (Menzies and Ramanoon, 2001). In cases where the mastitis appears to be localized to the udder one may proceed with treatment by first "milking out" the affected gland followed by infusion of the affected gland with a commercially prepared intramammary infusion product. In cases where the infection is systemic (throughout the body), administration of the intramammary infusion plus parentral antibiotics such as Ceftriaxone, penicillin, ampicillin, erythromycin or tetracycline. Antihistamines, anti-inflammatory agents, or fluid therapy may be required in severe cases (Shearer and Harris, 1992). Major techniques for the control of mastitis are: 1) post-milking teat dipping and 2) therapy at dry off (end of the lactating period) (Shearer and Harris, 1992).

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Knuckling due to Peroneal nerve Injury in a Kid

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(received 28/01/206 - accepted 27/03/2016)

Abstract :

A case of knuckling due to peroneal nerve injury, caused by faulty injections, is reported and discussed.

Key words : Kid, knuckling, peroneal nerve.

Introduction

Knuckling refers to flexion of fetlock joint leading to weight bearing on front of the pastern. Its etiology varies from injury to the spinal cord, the sciatic nerve or its branches, the gastrocnemius muscle or tendon to caprine arthritis encephalitis virus (CAE-virus). Though knuckling is common due to faulty injections, it has scarcely been reported in India. A case of knuckling due to peroneal nerve injury owing to faulty injections is reported and discussed.

History and Investigations

A two and half month old kid was referred at the hospital with the complaint of knuckling for 10 days. The knuckling started on the 2nd day of intramuscular injections (tetracycline, meloxicam, vitamin B complex with liver extract, and enrofloxacin) given in the right hind limb for the treatment of respiratory infection by the local Veterinarian. On failure to treatment the case was referred at the hospital.

Detailed clinical examination revealed knuckling of right hind limb , hyperflexion of right fetlock, overextension of hock and inability to extend digit, soft swelling on medio-lateral side of right thigh, weight bearing on the front of the pastern(

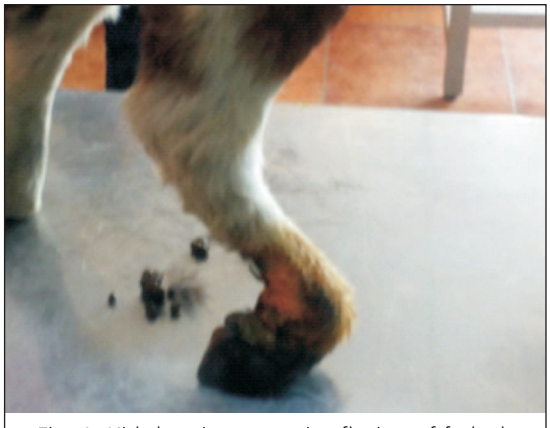

Fig. 1. Kid showing extensive flexion of fetlock and front of the hoof touching the ground

Fig.1) and normal temperature (102.80 F). There were poor skin sensory reflexes on the dorsal aspect of the right foot and cranial surface of the tibia and hock. Exploratory aspiration of the swelling revealed suppurating fluid.

Diagnosis and Treatment

Diagnosis of peroneal nerve injury was made on the basis of history and clinical picture.

The kid was treated with draining of the swelling; prednisolone (1.0 mg/kg PO OD for 3 days),Amoxicillin with clavulanate (12.5 mg/kg

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PO BID for 5 days); intramuscular injection of vitamin B- complex 2.0 ml daily for one week and bandaging the digit and pastern region.

Discussion

Development of unilateral knuckling in the right hind limb of the goat kid on 2nd day of intramuscular administration of tetracycline, enrofloxacin, meloxicam and vitamin-B complex with liver extract aroused suspicion of nerve injury due to faulty injections in gluteal region in the caudal thigh (Matthew, 2009). Young kids appear more prone to such injection injuries owing to small muscle mass. Injury to sciatic, femoral or peroneal nerve due to needle puncture or relatively large volume of irritating medicines near the nerve have been associated with knuckling in animals (Matthew,2009).Out of these Peroneal nerve is most frequently damaged by injection trauma resulting into paralysis of the muscles flexing the hock and extending the digit leading to knuckling at fetlock. Loss of cutaneous sensation on the anterior aspect of metatarsus and digit (Radostits et al.,2006) with dragging of hoof along the ground, observed in the present case, have been described as characteristic features of peroneal nerve injury. Multiple injections in caudal thigh region in kids increases the chance of damage to peroneal nerve (Fox et al.,2002).The peroneal nerve injury was differentiated from sciatic nerve injury(characterized by a loss of function in almost total hind limb, loss of skin sensation on lateral of tibial region, hock and below ,and upward and forward pulling of the leg by contraction of quadriceps muscle with each step- Matthews,2009) and from femoral nerve injury(characterized by dragging of the affected limb while hopping on the unaffected limb; and intact sensation on medial skin surface - Pugh and Baird, 2012). Enrofloxacin and tetracycline are known irritant in nature and might have caused inflammatory swelling near the nerve due to irritation and volume leading to pressure on the nerve. Draining of the swelling provided much sought relief and with the treatment adopted there was much improvement on 7th day. It appears that knuckling was temporary due to pressure on the peroneal nerve owing to volume of the drugs and infection introduced possibly through needle leading to minor injury.

Acknowledgements:

Thanks are due to the Chairman, Managing trustees and Board of trustees for providing necessary facilities at the hospital. .

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Retrieval of Intraluminal Oesophageal Foreign Body in a Buffalo

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(received 18/01/2016 - accepted 17/03/2016)

Abstract

A case of recently parturated buffalo was presented to Dept of Surgery, Veterinary College, Bidar, Karnataka, India with a history of regurgitation of feed and water since 12 hours through the mouth within 2 to 3 minutes after ingestion. Palpation of the left ventral cervical region and passing of a probang per-orally revealed obstruction at the mid-cervical region .Ultrasound and radiography revealed intra-luminal oesophageal obstruction. Mid cervical oesophagotomy was performed with local analgesia which was provided using 2% Lignocaine Hydrochloride. The oesophagus was closed using two layer suture pattern (the Mucosa was sutured using Cushing pattern with Polyglycoic acid No-1 suture followed by the Submucosa, the Muscularis and the Adventatia all together were sutured as a single layer with Lockstitch suture pattern using Polyglycoic acid No-1suture)and the muscles were sutured with simple interrupted suture pattern using Catgut No- 2. The skin was sutured with Nylon No- 2 using simple interrupted pattern. The animal had an uneventful recovery. The present paper reports a case of oesophageal obstruction at mid cervical region due to regurgitated piece of rexin.

Key words: Choke, Mid Cervical oesophagotomy, Rexin, Buffalo.

Introduction

Oesophageal obstruction (choke) is a life threatening, emergency which occurs when the oesophagus is obstructed by food or foreign objects which are too large in comparison to oesophageal lumen (O'Connors, 1965). The obstruction of oesophagus may be partial or complete. Usually the animal suffering from this condition are high yielders which are generally nutritionally deficient and are prone to eating foreign objects (Singh *et al.,* 1993). Shivprakash (2003) observed that oesophageal obstruction was more common in pregnant buffaloes due to high nutritional demand and pica, in addition to the basic nature of indiscriminate feeding habits. Haskell (2008) mentioned that four major oesophageal obstructional location which occurs most common in areas of decreased dilation where the cranial cervical oesophagus, thoracic inlet, base of the heart and cardia of the rumen. (Singh *et al.,* 1993 and Venugopalan, 2000) reported that the sight of oesophageal obstruction in ruminants can be anywhere between pharynx to cardia. Hofmeyr (1974) reported that the most common location of choke was the mid-cervical region. The present article discusses about a case of intra luminal oesophageal obstruction in a buffalo due to rexin material.

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History and Observation

A five years old recently parturated buffalo was presented to Dept. of Surgery and Radiology, Veterinary college, Bidar, Karnataka, India with a complaint of regurgitation of feed and water through the mouth within 2 to 3 minutes after ingestion since 12 hours. Clinical examination revealed frothy salivation. No signs of bloat were noticed, however the animal was slightly restless. The ventral cervical region on the left side was slightly swollen (Fig 1). The physiological parameters were within normal range. Palpation of the left ventral cervical region induced slight cough and presence of a slight hard structure could be felt. The case was tentatively suspected for intra-luminal oesophageal obstruction. Passing of probang (stomach tube) per-orally revealed obstruction at mid-cervical region (Fig 2). Ultrasound examination of the obstruction site revealed a hyper echoic round mass with shadowing below the mass (Fig 3). Further investigation with a survey radiograph of lateral cervical region revealed radioopaque foreign body at 3rd to 4th mid-cervical region (Fig 4). The case was confirmed to be choke and the case was subjected for mid-cervical oesophagotomy. Pre-

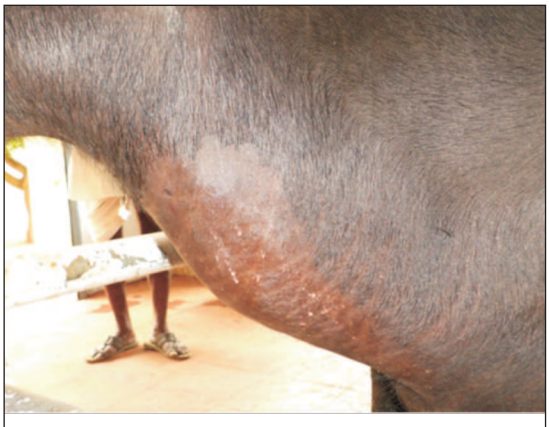

Fig 1: Swollen Left Ventral Cervical Region.

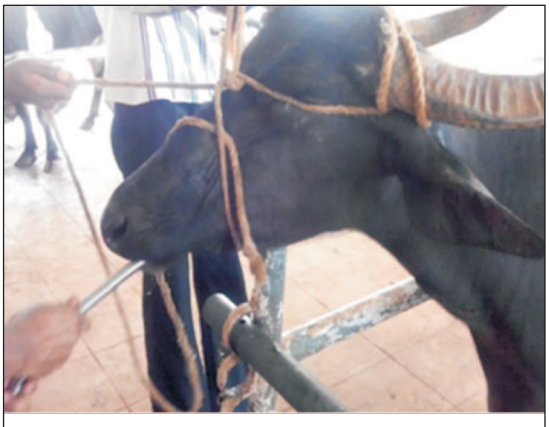

Fig 2: Passage of probang (stomach tube).

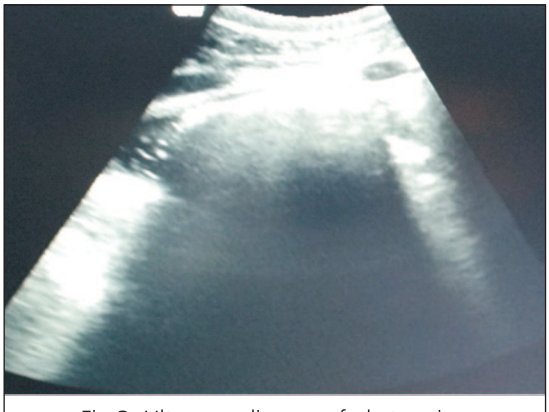

Fig 3: Ultrasoundimage of obstructing Rexin material.

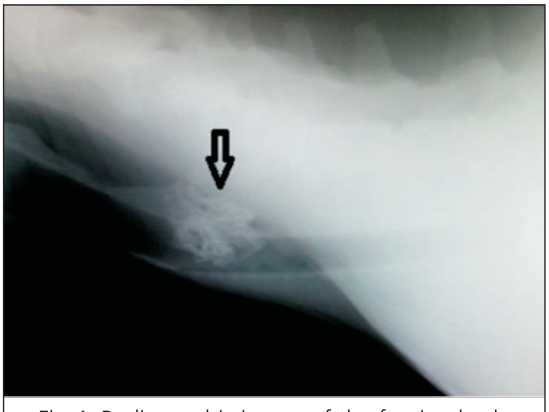

Fig 4: Radiographic image of the foreign body in the oesophagus.

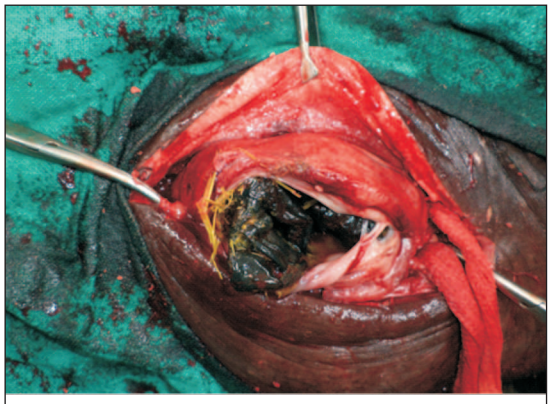

Fig 5: Rexin material in the oesophagus retrieved to the site of incision.

operatively the animal was administered with inj. Strepto- penicillin @ 5g i/m total dose and inj. Tolfenamic acid @ 2 mg/ kg b/w i/m. Three liters of fluid therapy with inj. RL and inj. DNS were administered intravenously to correct the status of dehydration, electrolyte and acid-base imbalances. The animal was restrained in right lateral recumbancy. Local analgesia was achieved using linear infiltration with 2% Lignocaine HCl at the site of obstruction. The site was surgically prepared. A linear incision was made on the skin at the site of the obstructing mass. Sternocephalicus and Sternohyoidous muscles were separated to expose the obstructive mass in the oesophagus. The area adjacent to the oesophagus at the obstructing site was packed with the sterile gauze pieces. A roll bandage was ligated proximally and distally to the site of obstruction in order to prevent any leakage from the saliva and the rumen respectively. An incision was made on the dorsal aspect of the oesophagus cranial to the site of obstruction. An Allis forceps was inserted into the oesophageal lumen and the foreign body was carefully retrieved (Fig 5). The site was adequately lavaged with Normal Saline solution. The oesophagus was closed using two layer pattern (the Mucosa was sutured using Cushing pattern with Polyglycoic acid No-1 the

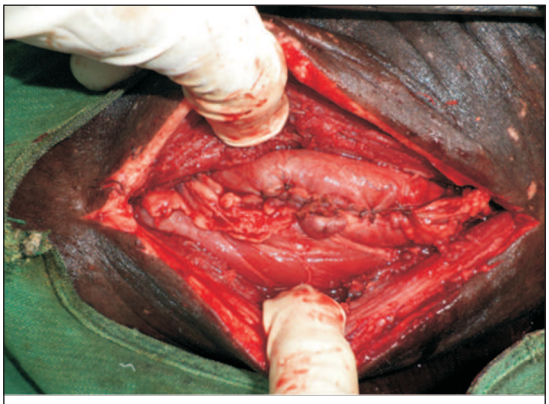

Fig 6: Sutured oesophageal lumen.

Submucosa, the Muscularis and the Adventatia (all together) were sutured as a single layer with Lockstitch suture pattern using Polyglycoic acid No-1) (Fig 6). About 20 ml of Normal Saline solution was injected intraluminally into the sutured site using a fine bore needle to check for leakage from suture site, if any. The proximally and distally ligated roll bandages were removed. The site was adequately lavaged with Normal Saline solution and the muscles were sutured with simple interrupted suture pattern using Catgut No-2. The skin was sutured using Nylon No-2 suture. Postoperatively the animal was kept off feed water for 5 days. Adequate fluid therapy

Fig 7: Rexin material retrieved from the Oesophagotomy.

(using inj. RL and inj. DNS combination) twice a day for five days along with the inj. Strepto-penicillin, inj. Tolfenamic acid and multi vitamin injections as per the standard recommended doses. The animal started to masticate on the second post operative day. The animal was offered drinking water from 5th post-operative day onwards and was gradually shifted to soft liquid diet followed by chopped green fodder. The animal recovered without any complications and regained normal feeding. The incisional wound was dressed until healing and the sutures were removed on the 14th post-operative day. Post operative exploration of the foreign material revealed it to be a rexin material (Fig-7).

Results and Discussion

Cases of oesophageal obstruction may sometimes resolve spontaneously. However each case should be treated as an emergency to decrease the inflammation and to reduce the incidence of further complications. Conservative treatment should be aimed at reducing the oesophageal spasms and to dislodge the obstruction by external or internal manipulations (Haskell, 2008). However, in present case ingested material was rexin which didn't cause any tear of the oesophageal mucosa and its wall. Tyagi and Singh, (2010) suggested that the obstruction in the cervical region can be easily palpated and when in doubt a probang may be passed into the oesophagus to locate the possible site of obstruction. However there was thinning and dilation of the site and change in color of oesophageal tissue. (Nigam et al., 1978 and Singh and Nigam, 1980). Survey radiograph and ultrasonography in the present case was helpful to locate and identify the nature and site of foreign body which was obstructed between the 3rd and 4th cervical region which was successfully retrieved with Allis forceps. Blikslager and Jones(2009)opined that ultrasonography as diagnostic aid would be an useful non-ionizing modality in diagnosing the cases of oesophageal disorders. Thrall,(2007) and Tyagi and Singh (2010), concluded that the contrast oesophagogram is a definitive diagnostic modality in cases of intra luminal cervical oesophageal obstruction. Surgical treatment is required if conservative treatment fails. Similar observations were made in the present study. The major complication of choke occurs when the struck objects presses hard enough against the oesophageal wall to cause necrosis. If the necrosis is severe enough then there will be ulceration and the oesophageal fluid leaks into the cervical and thoracic area. If healing occurs by scarring then a stricture or stenosis may develop, which results in permanently narrowing of oesophageal lumen and is likely to repeatedly cause the same problem (Tyagi and Singh, 2010). Surgery was indicated in the present case as the obstructing material was not smooth edged and has potential to cause further trauma and rupture of the oesophageal lumen. A foreign body can cause a complete or partial obstruction with the obstruction occurring most often at the site of oesophageal distension. (Veeraiah *et al.,* 2003;Haskell, 2008 and Tyagi and Singh, 2010) advised to with hold the feed and water post operatively and to gradually offer liquid, followed by semi solid feed until the healing has occurred. Haskell (2008) opined that the diet associated with oesophageal disorder is dependent on the type of severity of disease present. Choke in ruminants is often noticed due to foreign material such as rexin, towel, cloth material, gunny bags, synthetic leather (Shivprakash *et al.,*1998; Sreenu and Suresh kumar, 2001;Salunke *et al.,* 2003; Shivprakash, 2003; and Shivprakash and Usturge 2004). In the present case the obstructing material was Rexin. The nature and consistency of the material indicated that the obstruction might have not been during ingestion and would have probably been during regurgitation of the foreign body.

Fig 8: The post operative healing of the incisional wound.

The present case healed and recovered without any complications (Fig 8). The stitches were removed on 14th post-operative day.

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Spinal Shock in a Labrador sustaining an accidental neck trauma

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Abstract

Spinal shock was observed in an adult female Labrador immediately after sustaining accidental neck injury. Detailed investigations revealed unconsciousness, flaccid paralysis, intense pain, absence of stretch and flexor reflexes, dyspnoea, feeble pulse, arrhythmic heart and lateral recumbancy; fracture of 1st cervical vertebra injuring spinal cord; and sino-atrial blocks, wandering pace maker, R alternans and increased amplitude of T wave. Therapeutic interventions were futile.

Key words: Cervical fracture, electrocardiographic abnormalities, Labrador, Spinal shock.

Introduction

Traumas are common in humans and dogs either due to vehicle accidents or falls. Spinal shock develops immediately after sudden severe spinal trauma and is manifested by flaccid paralysis and shock. Cardiac abnormalities are well recognized post traumatic complications in humans. A few publications have indicated that cervical injury in dogs has profound effects on body systems involving both sympathetic and parasympathetic divisions of autonomic nervous system (Greenhoot and Mauck, 1972; Snyder *at al.,* 2001). The present report puts on record a case of spinal shock in a Labrador, due to spinal injury caused by fracture of 1st cervical vertebra.

History and Observations

A 21 month old female Labrador, weighing 30 Kg, was referred at the hospital in unconscious condition. The history revealed that the dog fell down and struck with table 4 hours ago.

Detailed clinical examination revealed, lateral recumbency, dyspnoea, groaning, elevated rectal temperature (103.5^{0}F), closed eyes, congested conjunctiva, swelling at the joint of neck and head with intense pain on palpation, mild swelling on nose, feeble pulse, arrhythmic heart, no response to vocal commands, flaccid paralysis of fore and hind limbs, absence of stretch and flexor reflexes and cutaneous sensitivity.

Haemogram (Hb-13.5 g/dl, Total Erythrocytes-7.49 x 10^6/mm^3, paked cell volume - 43%, Total Leucocytes-14.6x10^3/mm^3,Neutrophil-79%, Lymphocytes-14%, Monocytes-5% and Eosinophils-2% , and no blood parasite) was non committal.

Fracture of 1st cervical vertebra (Fig1) was detected on radiological examination. Electrocardiogram (Fig.2) revealed heart rate as 72 bpm with irregular rhythm (R-R interval from 0.44 to 1.2 second,), wandering pace maker (P

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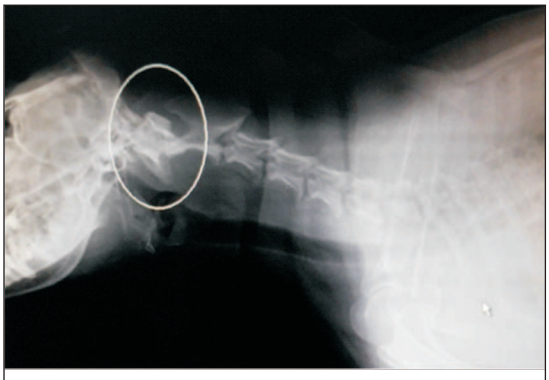

Fig.1. X-ray of an unconscious female Labrador showing fracture of 1st cervical vertebra.

amplitude varying from 0.075 to 0.25 mV, amplitude of P wave of the complex after the pause was low), normal duration of P wave (0.04 sec.), normal P-R segment (0.04 sec.) and P-R interval (0.08 to 0.10 sec.), R wave amplitude varying from 0.8 to 1.2 mV (electrical alternans 2:1 i.e. alternating 'R' showing change in amplitude), normal QRS (0.04 sec.), normal S-T segment (0.08 sec), and an increased amplitude of T wave (0.5-0.6 mV i.e. ¾ th to ½ of 'R' wave).

Diagnosis and Treatment

Diagnosis of spinal shock was based on the history, clinical signs, and radiographic evidence of 1st cervical fracture.

Emergency treatment was started with dexamethasone (2 mg/kg IV), mannitol (2.0 g/kg IV), pantoprazole (30 mg IV),diazepam (20 mg IV and 10 mg IM), Clindamycin (300 mg IM) , Ringer's lactate (1000ml fluid) with vitamin B complex (2ml) intravenously and neck support.

Discussion

Accidental neck trauma caused fracture of 1st cervical vertebra injuring the spinal cord making the dog unconscious as spinal shock develops immediately after severe injury to spinal cord. Cervical spinal cord injuries are associated with profound effects involving both sympathetic and parasympathetic divisions of the autonomic nervous system (Greenhoot and Mauck,1972). Pain, flaccid paralysis, absence of stretch and flexor reflexes and cutaneous sensitivity, and unconsciousness were consistent with the observations of other workers in cervical trauma (Silverstein and Hopper,2009). Sino-atrial blocks, wandering pace maker, electrical alternans of R wave and increased T wave amplitude (in relation to R waves) were the predominant electrocardio graphic abnormalities in the dog sustained cervical fracture through neck trauma. These changes might be due to parasympathetic stimulation owing to cervical fracture. Serious cardiac rhythm disturbances, S-T and T changes, and elevations of arterial pressure have also been observed in experimental cervical spinal injury in dogs (Greenhoot and Mauck, 2004). Electrical alternans in every other complex originating from same pace maker with normal heart rate, increased T amplitude and SA blocks denoted idiopathic pericardial effusion (Tilley,1986), myocardial hypoxia (Coulter et al. 1975) and irritability of vagus nerve (Tilley, 1985) respectively. Treatment was futile as the dog collapsed next day.

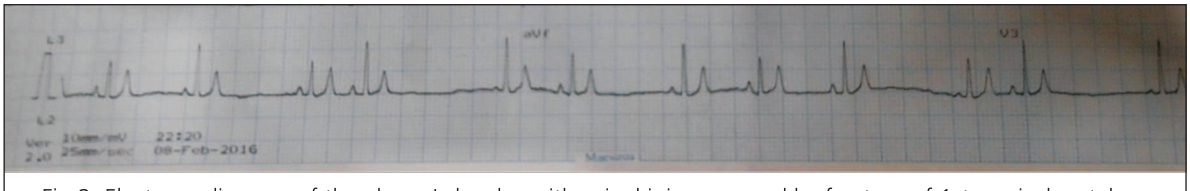

Fig.2. Electrocardiogram of the above Labrador with spinal injury, caused by fracture of 1st cervical vertebra, showing wandering pace maker, R- alternans ,Sino-atrial block and increase in T amplitude(relative to R).

Acknowledgements

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Clinical Management of Inguinal Hernia in a Dachshund pup

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Abstract

A 4 month old Dachshund male pup was presented to our TVCC with a pendulous swelling located on the caudal ventral abdomen. Diagnosis of Inguinal hernia(Epiplocele) was confirmed through radiography & ultrasonography. Herniorrhaphy was done under general anaesthesia. Case was followed up for 6 months,there was no incidence of recurrence.

Key words : Inguinal Hernia, Epiplocele, herniorrhaphy.

Introduction

Inguinal hernia refers to a swelling on the caudal abdominal region that occurs as a result of protrusion of abdominal contents through a defect in the inguinal ring. The causes of inguinal hernia can be classified as either congenital or acquired. Congenital inguinal hernias in dogs are rare and often co-exist with the umbilical hernia (Bellenger, 1996). Congenital inguinal hernia develop more often in male dogs than in females, possibly due to delayed narrowing of the inguinal ring as a result of late testicular descent (Waters et al, 1993). The most commonly predisposed breeds of dogs are Basenji, Pekingese, Poodle, Basset hound, Cairn terrier, Chihuahua, Cocker spaniel, Dachshund, Pomeranian, Maltese and West highland terrier (Hayes, 1974). Acquired inguinal hernias are relatively common in dogs and most often involve the middle aged intact bitches (Waters et al, 1993) and are mostly due to trauma that weakens the abdominal musculature resulting in abnormality of the inguinal ring. Clinical signs often reflect the size of the hernia and the hernial contents and range from a painless inguinal mass to signs related to incarcerated or nonviable small intestine (Alireza et al, 2009). Diagnosis of inguinal 3 hernia is accomplished by radiography and ultrasonography (Abdin and Ramadan, 2001). This report describes the diagnostic and the surgical treatment of inguinal hernia in a Dachshund pup.

Case history and Clinical observations

A 4 month old male Dachshund breed dog weighting 6 Kgs, was presented with a pendulous semi-circular and unilateral swelling on the left caudoventral abdominal region that had progressively increased in size for 2 months. Clinical examination revealed that the patient was emaciated, depressed and dehydrated. rectal temperature, femoral pulse and respiratory rate were 39^{0}C, 108 beats per minute and 20 breaths per minute respectively. The swelling was painless with a soft, doughy consistency and measured 3cm by 2cm in length and width

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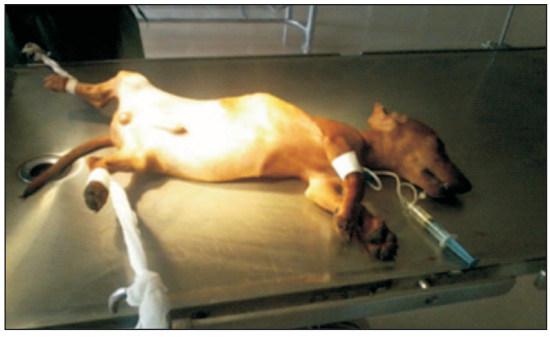
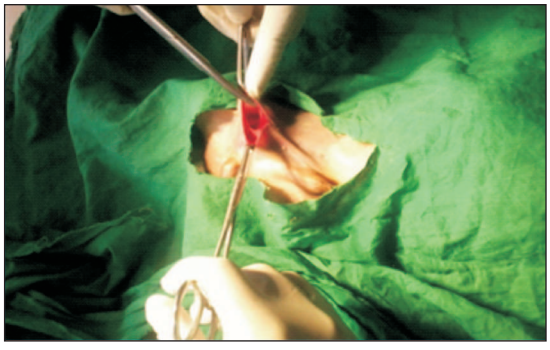
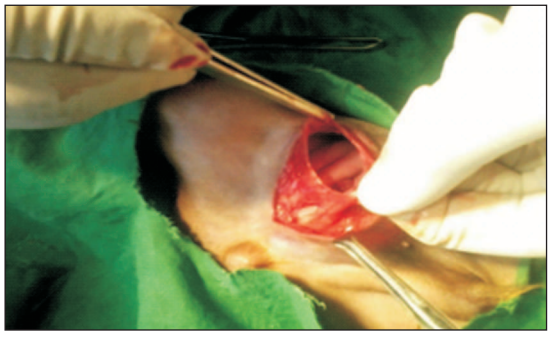
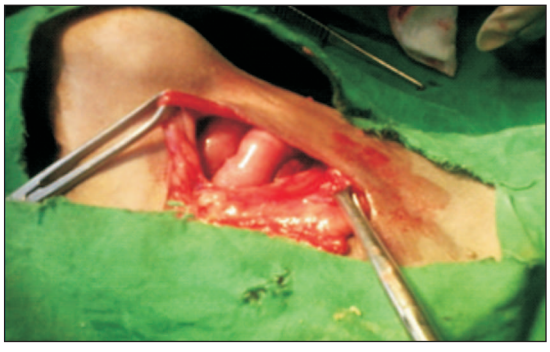
Surgical procedure of inguinal Herniorraphy images









respectively. The skin above the swelling was hyperaemic. The content within the swelling retracted back into the abdominal cavity upon placing the patient on dorsal recumbency and elevation of the hind limbs. Radiograph of the left lateral abdomen, revealed presence of radiolucent gas filled intestinal loops surrounded by radio dense content (fluid, probably due to sequestration from the intestinal loops) in the swelling. Ultrasonography of the swelling revealed intestinal loops in the swelling as indicated by hypoechoic circular areas (indicating intestinal lumen) and acoustic enhancement due to air and fluids in the intestines . Based on these findings, a diagnosis of a left unilateral inguinal hernia was made.

Treatment

Herniorrhaphy was performed under general Anaesthesia. The dog was first sedated using Triflupromazine* at a rate of 1mg/kg administered intramuscularly following which the caudal abdominal region including the swelling was prepared for an aseptic surgery.

General anaesthesia was induced using 1.25% Thiopentone Sodium **60mg administered intravenously to effect and maintained using the same. The patient was positioned for surgery in dorsal recumbency and dilute5% povidone iodine solution applied on the surgical site following which the area was draped.

A ventral incision on hernia mass was made by first making a sharp skin incision using scalpel blade. The linea Alba was tented using thumb holding forceps, a stab incision made using surgical blade and extended using scissors. The hernia sac was then exposed by blunt dissection. The inguinal canal was enlarged to allow

reduction of intestines and omentum into the abdominal cavity. Excessive hernial sac was excised and margins apposed using number 2/0 Prolene A in a simple interrupted pattern. The incision was closed using number 0 VicrylB in cruciate pattern. Excessive skin tissue was trimmed of, dead space reduced and the skin apposed using number 2/0 nylon in a simple interrupted pattern. Ceftriaxone*** at a rate of 25mg/kg and Meloxicam**** at a rate of 0.3mg/kg were administered intramuscularly immediately after surgery. Antibiotic ceftriaxone oral syrup at a rate of 30 mg/kg were administered for 5 days postoperative. The follow up study for two weeks revealed a complete recovery with no complications.

The case was followed up with Telephone call for 6months, there was no incidence of reoccurrence.

A-Polypropylene

B-Polyglactic acid-910

*SIQUIL

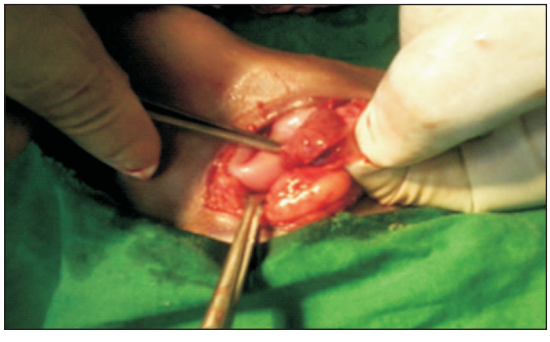
**THIOSOL-500

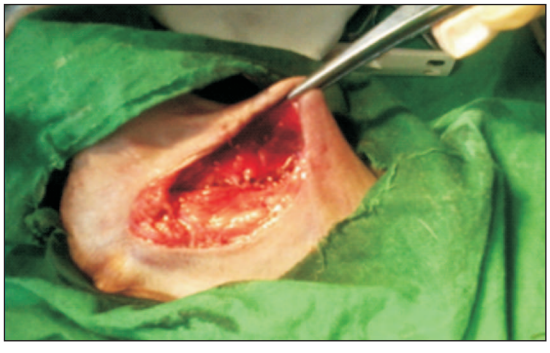
***INTACEF-250 (INTAS PHARMA)

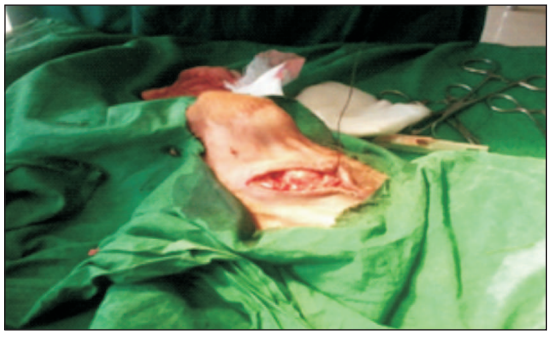
****MELONEX (INTAS PHARMA)

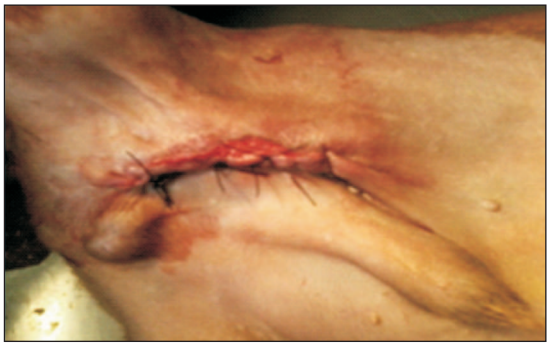
Discussion

In this case, the inguinal hernia was unilateral and contra lateral inguinal ring was not involved as reported by Alireza et al (2009). Diagnosis of inguinal hernia can be achieved using radiography and ultrasonography (Abdin and Ramadan, 2001). In this case, plain radiography and ultrasonography were used and intestinal loops appeared radioluscent in plain radiography and anechoic circular areas in ultrasonography. Intraoperative pneumoperitoneography has









been used as an alternative to surgical exploration in the detection of occult contralateral hernias in children (Harrison et al., 1990).

Surgical management of inguinal hernia consists of identification of the hernia sac, assessment of the viability of the hernia contents, surgical resection of nonviable tissue, herniorrhaphy, and, in some instances, neutering (Alireza et al, 2009). Herniorrhaphy by simple interrupted or mattress suture was safe and quick to correct inguinal hernia without any complications.

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Vaccine for parasitic diseases

Control of parasitic diseases has historically focused on the use of chemotherapy and chemoprophylaxis all over the world. However, this method of control is hindered by the development of drug resistance, high price of drugs, unavailability and growing concern about drug residues. In an attempt to develop commercial vaccines against economically important parasites, researchers have so far focused on identifying target antigens. In the near future the use of vaccination against parasitic diseases of animals in veterinary health services is expected to contribute significantly in promoting livestock productivity.

(Source: Kebede, et al., J VeterinarSci Techno 2016, 7:3)

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Cutaneous Papillomatosis in a Jersey cross bred cow - A Case Report

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Abstract

A case of cutaneous papillomatosis in a jersey cross bred cow was successfully treated by autohaemo therapy.

Key words: Cutaneous papillomatosis, Cow, Autohaemotherapy

Introduction

Bovine papillomatosis is a common viral disease of the skin, mostly of young cattle, manifested as benign tumours or warts, caused by bovine papilloma virus (BPV) that has six serotypes hitherto described (Olson, 1990). The common sites for the development of cutaneous warts are head, eyelids, ears, neck, dewlap, brisket, shoulders and legs, occasionally on the back, para-genital region and along the lower line of abdomen (Miller and West, 1972 and Smith, 1996). These neoplasms most often regress spontaneously. Different methods have been used to treat bovine papillomas. A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (Hunt, 1984; Süveges and Schmidt, 2003).

Case History and Observations

A jersey cross bred cow of about six years was presented to the teaching veterinary clinical complex, college of veterinary science, Hyderabad with a history of diffuse areas of marble sized masses all over the body. On clinical examination, all the physiological parameters were in the normal range. Grossly, pedunculated cauliflower like tumour tissue was composed of hyperplastic epidermis supported by thin, inconspicuous dermal stalks. The lesions were more concentrated in the neck and dewlap. By clinical lesions it was diagnosed as cutaneous papilloma /warts.

Treatment and Discussion

The cow was treated using its own blood. 20ml of venous blood was drawn from the Jugular vein using 18G hypodermic needle in a disposable syringe. 10ml of it was injected subcutaneously in the lateral neck region and 10ml was injected deep intramuscularly in the gluteal region by taking all sterile precautions. The treatment was repeated once in a week for four weeks continuously. Regression of papillomas in the present case occurred about 3 weeks after the beginning of treatment, and within 6 weeks all warts spontaneously disappeared and animal was completely recovered. No recurrence of papillomas has been observed.

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Figure 1 & 2 showing papillomatous growths on dewlap region

Rachel (2011) used a combination of autogenous vaccine, hemotherapy and homeopathy to treat oral papilloma in dogs. Halil *et al.,* (2003) treated bovine papilloma with a combination of autohemotherapy and autogenous vaccine within a period of 1.5-2 months. Vaccines are of some value as a preventive but are of little value in treating cattle that already have lesions (Merck Veterinary Manual, 2011). However in this report, without using any chemical agent, only autohemotherapy was employed to treat cutaneous papillomatosis.

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An outbreak of Avian Spirochaetosis in White Leghorn Chickens

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Abstract

Avian Spirochaetosis or Avian borreliosis is an acute infectious disease of bacterial origin caused by avian spirochaete *Borrelia anserina*. The present investigation reports diagnosis and control of Avian spirochaetosis in an organized poultry farm of White Leghorn chickens at Indore in Madhya Pradesh. The layer chickens were experiencing high fever and loss of body weight. There was 10% mortality in last two consecutive years in the flock of White Leghorn chickens. Close examination of cages of these chickens on removal of nuts and bolts used to fit cages, ticks were found which indicate involvement of tick borne bacterial infection. So, whole blood samples were collected at the farm and transported to laboratory. Two dead chickens were brought to the laboratory for examination. Whole blood samples were used to prepare blood smears on glass slide and stained by Wright's stain. Long spiral shaped organisms similar to *Borrelia anserina* were seen under the microscope. Detection of spirochaete in blood samples and ticks found on affected birds confirmed the outbreak of Avian Spirochaetosis in White Leghorn chickens. Thereafter, to control and minimize losses in the farm, diseased and susceptible chickens were culled from the poultry farm. Cages were disinfected by burning with kerosene to eliminate ticks from the cages before housing healthy chickens. The study reports presence of Avian Spirochaetosis and its successful control by management.

Key words: Avian Spirochaetosis, White Leghorn chicken, Borrelia anserina

Introduction

Avian Spirochaetosis or Avian borreliosis is an acute infectious disease of bacterial origin caused by avian spirochaete *Borrelia anserina. Spirochaetaceae* is one of the five families under the order *Spirochaetales*. The genus *Borrelia* causes tick borne disease in birds, cattle, sheep, deer and horses. Quinn et al. (2011) reported that *Borrelia anserina* infected domestic chickens exhibit fever, weight loss, anaemia and loss of egg production in layers. Natural hosts for Avian Spirochaetosis are chickens, turkey, ducks, geese, pheasants, canaries and other birds while crows, pigeons, sparrows and others are experimentally infected as per Sharma and Adlakha (1996). In India four serotypes of *Borrelia anserina* are reported. In the present investigation observations on an outbreak of Avian Spirochaetosis and its control in an organized poultry farm is reported.

Case history & observations

History : A total of 125 White Leghorn chickens were reared in cages at a commercial poultry

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farm of Indore in Madhya Pradesh in the year of 2012. In the last two consecutive years 10% mortality was observed in affected birds in the summer season. Fever, body weight loss and lethargy were noticed in affected birds.

Sample Collection : Blood samples were received from two affected White Leghorn chickens showing fever due to spirochaetemia. These freshly collected blood samples and two dead White Leghorn chickens were submitted to Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Mhow-453446, India for laboratory and post mortem examinations.

Laboratory Staining and Microscopy : Blood smears were prepared on glass slides and stained with Wright's staining method. Stained blood smears were examined under the oil immersion microscopy.

Pathological Findings : Post mortem examination was conducted on dead chickens as per standard procedure. Pathological changes were noticed and recorded.

Results and Discussion

India ranks 3rd and 5th in egg and poultry meat production, respectively in the world. Avian spirochaetosis is reported from the globe including India. Avian spirochaetosis is found mainly in the areas where the poultry ticks are found in Madhya Pradesh, India (Chauhan and Roy, 2000). In the present investigation, diagnosis and control of Avian spirochaetosis in White Leghorn chickens is reported. There was high fever in the affected chickens and birds appeared nervous. In post mortem examinations enlargement as well as mottling of spleen was observed. Yellowish liver was also noticed. There were necrotic foci on liver and also linear haemorrhages between proventricular glands. On close examination of cages of these poultry birds after disassembling of nuts and bolts of the cages ticks similar to Argas spp. were detected. Zaher *et al.* (1977) have studied *Borrelia anserina* in four species of Argas ticks. The stained blood smears revealed bacteria similar to *Borrelia anserina* (Barbour and Hayes, 1986) having spiral shape and findings are similar to McNeil et al. (1948) and Hougen (1995). Affected birds including rest of the susceptible birds were culled. The premises of the farm were properly cleaned and washed with disinfectants. Any cracks and crevises were identified and filled. Dead chickens and infected materials were disposed of by burying. Cages were disinfected by burning using kerosene before keeping healthy chickens.

Lisboa *et al.* (2009) reported implication of Argas ticks in biological transmission of *Borrelia anserina* to poultry birds naturally as well as experimentally. Burch et al. (2006) reported improvement in egg production (9.8%) and total egg weight (9.7%) and reduction in mortality (8.6%) when compared to untreated birds after tiamulin treatment in chronically affected field birds with avian intestinal spirochaetosis. The White Leghorn chickens in the present investigation did not receive antibiotic treatment before the outbreak. Fever and death in the birds was not established before. In the investigation disease outbreak was contained largely due to better management and basic hygiene practices. However appropriate antibiotics must be chosen after studying sensitivity to antibiotics for the prevalent *Borrelia* spp. in the area. Earlier resistances to antibiotics are observed against *Borrelia* spp. Jackson *et al.* (2007) reported resistance to high levels of erythromycin, spiramycin and the lincosamides by low-passage isolates of *Borrelia* spp. However in the study it was revealed that only elimination and control of ticks from the cages and premises, culling of affected and suspected birds and maintenance

of healthy flock without any specific antibiotic treatment were sufficient to contain borreliosis. Fukugawa *et al.* (1996) reported phylogenetic analysis of *Borrelia* spp. Aslam *et al.* (2012) reported presence of a unique cluster of Borrelia anserina in Pakistan on the basis of sequencing and phylogenetic analysis of flagellin gene fla B. Autoimmunity in case of experimentally induced avian borreliosis has been reported by Nikolov (2008). The present investigation warrants thorough investigations on poultry birds reared in and around the area for isolating Borrelia spp. to understand epidemiology of the Avian Spirochaetosis in poultry birds of Madhya Pradesh.

Acknowledgements

Blood samples from the affected poultry birds were provided by Dr. B.S. Dhodapkar, Retired Veterinary Practitioner at Indore.

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Hydrocephalus Monster as Cause of Dystocia in Mehsani Buffalo

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Abstract

A naturally mated three years old mehsani buffalo heifer in her first parity was presented with the history of straining severally since last 12 hours but failed to parturate. The case was handled at field level by local veterinarian but efforts were ineffective. Per rectal findings and per vaginal examination revealed the bulging of head, no fetal extremities were palpated and lack of space in birth canal. The case was diagnosed to be a case of fetal dystocia due to hydrocephalus monster. Under local anaesthesia caesarean section was performed and dead female hydrocephalic monster was removed. Buffalo was medicated with the parental antibiotics, analgesic and supportive therapy. Buffalo showed uneventful recovery without any post-partum complication.

Keywords: Hydrocephalus monster, Caesarean section, Mehsani buffalo

Introduction

Difficulty in parturition is generally encountered by livestock owners after rupture of water bags. The etiologies involved in raising the difficult parturition may be either maternal (25%) or fetal origin (75%) (Roberts, 1986). Foetal monsters arise from adverse factors affecting the foetus in the early stages of its development. The adverse factors are mostly of genetic origin but may also include physical, chemical and viral factors (Jackson, 2004). A foetal monster usually has severe physical damage that affects its appearance but may not cause its death in the uterus. The various types of monsters and congenital abnormalities in farm animals reported in literature include conjoined twins, Schistosomus reflexus, perosomus elumbis, hydrocephalus, foetal anasarca, foetal ascites and chodroplastic monsters. Hydrocephalus involves a swelling of the cranium due to an accumulation of fluid which may be in the ventricular system or between the brain and the

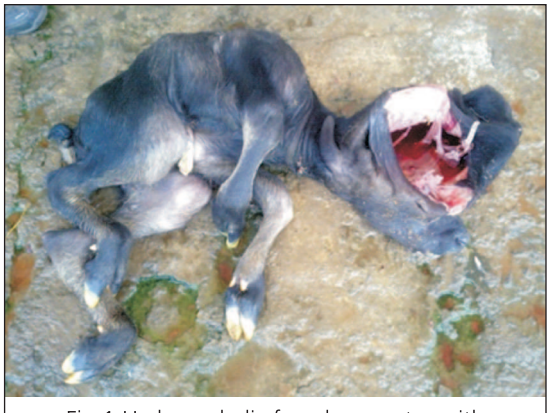

Fig.1 Hydrocephalic female monster with ankylosed limbs.

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dura. It affects all species of animals and is seen most commonly by veterinary obstetricians in pigs, puppies and calves (Arthur *et al.,* 1996). The present communication is based on the rare case of hydrocephalus monster as cause of dystocia.

History

A naturally served full term pregnant three year old mehsani buffalo heifer was presented with complain of straining severely since last 12 hours but failed to parturate. Owner concerned local veterinary but efforts are ineffective then case was referred to veterinary dispensary.

Clinical Observation

At time of arrival of case at clinic buffalo was alert and active with vital parameter, body temperature (102.2^{0}F), Respiration rate (20 per minute), heart rate (58 per minute) were within normal range and congestion of eyes and vaginal mucous membrane. Buffalo was straining severely with vaginal bleeding. The vulva was swollen and lacerated due to handling at field. Per-vaginal Obstetrical examination after proper lubrication revealed that the fetus was in anterior longitudinal presentation, dorso-sacral position. Repulsion and manual manipulation revealed the presence of bulging of the head of fetus with fluid filled and without any reflexes. On the bases of obstetrical examination the case was diagnosed as of Fetal dystocia due to hydrocephalus monster.

Surgical Treatment

The animal was restrained on right lateral recumbency and surgical site on left lower flank region-lateral and parallel to milk vein was aseptically prepared for operation. To achieve local analgesia Infiltration of 2% lignocaine hydrochloride was performed at incision site and twelve inch long incision was given for laparohysterotomy. Laparotomy was performed in standard manner to approach the gravid uterus at above described site. Hysterotomy was performed on grater curvature of gravid uterine horn parallel to the laparotomy incision. Dead hydrocephalic female fetus with ankylosed limbs was removed through laparohysterotomy and manually placenta was removed from uterus. Then uterus was evacuated completely, flushed with normal saline solution and four furea boluses were kept inside. The uterine incision was closed as per standard manner. Then afterwards, peritoneum, muscles and skin were sutured with standard manner. Tr. Benzoine seal was applied at suture line on skin. Post-operative buffalo was treated with Inj. DNS 2 lit. i/v, Inj. NS 2 lit. i/v, Inj. Oxytetracyclin 40ml i/m (Zydus AHL), Inj. Pitocin, 100 IU i/v (Pfizer Ltd), Inj. Calborol 450 ml i/v (Novartis), Inj. Dexal (Geevet, Remedies) 10 ml i/m, Inj. Chrome (Morvel Pvt. Ltd.) 10 ml, Inj. Melonex (Intas Pharmacuetical) 15 ml i/m, Inj. Avilin (MSD, Animal Health) 15 ml i/m.

After the completion of operation buffalo stood up and started drinking water and showing little in-coordination in waking because of time consuming surgery.

Post-operative Care

Owner was advised for giving treatment to animal on prescribed medicines for five days from the next day onwards. Owner was advised to give less quantity of feed and water to animal at regular interval. Owner was also advised to keep the incision site dry and clean. The antiseptic dressing of suture line twice a day was also advised. After twelve days of operation skin sutures were removed and advised to continue dressing with povidon iodine till complete healing of surgical wound.

Result and Discussion

Hydrocephalus is assumed to arise from disturbances in normal circulation of

cerebrospinal fluid resulting from its altered production or absorption (Fride, 1975). A simple autosomal recessive gene and autosomal dominant gene with incomplete penetrance has been known to be associated with hydrocephalus (Roberts, 1986). Hydrocephalus is either external or internal. In the external hydrocephalus, (Vidya Sagar *et al.,* 2010) fluid accumulates in the subarachnoid space exterior to the brain whereas in the internal hydrocephalus, (Balasubramaniam *et al.,* 1961) fluid accumulates in the ventricles of the brain. Death of the fetus is due to pressure on vital centers of the brain. The condition does not affect fetal development but may result in death of the fetus at birth or soon after birth. In the more severe forms of hydrocephalus there is marked thinning of the cranial bones. This facilitates puncture of the head with a trochar and compression of the skull is advocated to relieve dystocia, along with routine obstetric maneuvers. Trocarisation allows vaginal delivery (Salunke *et al.,* 2001). Where this cannot be done, the dome of the cranium may be sawn off with fetotomy wire or a chain saw. In the present case there was none availability of space in birth canal so, it was decided to go for laparohysterotomy which is collaborated with findings of Bugalia *et al.,* (1990). Caesarean section may be when the fetus is presented posteriorly or when hydrocephalus is accompanied by ankylosis of the limb joints (Arthur *et al.,* 1996). Similarly, in the present case there was ankylosis of the limb joints. Buffalo was survived uneventfully and after five month she was diagnosed with 2 month of pregnancy, indicating early diagnosis and timely treatment helps in maintenance of reproductive efficacy and laparohysterotomy had no harmful effects on reproduction.

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Haemonchosis in non-descript Sheep - Case report

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Abstract :

Haemonchosis in an important disease of sheep and Goat, exerts great economic effect in small ruminant farming. A case report of haemonchosis in a non-descript sheep was discussed in this article.

Key words: Haemonchosis, Sheep, *Haemonchus contortus*,

Introduction

Small ruminants play major role in rural economy. They are a source of additional income for small and marginal farmers as well as the rural farmers. Hence, they provide economic stability to these rural families by providing cash income through milk, meat, skin and wool. Like all domesticated livestock, small ruminants are susceptible to various internal parasites. Largest group of parasites that infect small ruminants are gastro intestinal nematodes (GIN).The blood sucking nematode, *Haemonchus contortus,* also known as barber pole worm or red worm, is the most pathogenic among all GINs. Haemonchosis in sheep is characterized by anaemia, hypoproteinemia and digestive disturbances. *Haemonchus contortus* is mostly prevalent in warm and humid seasons post monsoon. It is less prevalent in dry and hot summers in tropics. However a poor nutrition due to poor availability of quality feed and fodder is semiarid tropics a moderate infection of Haemonchosis may prove fatal if not diagnosed and treated. These nematodes colonize in the abomasal mucosa of sheep and feed on blood. The eggs of the parasite pass out through the faeces, hatch in the environment under proper temperature and moisture. These nematodes cause losses in terms of production, decreased weight gain and even mortality in lambs. The nematode is highly prolific and developes anthelmintic resistance against the all known anthelmintics very quickly. Hence we are putting this case on record.

Case history

Non-descript one year old female sheep with history of emaciation, anorexia, lateral recumbancy, pale conjunctiva mucus membranes and dark colour feces was brought for post mortem examination after death. It had

Fig.1 Showing pale conjunctival mucus membrane

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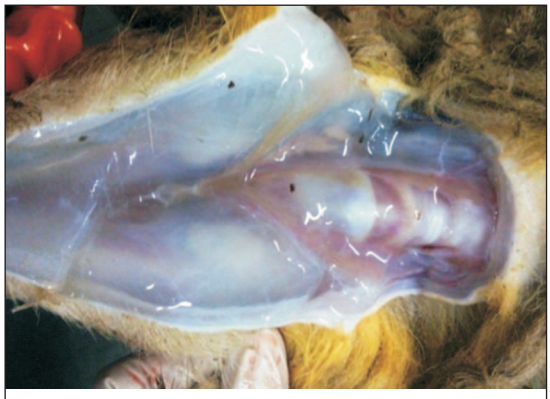

Fig. 2 Showing gelatinization of submandibular fat (Submandibular edema)

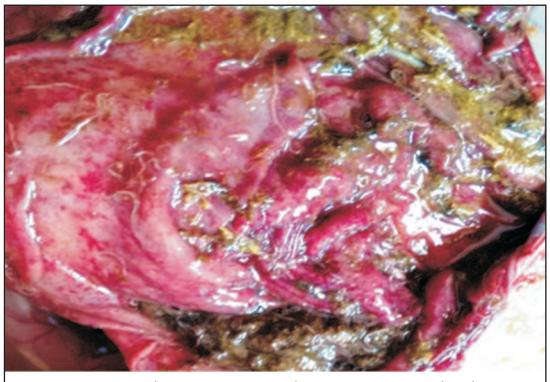

Fig. 3 Abomasum with severe petechial hemorrhages and wire like worms *(Haemonchus contartus)*

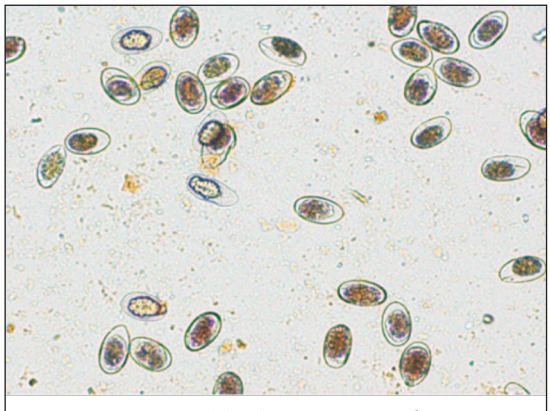

Fig. 4 Slide showing eggs of *Haemonchus contortus*

Fig.5 Slide showing vulval lips of *Haemonchus contortus*

been treated earlier by local veterinarian with suitable antibiotic (Enrofloxacin@ 2.5mg/kg BW) and anthelminthic (Albendazole@7.5 mg/kgBW) drugs. Animal succumbed despite treatment after four days of initiation of therapy. Necropsy was conducted at Post Mortem Examination Facility, Department of Pathology, KNPCVS, Shirwal for detail necropsy examination.

Clinical Observations:

Detailed external and internal examinations were carried out before and after opening the carcass, in order to ascertain the cause of death. Animal was examined carefully externally for any injury and parasite followed by internal organ examination and macroscopic lesions observed were systematically recorded. Faeces were collected in suitable container in formal saline for identification of parasitic eggs. Parasites in the gastrointestinal tract were collected in normal saline and transferred to 70% ethanol with 5% glycerine for identification of parasite for specific species identification.

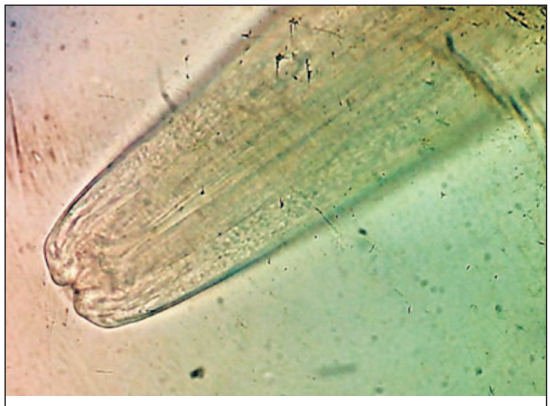

Fig. 6 Anterior end with lancet (black arrow) of *Haemonchus contortus*

Detail Necropsy findings and discussion

The musculature and visible mucous membranes of the carcass were pale in colour with hide bound condition at external examination. Internal examination showed gelatinization of peritoneal and epicardial fat. The quantity and quality of subcutaneous and peri-renal fat were poor. The prescapular lymph node was pale, edematous and gelatinization of fat around the lymph node was observed. The thoracic cavity had approximately 500 ml of fluid and abdominal cavity was filled with 750 ml straw yellow colour fluid. Lungs appeared pale and emphysematous. Abomasal mucosa was severely congested and showed numerous pin point petechial haemorrages. Numerous minute wire like motile nematodes were present in the abomasum. Abomasal content was watery and severe congestion and petechiae of abomasal mucosa were observed. Small intestinal had watery content, mucosa was congested and there was pinpoint to ecchymotic hemorrhages. Mesenteric lymph node was enlarged, edematous and pale in color. Caecal mucosa was congested; pin-point hemorrhages and few white coloured whip worms were attached with mucosa. Liver was swollen with rounded borders with severe congestion. Fecal examination was carried out following standard procedures for identification of eggs and it was identified to be of *Haemonchus contortus* depending on its morphological characteristics. Moreover, live parasites collected during postmortem were identified as *Haemonchus contortus* and *Trichurisovis* (Soulsby, 1982).

Haemonchus contartusis mainly involved in direct sucking of blood; each parasite can suck 0.05ml blood per day (Ijaz et al., 2009). Loss of blood due to blood sucking activity of the parasite along with losses due to seepage of plasma proteins from haemorrhagic lesions, increased permeability of abomasal mucosa because of inflammatory changes (haemorrhagic abomasitis) and improper digestion is responsible for anaemia. Similar findings were recorded by Kelkele et al. (2012).Abomasal digestion of proteins is due to enzyme pepsin which is secreted as precursor pepsinogen. Acidic pH of abomasum maintained by secretion of hydrochloric acid by the parietal cells is responsible for conversion of pepsinogen to pepsin. Haemonchosis is responsible for mucous cell metaplasia and hyperplasia where in the parietal cells are replaced by mucous secreting cells. This causes achlorhydria. Resultant increased abomasal pH makes the protein digestion difficult because of insuuficient quantity of pepsin (Bakers et al., 2007). This result is nutritional loss of proteins present in feed. The conditions of haemonchosis are accentuated if there is poor nutrition. Sheep on a plane of nutrition low in protein and poor iron reserves are likely to suffer more than their well-nourished flock mates. Hypoproteinemia is responsible for the accumulation of serous fluid in various body cavities which was the finding in the present study is in accordance with Swaminathan et al.,(2015).Congestion and petichae in intestinal mucosa was also reported by Teharani et al. (2012). Such observations may be due high amount of unutilized proteins passing from abomasum into small intestine

which are substrate for bacterial overgrowth. Such alterations Subsequently it leads to dehydration and hypovolemic shock (Kelkele et al., 2012). In the present case though animal was treated with the broad spectrum antihelminthic, it didnot respond to the treatment. It indicates the presence of resistance against antihelminthic, earliar similar reports are there indicating atihelminthic resistance (Mortensen et al., 2003)and it is a emmerging problem which need to be adress to prevent economic losses of farmers .

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Your problems ?

Expert's solutions

An expert

Management of Mastitis in Dairy Animals

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Dr. B.K.Bansal has published 90 research publications (18 International and 72 national) and presented 99 papers in different National as well as International conferences. He is having 27 years of professional experiences, guided 13 students directly and as major advisors for 24 students of Veterinary Medicine. He has handled 21 different research projects, received various awards and grants for his role in the field of Veterinary Medicine.

Q1. What is Mastitis? How does it affect the dairy industry?

In simple terms, the mastitis is defined as the inflammation of mammary glands. It occurs in two forms; subclinical and clinical. The average prevalence of subclinical mastitis in India has been found 47% of cows and 33% of buffaloes. The clinical mastitis is prevalent in 6% of dairy cows and buffaloes. Subclinical mastitis though in apparent causes 10-25% loss in milk production, whereas in clinical mastitis there may be total loss of milk. The annual economic losses due to mastitis in India have been estimated to be Rs. 7165 crore. Besides direct economic losses to the farmer, the presence of mastitis causing organisms and antibiotic residues in milk following therapy of mastitis poses a major threat to the consumer health. In mastitis milk desirable components such as lactose, casein, butterfat, solids not fat, calcium and phosphorus decrease while undesirable milk components such as lipase, whey proteins, immunoglobulin, sodium and chloride increase. Also, the high somatic cell count (SCC) in mastitis milk has a lipolytic effect on fat resulting in rancidity of milk and milk products. A Rancid milk with acid degree value > 1 could be detected at $400x10^3$ cells/ml. The milk loss during lactation with the increasing milk SCC is being graded as under:

Milk SCC (Cells/ml)	Milk Loss in lactation
< 300×10^3	Nil
500×10^3	6.0%
1×10^6	10.0%
2×10^6	16.0%
4×10^6	24.5%

Q2. How the mastitis does occur?

The disease is mainly caused by bacterial organisms, which are frequently present in the close vicinity of animals. The causative agents are categorized into two groups; the contagious organisms such as *Staphylococcus aureus* and *Streptococcus agalactiae* which frequently present on the teat and udder skin of animals, and transmitted from one animal to another animal at the time of milking through milking utensils, milker' s hands and cups of milking machine. The other group comprised environmental organisms such as coliforms and *Streptococcus uberis*, which are frequently present in dung, animal bedding, manure, soil, feed stuffs, uterine discharges and urine, etc., may be transmitted to animal at any time, even in-between the milking.

Q3. How the farmer can diagnose/ identify the mastitis quarters?

In its clinical form, disease may be diagnosed well by the classical signs of inflammation and visible alterations in milk consistency, colour and appearance, etc. The changes in levels at which certain components in the mammary secretion are present are commonly employed in identifying the disease at its subclinical level. A variety of diagnostic tests for mastitis are available which differ markedly with respect to sensitivity, specificity, simplicity, rapidity and cost. Among these, Bromothymol blue (BTB) card, Sodium lauryl sulphate (modified California mastitis test) and Electrical conductivity (EC) tests are simple and economical tests that can be performed as cow side tests at the field level.

Bromothymol blue card test:

It is based on the principle that in mastitis, the pH of milk rises due to entry of bicarbonate salts from the blood into milk. Depending upon the health status of the quarter and hence pH, the colour of the dye changes from yellow (normal) to greenish yellow (+), green (++) and blue (+++) when a drop of quarter milk is placed on the card. But, this test has comparatively less sensitivity.

Sodium Lauryl Sulphate (SLS) test:

It is based on the principle that reagent ruptures somatic cell releasing cellular Nucleic acid (DNA) that results in gel formation, and depending upon the degree of gel formation the reaction is scored as 0, Trace, 1, 2 and 3. Thus, this test gives the indirect estimate of milk somatic cell count. To conduct the test, 2-3 ml of milk sample from each quarter is drawn separately into the cups of the specially designed plastic paddle.

The reagent is added in equal quantity, and the contents are mixed by a rotatory movement of the hand. The reaction is scored while mixing the contents and by tilting the paddle in between. This test could be used with high accuracy for estimation of milk SCC (r = 0.84).

Electrical conductivity test (EC):

The ions in milk conduct electricity, such that any change in the concentration of ions is reflected as a change in conductivity. Dissociated, inorganic salts such as sodium, chloride and potassium are the main contributors to conductivity. The breed of animal markedly influences the ionic composition of milk and so milk EC. The mean EC value for healthy quarters was found much less in buffaloes (3.91 mS/cm) than in crossbred cattle (5.41 mS/cm). In general, absolute value of EC for healthy quarters in HFxSahiwal cows varied from 4.5-to 5.5 mS/cm. Due to this animal to animal variation, the within cow comparison between

quarters (relative conductivity) seems to be the best way to use conductivity measures in identifying mastitis quarter, and a inter quarter difference of more than 15% may be taken as indicative of mastitis.

Q4. What is the line of treatment of mastitis?

In vitro testing of milk samples revealed that the drug sensitivity pattern of mastitis organisms goes on changing from time to time and place to place or farm to farm. So, treatment should be given, preferably based on culture and sensitivity test. In acute or per acute cases, where there is no time for these tests, the therapy may be given based on the past data of herd infection and sensitivity reports. Moreover, it may also be made clear that there is no surety that in vitro sensitivity determination will correlate with the *in vivo* treatment results. For example, enrofloxacin that shows high *in vitro* sensitivity and is pharmacologically considered to distribute well in the udder clinically proved to be less efficacious against staphylococcal mastitis because of its inability to kill intracellular organisms. On the other hand, amino-glycosides (gentamicin and neomycin) that are considered to have poor distribution in the udder, *in vivo* proved very much effective in treatment of clinical mastitis. The organism involved in mastitis also affects the efficacy of treatment. Streptococci respond well, staphylococci less and coliform are difficult to treat due to severe per acute reaction.

For taking specific therapy, clinical mastitis is generally divided into three forms viz., per acute, acute and chronic form.

Per-acute mastitis:

It is generally caused by coliform and it occurs commonly around calving but may develop at any time during lactation. The disease is usually sudden in onset: the cow may appear normal at one milking and at the next milking shows pronounced signs, including anorexia, rise of temperature, depression, shivering and rumen stasis.

Inflammatory signs in the udder may be minimal at this time and swelling may be detectable only after the udder is milked out. Later, the quarter is swollen and hard, the teat may be thickened, oedematous, hot to touch and sensitive. In the early stages, the milk may appear normal or faintly watery. Subsequently, it may be serous and contain tiny particles. In severe cases it may become blood tinged. Recommended therapy includes the following:

- Removal of bacteria, toxins and inflammatory exudates from the mammary gland by frequent milking and even oxytocin injections (20-30 IU I/M) may be given
- Appropriate antibacterial therapy to start with systemic administration that may be later (after 12-24 h) supplemented with suitable intramammary infusion.
- Fluid therapy; dextrose saline solution (10-20 L in the first hour, up to 60 L in severe cases) to restore vital body fluids, dilute toxins and counteract acidosis. Even 5% sodium bicarbonate (150-250 G) with first 3-5 L of fluid may be given.
- Systemic glucocorticoids, Dexamethasone @ 1-3 mg/kg IV or IM once or may be repeated after 8-12 hours.
- Calcium borogluconate 20% @ 500 ml IV to counteract hypocalcaemia induced by endotoxin. Administer with care as such therapy may have damaging effects on the heart in animals that are in shock.
- Aspirin 30 grams P.O. reduces pain and inflammation, and restores appetite
- Antihistaminic drugs and multivitamins

Acute mastitis:

In this form there is no systemic reaction. Primarily changes are observed in milk, which may contain flakes, become watery or thick, and sometimes may

contain blood. The udder may become swollen and hard. The line of treatment includes use of antibacterial drugs plus calcium and multivitamin therapy. The important recommendations in mastitis therapy are (i) Use antibacterial on need for recommended time i.e. at least for 5 days (ii) Use appropriate dose and dosing interval (iii) Stick to the recommended milk withdrawal times. The combination therapy, i.e. intramammary plus parenteral works well in clinical mastitis. The Intramammary route is accepted route of choice for subclinical mastitis, the one caused by streptococci. It is advantageous to combine systemic and local therapy in treating acute clinical non gangrenous *S. aureus* mastitis. Dry cow therapy is reported to be much more successful curing staphylococcal infection than lactation therapy. Depending upon the sensitivity report following combination may be used in treating clinical mastitis.

Option	Parenteral	Intramammary infusion
1	Gentamicin sulphate 3-5 mg/Kg IV or IM twice daily	Gentamicin sulphate 100-150 mg twice daily or commercially available intramammary preparations of neomycin for 3 days
2	Ampicillin sodium 20 mg/Kg IV followed by 10mg/kg IM twice daily	Cephapirin sodium 200 mg at every milking for 3 times or cloxacillin sodium 200 mg at every milking for 6 times
3	Sulfadiazine/ trimethoprim 25 mg/Kg IV or IM once daily	Amoxycillin sodium 62.5 mg or Hetacillin potassium 62.5 mg every milking for 6 times
4	Erythromycin lactobionate or tylosin tartrate 10 mg/Kg IV followed by 5 mg/Kg IM twice daily	Erythromycin 300 mg at every milking for 6 times
5	Cefquinome sulphate 1 mg/Kg IM once daily for 2 days	Cefquinome sulphate 75 mg at every morning and evening after milking for 03 times (total dose per quarter)
6	Ceftiofur up to 1 G IM once daily	Ceftiofur 100 mg
7	Sulfamethazine sodium 100 mg/Kg IV followed by 50 mg/Kg IV	Option 3 or procaine penicillin 1 Lac IU at every milking for 6 times

Chronic mastitis:

A case is considered chronic when (i) there is formation of fibrotic cord inside the teat canal (ii) there is a thick pus discharge, not responding to treatment (iii) there is a frequent recurrence of mastitis in the same quarter. The treatment/surgery of chronic mastitis is not rewarding. Rather, such cases should be isolated from the milking herd or the affected quarter may be permanently dried-off by producing a chemical mastitis. Infusing 30-60 ml of 3% silver nitrate solution or 20 ml of 5% copper sulphate solution can do it. If a severe local reaction occurs, the quarter should be milked out and stripped frequently until the reaction subsides. If no reaction occurs, the quarter is stripped out 10-14 days later. Two infusions may be given.

Q5. How we can prevent the occurrence of mastitis at the farm?

Mastitis control is a comprehensive program that includes good milking and environment hygiene, use of properly functioning milking equipment, application of teat dipping, proper identification and treatment of mastitis cows, use of dry therapy and sound nutritional program. The important features of a successful mastitis control program are:

Minimizing the source of infection:

Infection can be prevented by maintaining optimal environmental and milking hygiene, segregation and prompt treatment of clinical mastitis cases, culling of carriers and drying off of chronically infected quarters. The adoption of hygienic measures depends upon the epidemiology of the causative organisms. For example, in case of contagious organisms, which are transmitted from one to another animal through the milking equipment and milker's hands, proper washing of udder, cleanliness of milker's hands/milking machine clusters in between each milking and post-milking teat dipping in germicidal solution will reduce the infection to a great extent. On the other hand, for the organisms that come from the environment, e.g. to prevent coliform mastitis animal environment should be kept clean by frequent removal of dung, proper drainage, and adequate milking and feeding space should be provided.

Elimination of existing udder infections:

It is achieved by dry therapy. The dry therapy is done at the end of lactation (after last milking) with a long acting antibiotic intramammary preparation like Cefalonium long acting maintains effective drug concentration for 6-8 weeks i.e. throughout the dry period. It not only eliminates the subclinical infections of previous lactation, but also prevents new IMI and increases the milk production by about 8-10%. In addition, it improves the milk quality at calving and prevents the occurrence of clinical mastitis cases during dry period and around calving. Studies conducted at this institute revealed dry therapy as very much effective and economical under Indian dairy conditions. Very recently, a dry cow therapy preparation "Cepravin Dry Cow (cephalonium 250 mg in long acting intramammary suspension)" has been introduced in Indian market.

Prevention of new intramammary infections (IMI):

It is achieved by Post milking teat dipping. The teats of all the lactating cows and dry cows (during the first 10-14 days of dry period) are dipped regularly after every milking in a germicidal solution. The recommended teat dips are

Iodine (0.5%) solution + Glycerine @ 15% of iodine solution

Chlorhexidine (0.5%) solution + Glycerine @ 06% of chlorhexidine solution

The iodine teat dip is found best as it also treats various types of teat lesions and injuries.

Increasing the udder resistance to mastitis:

Future trends in mastitis control are aimed at increasing the immunity of the udder to mastitis pathogens. This can be achieved by use of non-specific (cytokines, nutrition) and specific (vaccination) immunomodulators.

Cytokines:

Cytokines include interferons, interleukins, colony stimulating factors (CSF), and a variety of other proteins that modulate the activity of immune cells and thus enhance the

phagocytic cell functions in the udder. It has been shown that interferon treated cells exhibit significantly more phagocytosis and intracellular killing of *Staphylococcus aureus*. Interleukins enhance the production of local antibodies and accelerate the involution process that will further promote resistance to mastitis during the dry period. Similarly, the granulocyte-macrophage CSF significantly increases the chemotactic and bactericidal activities of mammary gland neutrophils.

Nutrition:

Even slightest deficiencies of certain vitamins (Vit E, C, A and ß-carotene) and micro-nutrients (Cu, Se, Zn, Co) are reported to have a detrimental impact on the efficient functioning of the immune system. Vitamin A is involved in maintaining a functional epithelium that provides a physical barrier to the entrance of pathogens. ß-carotene also referred as pro-vitamin A enhances the immune function and disease resistance. Zinc supplementation prevents the infection by strengthening the skin and stratified epithelium (keratinocytes) of the teat canal. The biological role of Cu is exerted through a number of Cu containing proteins, including ceruloplasmin and superoxide dismutase (SOD). These proteins protect the host tissues from membrane oxidation by acting as an antioxidant and scavenging oxygen free radicals produced during the inflammatory response. Similarly, vitamin E and the Se containing enzyme glutathione peroxidase (GSH-pX) also act as integral part of the antioxidant system.

Studies have shown that supplementation of cows during dry period and around calving (first 8-10 weeks) with the following nutrients per head per day proved beneficial in preventing mastitis/ lower milk SCC.

- Vitamin 53000 IU + Beta- carotene 300 mg
- Zinc-methionine (180-360 mg Zn, 360-720 mg methionine)
- Copper @ 20 ppm i.e. about 200 mg
- Vitamin E 1000 IU during dry period and 500 IU for lactating cows
- Selenium is recommended as 3 mg during dry period and 6 mg during lactation

Vaccination:

The effective immunization against mastitis has been a goal of mastitis researchers for many years. But, the nature of disease creates a number of unique challenges for the production of successful immunity against mastitis. Commercially, few mastitis vaccines are currently available in the developed world for immunization against mastitis caused by *Staphylococcus aureus* and *E. coli*. Several studies have evaluated these; the outcomes have been inconsistent and confusing to interpret.

However, it is generally accepted that *S. aureus* vaccine has limited ability to prevent new infections and clinical mastitis cases. The best use of the vaccine is the reduction of chronic infections rather than prevention of new infections. The use of vaccine against coliform mastitis has been considered efficacious even though the rate of intramammary infection is not significantly reduced in vaccinated animals but because they significantly reduced the severity of clinical disease. The farmer may expect a mastitis vaccine to eliminate existing infections, prevent new mastitis cases and reduce the severity of mastitis. While these expectations seem reasonable, it is unlikely that any one vaccine will be able to achieve all of these outcomes. So, we may expect the role of vaccination as one of the components of mastitis control program, but alone its use may not be giving much encouraging results.

News...National...

Global warming may impact milk production: NDDB

Dairy business provides livelihood to 60 million rural households in India and the country continues to be the largest producer of milk in the world, but global warming could result in adversely impacting the overall output in the coming years. Indian dairy scientists estimate that climate change will lead to decline in milk production by over 3 million tonnes (MT) per year by 2020. The projections, shared by the National Dairy Development Board (NDDB) with the agriculture ministry, should be cause for worry considering the growing demand for milk in the country, estimated at 200 MT by 2021-22.

Though milk production has been steadily increasing with 2015-16 recording an output of 160 MT, the impact of rising temperatures, especially on cross-bred cows, will make the task of meeting domestic demand difficult and could eventually lead to a decline in percapita consumption. At a time when the world's major producers, including the US, Brazil and Australia, are importing Indian milch animals to develop heat-resistant species, the government is focusing on indigenous breeds by introducing various schemes through its ambitious National Gokul Mission programs. Up to now, the central government has approved setting up 14 Gokul Grams in different states under the National Gokul Mission

Source : Internet

Government preparing action plan to attract more FDI in dairy sectors

Aiming to double farmers' income in the next five years, the Centre is chalking out a national action plan to attract more foreign investment in the dairy sector. The Department of Animal Husbandry under the aegis of Agriculture Ministry is holding a series of discussion with private players in this regard. The plan is to increase foreign investment in the dairy sector in the next five years from the current level of around Rs 141 crore. Ministry held the first round of discussion on the issue with private players including Amul, Mother Dairy, Paras and others.

At present, FDI is allowed in most aspects of dairy sector, including machines and equipment. Recently, the government relaxed norms for FDI in animal husbandry by allowing research in non-controlled conditions as well. The government, which is aiming to double farmers' income by 2022, wants to supplement farm income by focusing on allied activities of agriculture like dairy, poultry and fishery. It is also focusing on increasing the milk productivity of cows and buffaloes, for which the government has set aside Rs. 104 crore for the current fiscal on breeding program.

Source : Internet

Know the prestigious Institute

ICAR-National Research Centre on Meat, Chengicherla, Boduppal, Hyderabad 500092

The ICAR-National Research Centre on Meat was established during IX plan and functioning from the present location since 2007. This institute has mission to develop modern, organised meat sector through meat production, processing and utilization of technologies. The institute has well established laboratories with research facilities related to meat quality and safety. Over the years, the institute has contributed substantially to the growth and development of meat sector through its research activities on meat quality improvement, meat species identification, traceability in meat value chain, organic meat production, analysis of chemical residues, development of value added and shelf-stable meat products, packaging and enhancement of shelf-life.

Infrastructure

Experimental animal sheds : CPCSEA registered experimental animal rearing facilities for small ruminants (capacity of 40 animals) and poultry (capacity of 200 birds) have been established for conducting research on pre-harvest interventions on meat quality. Currently projects on "Production of designer meat through nutrient supplementation in small ruminants" and MoFPI sponsored project on "Production of selenium enriched functional meat through nutrient supplementation in sheep" are being carried in these facilities.

Experimental abattoir : Model experimental abattoir for sheep/goat is established with facilities for unloading, lairage, stunner, overhead rail, carcass splitter, chiller, deboning hall and cold storage. This facility is being utilized for imparting training on clean meat production to butchers and also to provide refresher training course to veterinary officials working in slaughterhouses, in addition to the experimental activities of the institute.

Products processing plant : NRC on Meat has meat products processing plant for research and development of value added meat products, demonstration to students, faculty and

entrepreneurship training. Range of imported and local meat processing machineries including slicer, mincer, bowl chopper, planetary mixer, vacuum tumbler, multi-needle injector, blade tenderizer, patty making machine, batter applicator, sausage stuffer, smoke chamber, vacuum packaging machine etc. are available in the plant. The centre is providing various technologies for value addition of meat and meat products to entrepreneurs. Various value added meat products were developed at NRC on Meat. The centre is also providing consultancy and bankable project reports for establishment of meat products processing units. A separate Retort Processing unit has been created for the development of thermally processed meat products.

Laboratory facilities

Laboratory for meat animal production for quality and safety : In this section, projects works viz., organic meat production system for sustainable sheep husbandry and promotion of consumer health, production of designer meat through nutrient supplementation in small ruminants and MoFPI sponsored project on production of selenium enriched functional meat through nutrient supplementation in sheep are being carried out. This laboratory is equipped with atomic absorption spectrometer (AAS) with hydride generator for estimation of mineral content in meat and feed and bomb calorimeter for estimation of energy content in different foods and feeds. Apart from these, the lab has facilities for analysis of proximate composition of meat and feed.

Laboratory for meat inspection : This lab is engaged in conducting research projects on various aspect of meat borne infection. Currently APEDA sponsored project work on "Prevalence of zoonotic sarcocystosis in export buffalo meat is being carried out. In addition, awareness on prevention of sarcocysts in buffaloes is also carried out through awareness camps.

Nutrients and chemical residues analytical lab : Gas chromatogram (GC) equipped with ECD, NPD and FID detectors has been employed for estimating pesticides residues in meat samples. Projects entitled "Estimation of pesticide residues in poultry feed and foods and Estimation of pesticide residues in fishes collected from Kolleru Lake" were carried out in this laboratory. Three post graduate students of veterinary college have carried out their research projects in this lab. Officers of "Quality Control Laboratory", Dept. of Animal Husbandry, Andhra Pradesh have under gone 5 days hands on training. Fatty acids profile of meat and meat products also carried out in this lab. A high performance liquid chromatogram (HPLC) with UV, PDA and fluorescence detectors is being utilized to estimate the levels of antibiotic residues in meat samples.

Fresh meat quality lab : This lab is conducting research projects on colour, texture, functional and sensory properties of meat and meat products. Composition analysis and presence of CLA in meat and fat is also being studied in this laboratory. Major facilities in this lab includes texturomter, colourimeter, composition analysis instruments and facilities for chemical analysis of meat and meat products.

Meat products quality evaluation lab: Experiments to enhance the shelf-life of meat and meat products through various natural antioxidants, antimicrobials and packaging mediated strategies being carried out in this laboratory. This lab has developed the vacuum packaging and super-chilling technology for extending the shelf-life of buffalo meat, mutton and chicken without freezing. Studies were also carried out on emu meat quality and product development. The laboratory has also completed contract research project for characterizing new source of antioxidants in meat and meat products. Presently working on "Purification and characterization of important bioactive peptides from slaughterhouse by-products".

Proteomics lab : The proteomics lab is equipped with Flash chromatography, SDS-PAGE, Two-dimensional gel electrophoresis apparatus, 2DE gel scanner and image analyzing software and OFFGEL fractionator for protein enrichment. The laboratory has been working towards understanding the molecular mechanisms influencing buffalo and goat meat colour, proteomics of lipid-protein interactions and use of proteomic tools for identification of species-specific peptide biomarkers. The laboratory has identified the peptide biomarkers for differentiating meat from young and old buffaloes and also from tender and tough muscles. Protein identification and determination of molecular mass through peptide-mass fingerprinting using tandem mass spectrometry was carried out. The laboratory has completed a project funded by Department of Science and Technology, Govt. of India and presently handling Department of Biotechnology, Government of India funded project on "Identification of species-specific peptide biomarkers using high-throughput proteomic approaches".

Meat microbiology lab : This lab carries out research work in the area of microbial quality of meat and meat products. Currently project work entitled "Study on prevalence, characterization and antibiotic resistance of Campylobacter, Salmonella, *E. coli* and *L. monocytogenes* in raw meat and ready to eat meat products" is being carried out. This project is undertaken to create baseline data regarding the prevalence of important food borne pathogens in raw and ready to eat meat products and their antibiotic resistance pattern. The lab is equipped with biosafety cabinet, headspace gas analyzer, incubator shaker and other equipment related to microbial quality analysis.

Meat speciation laboratory : The authentication of meat species is a major concern for economic, religious and health reasons. Adulteration of high priced meat with cheaper meat is the most common fraudulent practice prevalent in meat trade. Moreover, the restriction/ban on slaughter of certain species/class of livestock in various states warrants authentication of species of the meat. Genomics plays an important role in identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including tracking the source of meat (traceability) and meat/fat species identification (adulteration). Projects entitled detection and quantification of animal body fat (tallow)/vegetable fat in milk fat/ghee (MOFPI sponsored project), food testing laboratory - species and sex identification of meat (MOFPI sponsored project) and species identification to check adulteration of cheaper quality meat in meat (FSSAI sponsored project)are being carried out in this lab. The lab is equipped with PCR machine, Real Time PCR machine, Gel documentation system and Agarose gel electrophoresis apparatus.

Pioneer's Profile

Prof. (Dr.) M. L. Madan
Convener, BSS committee for Breeding policy of Bovines In India. NAAS New Delhi.
Member , Planning Board , kamadenu University, Govt. of Gujarat.

Professional Experience

Dr. Motilal Madan, former Chairman of the livestock sub-group of Haryana Farmer's Commission, is a nationally and globally renowned Veterinarian and Animal Biotechnology scientists. He is renowned for his varied and distinguished work in education, research and developmental management/administration, which has had a meaningful impact on individuals, institutions and ecosystems. Dr Madan's significant achievements and outstanding scientific leadership in Science and Technology contributed significantly and impacted National Development He began his professional carrier from Haryana after completing advanced study in the United States and has always been focused on the betterment of people, animals and the environment, nationally and internationally and in ecologically different states in India.

As a professional Veterinarian with a total experience of over 50 years in National Agricultural Research and Development System in India and developing countries. Dr. Madan was involved in; Research, Teaching, Extension, Research Management, (planning, coordination and direction), infrastructure development in the area of Agriculture, Livestock, Veterinary health & production and Agricultural Production Systems; National and international coordination of agricultural research among Ministries, Departments, Universities and Research Institutions in Public/Private Sectors; Research, Teaching, Extension and media reporting in the area Veterinary Science, Natural resource management and Human resource development.

As research investigator, Reproductive Biologist and Biotechnologist he is credited with Scientific advances in the areas of, semen production and preservation, AI, in-vitro fertilization and cloning technologies, neonate physiology, draught evaluation,

veterinary education, human resource development and research and field management of development programs.

As Vice Chancellor and CEO of two large Universities Of Veterinary and Agricultural sciences, initiated comprehensive developmental agenda for the Institutions including taking the academics in the university to high standards and providing an enabling atmosphere for students through a unprecedented infrastructural and academic development.

He initiated and developed National and International Research and Development programs in the country and within ICAR Institutes, Agriculture Universities and Non-governmental organizations and state veterinary and animal husbandry departments. He pursued knowledge and through scientific innovation provided answers to the questions of livestock development in a career that spanned half a century. His work has advanced science, created future leaders and improved livestock production and development in India, as well as in different countries of the world.

As chairman of DBT Task force, Dr Madan managed funding and evaluation of national biotechnology research projects in Animal biotechnology.. He developed a comprehensive policy Document for the breeding Policy for Bovines in India under NAAS and developed and submitted a comprehensive LIVESTOCK Development POLICY report for the State of Haryana as chairman of the Livestock group of Haryana Farmer's Commission.

Outstanding Research Achiements

His outstanding research contribution include developing quick microquantitation techniques of hormone estimations by RIA, ELISA among bovines particularly in buffaloes for the first time, Treatment of anaestrus, Evaluation of draught and measurement of fatigue among work animals, Embryo transfer in cattle and buffaloes, Quick pregnancy detection kit, and production of antisera for several Protein and Steroid hormones. These contributions have resulted not only in the advancement of science but have significantly impacted Livestock production and National Livestock Development.

Dr Madan pioneered Embryo transfer technology among large ruminants. Through ETT he has produced as many as 10 calves from a single elite cow in a year's time, demonstrating this fast multiplication technology for elite animals. He has also produced worlds First In-vitro fertilized (TEST TUBE) Buffalo calf "PRATHAM" and innovated this technology among bovines for which he has received worldwide

scientific acclaim.

These technologies have been of great importance to the Nation for germplasm development and reproduction augmentation among dairy animals particularly buffaloes, and animal energy evaluation of work animals. The technologies developed have also been utilized in the Developing world where Dr. Madan has provided professional consultancies through FAO. The Biotech laboratory and the research team initiated by him have been continuing with the legacy of the technology innovation in producing the first Cloned buffalo calf in India and the world.

Recognitions and awards

Dr. Motilal Madan is recipient of the most prestigious National and International recognition and awards, like B P Pal Award (2006), Bhasin Award (2002), Rafi Ahmed Kidwai Award (1992), Hari Om Award (1990), Ogouri Biotechnology Award from Japan (1995), SAPI Honorific Award (2002), Malika Trivedi Award (1997), D. Sundaresan Award (1989), Nirmalan Memorial Award (1994-95), International Science Pioneer Award (1985), AJAS Purina Award (1999), and Rotary Service Excellence Award (2001). The Indian Association for Advancement of Veterinary Research (IAAVR) has honored Dr.M.L.Madan with Distinguished Veterinarian Award, 'DIVA' for the year 2002, and Indian Society for Reproduction and Fertility with Life Time Achievement Award (2011) for outstanding contributions in the field of reproductive health.

The citation for Bhasin Award recognized the "significant achievements and outstanding scientific leadership of Dr Madan in Science and Technology contributing to a significant impact on National Development". The award was conferred to Dr Madan "for his major research in innovative technologies, which have not only been translated into field application but have greatly helped animal husbandry development agencies in the country". B P Pal Award recognition came to Dr. Madan for singular overall distinguished contribution to Agriculture.

Dr. Madan attracts audiences with great attention and respect. His knowledge, personality, authority, sense of humor and command over language coupled with perfection in professional attire builds an incredible image of the Profession in the contemporary society. He has delivered numerous KEYNOTE lectures on diverse topics and is a leading public speaker.

Dr. Madan has credit of having 473 research publications, which include 235 Original Research Articles in referred journals, 28 Reports and 26 Bulletin/Book chapters.

Dr. Motilal Madan has attended several global professional seminars and interactions at Austria, Australia,

Afghanistan, Brazil, Bulgaria, Canada, China, Egypt, France, Indonesia, Iran, Nepal, Netherlands, New Zealand, Pakistan, Thailand, USA and USSR

and has worked as International consultant for FAO of United Nations.

Besides his scientific and research management and development accomplishments Dr. Madan has played a leading role as an environmentalist. He developed a biodiversity park at Nagpur (MS), having unique collections of fruits including Citrus and Mango, and ornamental plants. A collection of 350 medicinal and aromatic plants was reared at Nagarjuna Park at Akola, Maharashtra. He had also unique distinction of having 100,000 plantations done in a single calendar year under green campaign. The infrastructure developed by him for cutting edge technology under ICAR and agriculture universities has greatly paved way for scientific advancement that led to productivity increase in the livestock sector

Guidelines To Contributors

The contributions to the journal are accepted in the form of review articles, research articles (clinical / field studies), case reports, other information pertaining to animal health and production. The decision of the Editorial Board members will be final regarding acceptance of the article for publication. The manuscript should be typed on one side of the paper with double spacing except for footnotes and references for which single spacing be used. The style of reference citing should be followed as shown below.

The manuscript should be arranged in the following order:

Title:

Name/s of author/s:

Place of work :

Abstract :

Key words :

Introduction :

Material and Methods : (In details)

Results and Discussions :

Summary / Conclusions :

Acknowledgment : (If necessary)

References :

Periodical/s : Surname/s and initial/s of author/s, year of publication in parenthesis, title, abbreviated name of journal (italics), volume number, (Bold), Issue number first and last page number/s.

Books : Name/s of author/s., year of publication in parenthesis, title of the book, edition (Bold), name of publishers (Italics) and place.

Tables and Figures: Tables are to be numbered in Roman numbers (1 II and so on). Each table should have a clear title. Figures should be of good quality and numbered in Arabic numbers (1,2,3 and so on).

Clinical articles and short communications: Not exceeding 3 to 4 typed pages. In case reports, history, observation, tentative and confirmatory diagnosis, line of treatment and follow up on the case should be given. Trade names of drugs should be given in the Material & Methods and their details like composition, manufacturer etc. as a footnote.

We would appreciate if you kindly send us your manuscript (technical article) in Word File through e-mail.

Authors are requested to confirm that the paper has not been published elsewhere and also to indicate details of postal address for communication with STD code, telephone/fax number, mobile & email.

All manuscripts should be mailed to the following address:

E-mail : bluecrossbook@merck.com

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MSD Animal Health,
Intervet India Pvt. Ltd.
Intervet House, 33, Pune-Nagar Road, (Behind Eden Gardens), Pune - 411014, India
Tel. (Direct): +91-20 66294723. Fax: +91-20-66050403,
Mobile : 09890623470.

MSD
Animal Health

The Science of Healthier Animals™

For more information,
Please visit www.msd-animal-health.co.in

NEW INTRODUCTION

Introduces...

The Best Weapon to Improve Fertility –

VM^{all}™ Chelated

Promotes Health Improves Fertility

1st time in India with 6 Chelated Minerals

Zn Cu Co Se Fe Mn

Available in 1 kg & 5 kg

Nutritional Value per Kg:

Vit A	20,00,000 IU
Vit D_3	2,00,000 IU
Vit E 50%	3,000 IU
Vit B_3 (Niacin)	1,000 mg
Calcium	230 g
Phosphorus	115 g
Zinc	9,600 mg
Magnesium	6,000 mg
Copper	4,500 mg
Manganese	3,900 mg
Iron	1,500 mg
Iodine	500 mg
Cobalt	200 mg
Selenium	20 mg

Direction for use

After calving : from day 5 to day 60

Feed VM^{all} Chelated 25g to 50g/day/cow

Or mix 100g VM^{all} Chelated per 10kg feed

NEW INTRODUCTION

Global Leading Brand in Dry Cow Therapy

Cepravin®

Dry cow mastitis protection

Broad Spectrum — Action against all major mastitogens includes *Staphylococcus aureus , E.coli, Streptococcus uberis , Streptococcus dysgalactiae*

Long Acting for +/- 60 days — Cepravin' s long acting formula treats the existing infection and prevents new infection throughout the dry period

- Significantly lowers somatic cell count into following lactation
- The Ideal choice for dry cow therapy

Composition:

Each syringe contains 250 mg Cefalonium dihydrate as active ingredient

Indication:

For routine dry cow therapy to treat existing sub-clinical infections

Prevent new infections during dry period

Dosage:

One syringe should be infused into the teat canal of each quarter immediately after the last milking of lactation

Withdrawal period:

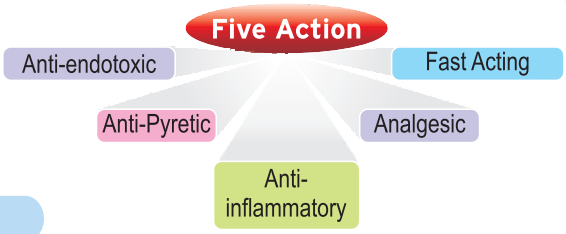
Milk : 54 days after last treatment 96 hours after calving.

Meat & offals : Zero days

MSD Animal Health

NEW INTRODUCTION

Finadyne™



- Fast acting, most potent NSAID
- Manages endotoxemia and inflammation

Composition :

Each ml contains:

Flunixin Meglumine IP	83 mg
Equivalent to Flunixin	50mg

Indications:

In Cattle, Sheep, Goat, Camel - for the control of inflammation and pyrexia associated with mastitis, respiratory disease and metritis

In Horse: For the alleviation of inflammation and pain associated with musculo-skeletal disorders

In Dogs: For use to alleviate Fever, Inflammation, endotoxemia or Sepsis

Withdrawal period:

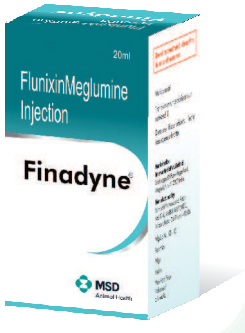
Cattle - Milk: 24 hours after last treatment
Meat : 5 days from the last treatment Horse
Horse - Meat : 7 days from last treatment Pigs
Pig - Meat : 22 days from last treatment

Dose and Administration:

Cattle, Sheep, Goat and Camel: 1.1 mg to 2.2 mg Flunixin per kg body weight or 1 to 2 ml of Finadyne® injection per 45 kg body weight given by slow intravenous or intramuscular administration.

Horses: by slow intravenous injection for Musculo-skeletal disorder at rate of 1ml per 45 kg bodyweight (1.1 mg Flunixin/kg) once daily for up to 5 days

Dog: by intramuscular or slow intravenous at dose rate of 0.5-1 mg/kg body weight as a single dose or if necessary once a day for not more than 3 days.



NEW INTRODUCTION

CHIKVIT Liquid (VET)

COMPOSITION

Consists of Vitamin A, Vitamin B complex and Vitamin D along with Essential Trace minerals. It also contains sorbitol as an instant energy source

BENEFITS

Helps in releiving the stress during transport

DOSE & ROUTE

Regular Supplementation 0.5ml/lt for 3 to 7 days through drinking water
In stress condition
1 ml/lt through drinking water

PRESENTATION

1 lt

RECENT INTRODUCTION

KNZ™

Globally accepted scientific way to provide salt

Free choice salt and mineral licks

UNIVERSAL MULTI
Daily support

Ensures a daily balanced intake - with iron

Available in 4 x 5 kg lick

Component	Value
Sodium chloride	> 99%
Magnesium	2000 mg/kg
Zinc	810 mg/kg
Iron	3000 mg/kg
Iodine	50 mg/kg

FERTILITY
Stimulates fertility

Stimulates fertility with higher level of selenium, iodine and vitamin E-plus yeast selenium for higher effectiveness

Available in 4 x 5 kg lick

Component	Value
Sodium chloride	> 99%
Magnesium	2000 mg/kg
Selenium	23 mg/kg
Selenium as yeast	2 mg/kg
Vitamin E	1000 IU
Iodine	300 mg/kg

One 5 kg lick may be consumed by one animal in approximately 6 months.
(However the consumption depends on more than one factor).

Transmix™

- Eases the calving stress
- Improve immunity and waning the chances of retained placenta and metritis
- Optimises milk production

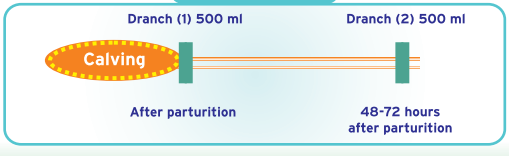
Instant & Sustained

Nutrients supplementation for maximizing profits in transition period

Precaution :

Take necessary precaution to avoid accidental entry into Trachea, Lungs & contact nearest veterinarian if animal exhibits any signs of discomfort

After calving



Recommendation

- Ketosis
- Negative energy balance
- Hypocalcemia

Floxidin™ LA (Vet)

(®Enrofloxacin 10%)

First Line Single Shot Therapy

- Broad spectrum action against gram positive and gram negative bacteria
- Antibiotic property remains for 48-78 hours.

Presentation: 50 ml

WITHDRAWAL PERIOD :
Milk : 84 hrs.
Meat : 14 days

Indications

- **Systemic Infections** - Mastitis, Metritis, Pneumonia, Gastro-intestinal infections
- **Soft Tissue infections** - Wounds, Post Surgical recovery, supportive treatment in cases of FMD

Dose of Floxidin™ LA (VET)

Body wt(Kg)	Floxidin™ LA (Ml)
30	3
50	5
100	10
200	20
300	30
400	40
500	50

At the dose rate of 1ml/ 10 Kg BW

HORMONES

Receptal® VET.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Buserelin acetate 0.0042 mg equivalent to 0.004 mg buserelin.	• True anoestrus	5 ml, IM	Vial of 10 ml and 2.5 ml
	• Improvement of conception rate (at the time of AI)	2.5 ml, IM	
	• Ovarian cyst (Follicular), Irregular oestrus, Nymphomania	5 ml, IM	
	• Delayed ovulation & Anovulation	2.5 ml, IM	
	• Improvement of pregnancy rate (11-12 days post AI)	2.5 ml, IM	WITHDRAWAL PERIOD Milk : 0 (Zero) days Meat : 0 (Zero) days
	• Improvement of post partum fertility (10-15 days post-calving)	5ml, IM	

CHORULON®

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each vial contains human Chorionic Gonadotrophin (hCG) 1500 IU as freeze dried pellet of natural glycoprotein human Chorionic Gonadotrophin	• Improvement of conception rate (cows/buffaloes)	1500 IU at AI or mating, IM or IV	Box containing 5 vials (1500 IU each) with 5 vials of solvent
	• Enhancement of luteal function post AI	1500 IU, 4-6 days post AI, IM	
	• Cystic Ovarian Disease (anoestrus, prolonged estrus, nymphomania)	3000 IU, IV	
	• Induction of ovulation (mares)	1500-3000 IU, IM or IV, 24 hours before AI/mating	WITHDRAWAL PERIOD Milk : 0 (Zero) days Meat : 0 (Zero) days

FOLLIGON®

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each vial contains Pregnant Mare Serum Gonadotrophin injection (Freeze dried) 1000 IU	Females:	Cow/Buffalo	Box containing 5 vials (1000 IU each) with 5 vials of solvent
	• Anoestrus	Anoestrus : 500 - 1000 IU IM	
	• Super ovulation	Super ovulation: 1,500-3,000 IU, IM between day 8-13 of cycle	
	• Increase of fertility rate after progestagen pre-treatment	300-750 IU, IM, at the end of a progestagen treatment	WITHDRAWAL PERIOD Milk : 0 (Zero) days Meat : 0 (Zero) days

Making calf a year a reality

Oestrus Management in Dairy Cattle

Goal: One Calf per Cow per Year

Receptal® 2.5 ml/5 ml

Estrumate™ 2 ml

Receptal® 2.5 ml

Fixed time AI

7 days | 48 h | 16-24 h

Suggested times

Day 0 At any time

Day 7 4 PM

Day 9 4 PM

Day 10 8 AM to 3 PM

Synchronization of oestrus

Synchronization of ovulation

ANTI-INFECTIVE

COBACTAN® 2.5% ACHIEVE MORE

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml of suspension contains 29.64 mg Cefquinome Sulphate (equivalent to 25 mg Cefquinome).	Cattle • Respiratory disease caused by *Pasteurella multocida* and *Mannheimia haemolytica* • Digital dermatitis, infectious bulbar necrosis and acute interdigital necrobacillosis (foul in the foot) • Mastitis Calf • *E. coli* septicaemia	1 mg cefquinome/kg bw MI (2ml/50 kg bw) 1 mg cefquinome/kg bw MI (2ml/50 kg bw) 1 mg cefquinome/kg bw MI (2ml/50 kg bw) 2 mg cefquinome/kg bw MI (4ml/50 kg bw)	50 ml multidose vial. WITHDRAWAL PERIOD Cattle : Meat : 5 days, Pig : Meat : 3 days Milk : 1 day

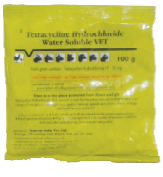
COBACTAN® LC ACHIEVE MORE

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each syringe of 8 gm contains 75 mg Cefquinome sulphate as active ingredient.	For the treatment of clinical mastitis in lactating cows caused by *Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli* & other entero-bacteria susceptible to cefquinome.	Gently infuse the contents of one syringe into the teat canal of the infected quarter every 12 hours after each of 3 successive milkings. Milk out the affected quarter (s). After thoroughly cleaning & disinfecting the teat & teat orifice, gently infuse the contents of one syringe into affected quarter. Disperse the product by gently massaging the teat & udder of the affected animal.	Box of 3 injectors with 3 isopropyl alcohol soaked towels WITHDRAWAL PERIOD Milk : 84 hours (7 milkings) Meat : 2 days

Floxidin™ VET

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Floxidin 10% injection : Each ml contains - Enrofloxacin I.P. 100 mg	- Alimentary canal e.g. Enteritis, calf scours. - Respiratory tract e.g. Pneumonia - Urogenital system e.g. Metritis, cystitis - Skin e.g. Bacterial dermatitis, pyodemra. - Mastitis, & Haemorrhagic Septicaemia.	Floxidin can be given once daily, for 3-5 days. Cattle, Sheep & Goat 2.5-5 mg/kg body weight IM Dog/Cat (adult) 5 mg/kg body weight IM Camel 2.5 mg/kg body weight IM	15 ml, 50 ml Now also available 100 ml WITHDRAWAL PERIOD Milk : 3.5 days Meat : 14 days

Tetracycline WSP VET



COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each gm contains Tetracycline Hydrochloride I.P. 50 mg	In Sheep & Goat : Pneumonia, Joint ill, Anthrax, Septicaemia, Contagious Caprine Pleuro-Pneumonia, Scours, Acute Mastitis, Acute Metritis, In Cattle : Infectious diseases like Haemorrhagic septicaemia, Anthrax, Black Quarter, Leptospirosis, Foot Rot & Contagious Bovine Pleuro-Pneumonia, Calf Scours, Calf Diphtheria, Pneumonia, Septicaemia, Acute Metritis, Acute Mastitis.	Sheep & Goat : 1 gm/kg body weight Cattle : 2.5-5 gm/15kg body weight for 5 days	Sachet of 100 grams WITHDRAWAL PERIOD Milk : 7 days Meat : Cattle-15-22 days, Poultry-5 Days

METRICEF™

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each single dose syringe of 19 g contains: Cephapirine Benzathine intrauterine suspension in pre filled syringe-500 mg	• Subacute/chronic endometritis in cows over 14 days postpartum • Repeat breeders (3 or more unsuccessful inseminations).	Single dose syringe to be administered intra-uterinely	Single dose (19 g) syringe provided with a separate disposable catheter and a glove. WITHDRAWAL PERIOD Meat & offals : 24 hours Milk :0 (Zero) hours

PARASITE CONTROL

butox® Vet

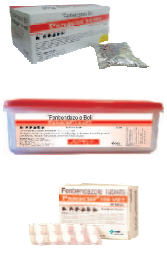
Highly effective & safe ectoparasiticide only for external use.
Ideally suited for control of ticks, mites, lice & flies of livestock, poultry, dogs & farm houses.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Deltamethrin I.P. 12.5mg	To control the ectoparasites in cattle, sheep, goats, horses, camels, dogs & farm houses.	Spray or dip : Ticks : 2 ml/lit Mites : 4 ml/lit Flies : 2 ml/lit Lice : 1 ml/lit	Aluminium container of 5 ml, 15ml, 50 ml, 250 ml and 1 lit with plastic measuring cup WITHDRAWAL PERIOD Milk : 0 (Zero) day Meat : 20 days

Taktic® 12.5% EC

Broad spectrum ectoparasiticide against ticks, mites, lice & keds

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Amitraz I.P. (Vet) 125 mg	1. For prevention & control of ectoparasitic infestation like ticks, mites, lice & keds in cattle, sheep, goat, camel & pig. 2. Taktic kills tick, mite and lice. 3. Taktic kills organochlorine, organophosphate & pyrethroid resistant strains of ectoparasites.	Taktic 12.5%/lit of water for ticks : Cattle/Buffaloes/Camel: 2.0 ml Sheep/Goat : 4.0 ml Pigs : 4.0 ml Taktic 12.5%/L of water for mites and keds : Cattle / Camel : 2.0 ml Sheep/Goat : 4.0 ml Pigs : 4.0 ml	Tin Container of 6 ml, 15 ml, 50 ml & 250 ml with plastic measuring cup. WITHDRAWAL PERIOD : Milk : 4 milking/2 days Meat : 1 day for cattle & goat 7 days for sheep & pig



Panacur® VET

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
The active ingredient of Panacur is Fenbendazole which is the research product of Intervet/Schering-Plough Animal Health. Each 1.5 g Bolus contains 1.5 g of active Fenbendazole. I.P. Each 150 mg tablet contains 150 mg of active Fenbendazole. I.P.	Infestation of cattle, buffaloes, sheep, goat & horses with gastro-intestinal nematodes, lungworms & tapeworms such as *Haemonchus spp., Ostertagia spp., Trichostrongylus spp., Cooperia spp.* and *Nematodirus spp.*	Recommended for cattle, sheep, goat, horses & pigs. Panacur 150 mg tablet per 30 kg body weight & Panacur 1.5 gm bolus per 300 kg body weight (5 mg Fenbendazole per kg body weight). Dose for horses : 7.5mg/kg bw	Box of 1.5x2'-1.5 gm bolus Box of 5 x 2'- 3 gm bolus Box of 5 x 10'- 150 mg tablets. WITHDRAWAL PERIOD Milk : 4 days Meat : 8 days for large animals 14 days for sheep & Goat

Panacur® 25% Wettable powder (vet)

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each gram contains Fenbendazole I.P 250 mg	Infestations of cattle, buffaloes, Sheep & goats with gastro-intestinal nematodes, lungworms & tapeworms such as *Haemonchus spp., Ostertagia spp., Trichostrongylus spp., Cooperia spp., Nematodirus spp., Neoascaris vitulorum, Oesophagostomum spp., Chabertia spp., Bunostomum spp., Gaigeria pachyscelis, Capillaria, Trichuris spp., Strongyloides spp., Dictyocaulus filaria, Dictyocaulus viviparus, Moniezia spp., Infestation of dogs with Ancylostoma spp., Infestation of horses with strongyles, Ascarids, Ascarids (Parascaris), Oxyuris & Strongyloides Infestation of pigs with Hyostrongylus rubidus, Oesophagostomum spp., Ascaris suum, Trichuris suis & Metastrongylus spp.*	Recommended for cattle, sheep, goat & pigs. Infestation with gastrointestinal nematodes & lungworms : (5 mg Fenbendazole per kg body weight) Suspension to be made by mixing clean water as: 6 g with 100 ml 60 g with 1 lit. 120 g with 2 lit.	6 g sachet, 60 g & 120 g container WITHDRAWAL PERIOD Milk : 4 days Meat : 8 days for large animals 14 days for sheep & Goat

Panacur® 2.5% Suspension (VET)

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains 25 mg of Fenbendazole I.P.	Infestation of cattle, buffaloes, sheep & goats with gastrointestinal nematodes lungworms & tape worms such as *Hoemonchus spp., Ostertagia spp., Trichostrongylus spp., Cooperia spp., Nematodyrus spp.,*	Dose recommended for cattle, buffaloes, sheep, goats & pigs' infestation with gastrointestina nematodes & lungworms: (5 mg Fenbendazole per kg body weight)	450 ml and 1 lit HDPE bottle pack of Panacur 2.5% suspension. WITHDRAWAL PERIOD Milk : 4 days Meat : 8 days for large animals 14 days for sheep & Goat

Tolzan® Plus-L

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Oxyclozanide I.P. - 3.4% w/v Levamisole Hydrochloride I.P. - 2.5% w/v	• Tolzan Plus-L treats the round worms and liver flukes in cattle, sheep and goats • Tolzan Plus-L controls adult and immature stages of conical flukes also (Paramphistomum spp.) Tolzan Plus-L can be used safely in pregnant animals during all stages of pregnancy. • Tolzan Plus-L can safely be given to all cattle, sheep and goats • without any pre-dosing, starving or change of diet.	Cattle: 90 ml for 300 kg live mass PO Sheep and goats: 9 ml for 30 kg live mass PO	120 ml HDPE bottle, 1 Ltr can WITHDRAWAL PERIOD Milk : 0 (Zero) days Meat : 14 days

Tolzan® F VET

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml of suspension contains Oxyclozanide I.P suspension of 3.4% w/v	1) Tolzan -F is used in the treatment of acute & chronic Fascioliasis in cattle, buffaloes, sheep & goats. The important species are : a) *Fasciola hepatica* b) *Fasciola gigantica* 2) Tolzan -F is also used to treat paramphistomiasis. The species involved are : *P. microbrothriodes, P. microbrothridium, P. gotal, P. orthocoelium* 3) Tolzan -F also acts on *Monezia* tapeworm in sheep.	Cattle & Buffalo : Orally 10-15 mg/kg body weight Sheep & Goat: Orally 15 mg/kg body weight	90 ml HDPE bottle & 1 ltr jerry can. WITHDRAWAL PERIOD Milk : 0 (Zero) days Meat : 14 days

Berenil® VET 7% RTU

As treatment & control therapy of Babesiosis, Trypanosomiasis and Theileriosis

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Diminazine Aceturate 70 mg Phenazone B. P. 375 mg	Babesiosis & Trypanosomiasis, Tenacious Trypanosomiasis, Theileriosis & mixed infections, Pyrexia of Unknown Origin	Babesiosis and Trypanosomiasis at 5-10 ml per 100 kg b.w. Resistant strains of Trypanosomiasis at 10 ml per 100 kg b.w. Theileriosis & Mixed infections at 5 -10 per ml 100 kg b.w. along with antibiotic (3-4 antibiotic injections on alternate days)	Amber coloured vials of 20 ml, 30 ml and 90 ml WITHDRAWAL PERIOD Milk : 3 days Meat : 20 days

SUPPORTIVES

Tonophosphan® VET

Injectable phosphorus preparation for improving metabolism, milk production & fertility in livestock. Its content of organically bound phosphorus is 20%.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Sodium salt of 4-dimethylamine, 2-methylphenyl-phosphinic acid 0.2 g	As a tonic in general metabolic disorders, debility, exhaustion, repeat breeding & infertility due to phosphorus deficiency. For disorders of bone formation as in rickets & osteomalacia. To promote callus formation in fractures in combination with calcium & vitamin D. For treatment of tetany & paresis resulting from calcium, magnesium & phosphorus imbalance (as in milk fever).	Large Animals : 5-20 ml. Small Animals : 1-3 ml. In chronic conditions- Large Animals : 2.5-5 ml Small Animals : 1-2 ml.	Vial of 10 ml and 30 ml Now also available 100 ml vial

VM^{all}™

CONTENTS PER KG	BENEFITS	DOSAGE	PRESENTATION
Each Kg contains a nutritional value of : Cobalt 120mg, Copper 1000mg, Magnesium 5000mg, Iron 2500mg, Potassium 100mg, Manganese 2000mg, Flourine 60mg, Calcium 150g, Selenium 10mg, Vit A 1200000 IU, Vit D3 120000 IU, Sulphur 0.70%, Vit E 1200 IU, Iodine 300mg, Zinc 5000mg, Phosphorus 60g, Niacinamide 4g, Vit K 200mg, Sodium 8mg.	To improve on fertility. To safeguard health and growth. To optimize milk yield and fat.	Ruminants Mix 100-200 g per 10 kg of feed depending on the availability of other fodder/feed. For direct feeding, Cow and Buffalo: 25-30 g/head/day Calf, Sheep and Goat: 15-20 g/head/day Aqua: Mix 100g to 10 kg of fish feed.	1 kg Zip-Locked pouch with measuring spoon. 5 Kg & 25 Kg bag

VM^{all}™- P

CONTENTS PER KG				BENEFITS	DOSAGE	PRESENTATION
Each KG contains a nutritional value of (When packed):				• To improve on fertility • To safeguard health and growth. • To optimize milk yield and fat.	Ruminants Mix 100-200 g per 10 kg of feed depending on the availability of other fodder/feed. For direct feeding, Cow and Buffalo: 25-30 g/head/day Calf, Sheep and Goat: 15-20 g/head/day Aqua: Mix 100g to 10 kg of fish feed.	25 kg Sealed bag Now also available 5 Kg bag
Cobalt	150 mg	Vit A	1200000 IU			
Copper	2200 mg	Vit D3	120000 IU			
Iodine	325 mg	Vit K	200 mg			
Iron	2500 mg	Vit E	500 IU			
Magnesium	6000 mg	Calcium	225 g			
Manganese	2200 mg	Phosphorus	90 g			
Potassium	100 mg	Niacinamide	1000 mg			
Sodium	8 mg	Biotin 2%	500 mg			
Sulphur	1%	Bioactive				
Zinc	9000 mg	chromium	65 mg			

SUPPORTIVES

Rumicare® (Vet)

Normalises milk production by restoring ruminal activity.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each gm powder contains : Calcium Propionate 480.00 mg Methionine 40.00 mg Picrorhiza Dry Extract 2.00 mg Cobalt Gluconate 0.32 mg Vitamin B_6 IP 0.32 mg Dextrose Anhydrous IP 428.00 mg	Bloat, digestive disorders caused by decreased activity of reticulum & rumen or sudden dietary changes &/ or intoxication. As a supportive therapy in diseases caused by foreign bodies & hypo-glycaemic conditions in cattle, calves, sheep & goats.	Adult Cattle : 125 gm sachet twice daily, (once in 12 hours Young Animals : 65 gm (approx) once or twice daily Sheep & Goat : 32 gm once or twice daily	125 g sachet

Avilin® Vet

For quick relief from allergic manifestations.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains: Pheniramine maleate IP 22.75 mg.	Itching due to eczema, dermatitis, urticaria, skin oedema, insect bites, photo-dermatitis, rhinitis, tail eczema in horses, stomatitis & inflammation of the hooves of cattle, serum sickness, paresis during pregnancy, toxaemia & retention of placenta, pulmonary oedema in cattle, pulmonary emphysema in horses.	Large animals : 5-10 ml. Small animals : 0.5-1 ml. or more. By IM or IV route	Amber coloured vial of Avil 10 ml and 33 ml WITHDRAWAL PERIOD Milk : 2 days Meat : 7 days

Prednisolone Acetate Injection

For quick relief from ketosis.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Prednisolone acetate I.P. 10 mg	Prednisolone is indicated in ketosis in dairy cattle, shock, inflammations (especially rheumatic arthritis, dermatitis, bursitis) and allergic conditions of livestock	Cattle, horses : 5-20 ml. Calves, pigs : 2.5-5ml. Piglets, dogs, cats :1-3 ml. or as recommended by Veterinarian.	Vial of 10 ml WITHDRAWAL PERIOD Milk : 3 days Meat : Cattle Sheep & Goat : 5 days Pig : 28 days

Vetalgin® VET

Highly effective analgesic, antispasmodic, antirheumatic & antipyretic agent.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Analgin I.P. 0.5 g Chlorbutol (as bacteriostat) 0.4% w/v	For relief from pain, fever, labour, spastic condition of cervix during parturition, rheumatic conditions, neuritis, neuralgia, retention of placenta, dysentry, bloat & gastritis in domestic animals.	Preferably intravenous, otherwise intramuscular or combination of IV/IM injection. Horse : 20-60 ml Cattle : 20-40 ml Foal, Calf : 5-15 ml Sheep, Goat : 2-8 ml Pig : 10-30 ml Dog : 1-5 ml	Vial of 33 ml WITHDRAWAL PERIOD Milk : 2 days Meat : Cattle 12 days/Pig 3 days & Horse IV 5 days

RUMINANT BIOLOGICALS

BOVILIS™ Clovax

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 2 ml dose contains inactivated & concentrated FMD antigen of FMD virus serotype O, A, Asia-1, NLT $3PD_{50}$ for each serotype	For the active immunization of cattle, buffalo, sheep and goats against Foot and Mouth Disease.	Cattle, Buffalo & Calves: 2 ml, Sheep & Goat: 1 ml by deep intramuscular route	Vials of 25 doses (50 ml).

BOVILIS™ HSBQ

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 2 ml dose contains formaldehyde inactivated anacultures of pasteurella multocida P52, sufficient antigen to give 4 PU in mice potency as per I.P.	For the prophylaxis against Haemorrhagic septicaemia and Black quarter disease in cattle and buffaloes	2 ml of vaccine per animal by deep intra-muscular route	Vials of 100 ml(50 dose)

BRUCELLA ABORTUS (STRAIN 19) VACCINE LIVE Freeze dried I.P.

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 2 ml dose contains not less than $4x10^{10}$ colony forming units of Live attenuated Brucella abortus strain 19 organisms	For the active immunization of female calves of cattle and buffaloes against Brucella abortus infection	2 ml of reconstituted vaccine per animal by subcutaneous route only	Vials of 5 doses with sterile diluent

BOVILIS™ ET

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 2 ml dose contains inactivated bacterial anacultures of *Clostridium perfringens* Type D, NLT 1500 MLD_{100} per dose.	For active immunization of sheep and goats against Enterotoxaemia type D	Sheep/Goats - 2 ml by subcutaneous injection only.	Vial of 50 doses (100 ml)

Clostridium Perfringens Vaccine Inactivated IP

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 2 ml dose contains inactivated *Clostridium perfringens* Type B 250 MLD_{100} per dose Type C 250 MLD_{100} per dose Type D 1500 MLD_{100} per dose	For active immunization of sheep and goats against Lamb dysentery, struck & Enterotoxamia	2 ml per animal by subcutaneous route	Vials of 25 doses (50 ml).

Ovilis® PPR

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Freeze dried vaccine after reconstitution with diluent Contains Live attenuated PPR virus NLT 2.5 $TCID_{50}$ per single dose (1 ml).	For the active immunization of sheep and goats of 4 months and above age against PPR disease.	1 ml per animal by subcutaneous route.	Vials of 100/50/ 25 doses.

COMPANION ANIMAL

Nobivac®:Puppy DP

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 1 ml dose contains : live infectious canine distemper virus strain Onderstepoort minimum 5.0 $\log_{10}$ $TCID_{50}$ Live infectious canine parvo virus strain 154 minimum 7.0 $\log_{10}$ $TCID_{50}$	Active immunization of dog against CDV and CPV.	Reconstitute one vial of Nobivac Puppy DP in one vial of Nobivac Solvent & inject subcutaneously.	One box contains 10 vials of 1 dose.

Nobivac®:DHPPi

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 0.5 ml dose contains : Live infectious canine distemper virus (CDV) strain Onderstepoort at least 4.0 $\log_{10}$ $TCID_{50}$ Live infectious canine adeno virus type 2 (CAV_2) strain Manhattan LPV_3 at least 4.0 $\log_{10}$ $TCID_{50}$ Live injections canine parvo virus (CPV) strain 154, at least 7.0 $\log_{10}$ $TCID_{50}$ Live injections canine para-influenza virus (CPI) strain cornell at least 5.5 $\log_{10}$ $TCID_{50}$	Vaccination against CDV, CAV2, CPV & CPi. Besides providing protection against CAV2 disease entities such as respiratory tract infections, the vaccine also protects against infectious canine hepatitis (ICH) caused by CAV1.	Reconstitute the contents of one vial of Nobivac DHPPi in one vial of Nobivac Solvent, Nobivac Lepto, Nobivac Rabies or Nobivac RL immediately prior to use & inject subcutaneously.	One box contains 10 vials of 1 dose.

Nobivac®:Lepto

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each dose contains inactivated strains of : Leptospira interrogans serotype Canicola strain Ca-12-000 $\geq$ 957 units/ml and Leptospira interrogans serotype Ictero haemorrhagiae strain 820K $\geq$ 625 units/ml	Active immunisation against Leptospirosis caused by *L.icterohaemorrhagiae* & *L.canicola of Leptospira interrogans.* Animals are protected against clinical disease, & also against becoming renal carriers after challenge.	Inject 1 ml of Nobivac Lepto subcutaneously. Nobivac Lepto can also be used to reconstitute Intervet's freeze dried vaccines Nobivac Puppy DP & Nobivac DHPPi.	One box contains 10 vials of 1 dose

Nobivac®:Rabies

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 1 ml dose contains rabies virus (Pasteur RIVM Strain) inactivated $\geq$ 2 IU	For the active immunisation of healthy dogs, cats, cattle, sheep, goats, horses and in principle all healthy mammals against Rabies & can be used for both (prophylactic immunisation & post bite therapy.	1 ml by subcutaneous or intramuscular injection. Shake well before use.	One box contains 1 ml x 10 vials or one box contains 10 ml x 10 vials

Nobivac®:RL

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each 1 ml dose contains : Rabies virus inactivated antigen suspension $\geq$ 3.0 IU Leptospira interrogans sero group Canicola $\geq$ 40 hamster PD_{80} Leptospira interrogans sero group icterohaemorrhagies $\geq$ 40 hamster PD_{80}	For the active immunisation of dogs against rabies, and canine leptospirosis caused by *L.interrogans* serogroups *canicola* and *icterohaemorrhagiae*.	1 ml by subcutaneous injection. Can be used to reconstitute Nobivac DHPPi. Intended for dogs from 8 weeks of age onwards.	One box contains 1 ml x 10 vials.

Nobivac® KC

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each (0.4 ml) dose Contains Brodetella bronchiseptica strain B-C2 - > 108.0 CFU and canine para influenza virus stain Cornell > 103.0 TCID50	Active immunization of dogs against Kennel Cough.	Nobivac KC aims to make administration as easy as possible: ● Low 0.4 ml dose ● Single nostril only Can be used with or without applicator	One box contains 5 vials of dose and 5 vials of diluent along with one applicator

Taktic® 5% EC

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Amitraz I.P. 50 mg	It is indicated for the topical treatment of Demodectic & Sarcoptic Mange, ticks & lice in dogs.	Mixing Rate / lit of water: Ticks & lice - 6 ml Mites - 10 ml 3-5 applications for mange and 2 applications for ticks & lice at weekly intervals. Taktic to be used as dip or spray	Glass bottle of 25 ml with plastic measuring cup

Taktic® 12.5% EC

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Amitraz I P 125 mg	It is indicated for the topical treatment of Demodectic & sarcoptic Mange, ticks & lice in dogs	Mixing Rate/ lit of water Demodectic Mange – 4 ml Sarcoptic Mange – 2 ml Ticks & Lice – 2 ml In severe cases of infestation a second treatment is recommended 5-10 days after the first.	Glass bottle of 25 ml with plastic measuring cup

San Coat®

NUTRITIONAL VALUE	BENEFITS	DOSAGE	PRESENTATION
Essential Fatty Acids (Linoleic Acid, Alpha Linolenic Acid, Gamma Linolenic Acid, Eicosapentaenoic Acid and Docosahexaenoic Acid) Vitamins (Vitamin A and E, Biotin and Pyridoxine) Zinc and Inositol Omega 6 and Omega 3 fatty acids in 6:1 ratio	San Coat is indicated as an aid in the management of allergic and inflammatory skin conditions like alopecia, dull and dry hair coat, pruritis, atopic dermatitis, *Malassezia pachydermatis*, pyoderma, mange etc. in dogs.	Pour measured dose on food once daily according to the following schedule. 0.3 to 1.0 ml per kg body weight. Under 7 kg - 3.75 ml 7 - 23 kg - 7.5 ml Over 23 kg - 15.0 ml	Container of 150 ml (bettix shape)

DELVOSTERON®

COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains proligestone Injection 100 mg	Suppression & postponement of oestrus in the bitch, treatment of pseudo pregnancy in the bitch, suppression and postponement of oestrus in the queen and suppression and postponement of oestrus in the ferret.	Dogs Body weight – Dosage < 3 kg – 1.0 ml 3-5 kg – 1.0-1.5 ml 5-10 kg – 1.5-2.5 ml 10-20 kg – 2.5-3.5 ml 20-30 kg – 3.5-4.5 ml 30-45 kg – 4.5-5.5 ml 45-60 kg – 5.5-6.0 ml > 60 kg – 1 ml/ 10 kg	20 ml Vials

DERMA STRENGTH™

NUTRITIONAL VALUE		BENEFITS	DOSAGE	PRESENTATION
Active Ingredients per 1 tablet :		• Collagen production • Skin texture • Circulation • Immune system response and circulation • Tissue recovery • Normal histamine levels • Provides support during allergy season	Directions for use or as directed by a veterinarian : Give 1 tablet per 10 kg of body weight daily. If giving more than 1 tablet daily, divide between AM and PM.	30 tablet
Methylsulfonylmethane (MSM)	75 mg			
N, N-Dimethylglycine Hcl (DMG	50 mg			
DL-Methionine	50 mg			
L-Cysteine	50 mg			
Grape Seed (Vitis vinifera) Extract	30 mg			
Ascorbic Acid (Vitamin C)	25 mg			
L-Proline	25 mg			
Perilla (Perilla frutescens) seed Extract	20 mg			
dl-alpha Tocopheryl Acetate (VitaminE)	10 IU			
Zinc (Zinc Citrate)	5 mg			
Hyaluronic Acid (HA)	5 mg			
Niacinamide (Vitamin B3)	4 mg			
Retinyl Acetate (Vitamin A)	37 IU			

CANINE PLUS™

NUTRITIONAL VALUE		BENEFITS	DOSAGE	PRESENTATION
Guaranteed Analysis Represents Minimum Levels per Tablet Unless otherwise Specified :		• Enhances immunity, support bone formation. Blood formation • Nerve formation, skin health, general health, antistress and antioxidant function	Directions for use or as directed by a veterinarian : Under 20 kg : 1 tablet daily Over 20 kg : 2 tablets daily When more than one tablet per day is required, dividing between AM and PM is optional.	30 and 60 tablet presentation
Moisture (max	5.655%			
Methionine	3.75 mg			
Calcium (6.25%)	37.5 mg			
Phosphorus (3.13%)	18.75 mg			
Potassium (0.03%)	0.187 mg			
Magnesium (3.13%)	18.75 mg			
Iron (3750 ppm)	2.25 mg			
Copper (3.33 ppm)	0.002 mg			
Zinc (1250 ppm)	0.75 mg			
Iodine (10 ppm)	0.006 mg			
Selenium (3.33 ppm)	0.002 mg			
Vitamin A	450 IU			
Vitamin D3	37.5 IU			
Vitamin E	3.75 IU			
Thiamine (Vitamin B1)	3.75 mg			
Riboflavin (Vitamin B2)	1.875 mg			
Panthothenic Acid	3.75 mg			
Niacin	3.75 mg			
Vitamin B6	1.875 mg			
Folic Acid	0.001 mg			
Vitamin B12	0.001 mg			
Choline	3.75 mg			
Biotin	0.001 mg			
Ascorbic Acid (Vitamin C)	9.375 mg			
Bromelain (Pineapple)	0.675 GD Units			

BLADDER STRENGTH

NUTRITIONAL VALUE		BENEFITS	DOSAGE	PRESENTATION
Active Ingredients per Tablet :		• Deals with urinne incontinence problems in male and female dogs which is due to less level of estrogen on testosterone. • These dogs are basically geriatric dogs, bitches post sparying , animals with poor anatomical disposition or having urinary tract infection.	Give one tablet per 14 Kg or 30 ponds of body weight. half tablet for animal - less than 30 Ponds of weight If giving more than one tablet, divide between AM and PM	30 tablets presentation
Pumkin Seed Powder	150 mg			
Rehmannia glutinosa (root) Powder	150 mg			
Wild Yam Extract	150 mg			
Soy Protein Extract	100 mg			
Corn Silk Powder	60 mg			
Saw Palmetto Extract	60 mg			
OliveLeaf (15% Oleuropein) Extract	50 mg			
Pyridoxine HCl (Vitamin B6)	25 mg			

CARDIO STRENGTH™

NUTRITIONAL VALUE		BENEFITS	DOSAGE	PRESENTATION
Active Ingredients per Capsule :		• Dogs and cats with pre-existing sub-optimal cardiovascular functions • Breeds of dogs and cats that are predisposed to cardiovascular stress • Support of geriatric patients	Directions for use or as directed by a veterinarian : Cat : Give 1 capsule daily. Dogs : Give 1 capsule, per 10 kg of body weight, daily. If giving more than 1 capsule, divide between AM and PM.	30 and 60 tablet
L-Carnitine HCl	125 mg			
L-Taurine	125 mg			
N, N-Dimethylglycine HCl	25 mg			
d-alpha Tocopheryl Succinate (Vitamin E)	30 IU			
Coenzyme Q10	10 mg			
Folic Acid	0.9 mg			
Magnesium (as Magnesium Citrate)	0.5 mg			
Potassium (as Citrate/Malate)	.01 mg			
Selenium (as Sodium Selenite)	0.007 mg			

GLYCOFLEX®

NUTRITIONAL VALUE		BENEFITS	DOSAGE	PRESENTATION
Active Ingredients per Tablet :		• Glyco FLEX Canine represents our comprehensive support for dogs needing moderate joint support. • These delicious chewable tablets are also recommended for adult and maturing dogs, sporting and working breeds as well as support normal recovery after orthopedic surgery.	Directions for use or as directed by a veterinarian : Up to 15 kg :½ tablet daily 15.5 kg-30 kg : 1 tablet daily 30.5 kg-45 kg : 2 tablet daily 45.5 kg & over : 2 ½ tablets daily If giving more than 1 tablet, divide between AM and PM.	30 and 60 tablet presentation
Glucosamine HCl (Shrimp and Crab)	375 mg			
Pena Canalicus (Glycomega™ brand Green Lipped Mussel)	300 mg			
Methylsulgonylmethane (MSM)	250 mg			
N, N-Dimethylglycine HCl (DMG)	50 mg			
Manganese (as Mn Proteinate)	5 mg			

RENAL ESSENTIALS

NUTRITIONAL VALUE		BENEFITS	DOSAGE	PRESENTATION
Active Ingredients per Tablet :		• Renal circulation • Immune and antioxidant defense system function • Homocysteine balance • Normal fluid retention • Stress management • Kidney and liver function • Normal detoxification	Directions for use or as directed by a veterinarian : Give 1 tablet per 10 kg of body weight, day For dogs less than 7 kg, give 1/2 tablet daily If giving more than 1 tablet, divide between AM and PM.	45 tablets presentation
Astragalus Root Powder	60 mg			
Rehmannia glutinosa Root Extract	50 mg			
Nettle (Urtica dioica) Seed Extract	50 mg			
Cordyceps sinensis Extract	50 mg			
Lecithin	50 mg			
L-Arginine	50 mg			
N, N-Dimethylglycine HCl (DMG)	25 mg			
Potassium (K Gluconate)	8.25 mg			
Inositol	8 mg			
Pyridoxal 5-Phosphate (Vitamin B6)	8 mg			
Thiamine (Vitamin B1)	4 mg			
Riboflavin (Vitamin B2)	4 mg			
Choline	4 mg			
Folic Acid	0.15 mg			
Methylcobalamin (Vitamin B12)	0.05 mg			

POULTRY PRODUCTS

Live Vaccine

Nobilis® Gumboro 228E

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Live Gumboro disease virus strain 228E at least 2.0 log_{10} EID_{50}	The vaccine is recommended for active immunization of chicken against Gumboro Disease (IBD)	One dose per bird through drinking water	1000 ds 2500 ds

Nobilis® Gumboro D78

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Live Gumboro disease virus strain D78 at least 4.0 log_{10} $TCID_{50}$	The vaccine is recommended for active immunization of chicken against Gumboro Disease (IBD)	One dose per bird through drinking water	1000 ds 2500 ds

Nobilis® ND Clone 30

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Live Newcastle Disease strain Clone 30 at least $10^{6.0}$ ELD_{50}	The vaccine is recommended for active immunization of chicken against Newcastle Disease	One dose per bird through drinking water, spray, intranasal/intra ocular	1000 ds 2500 ds 5000 ds

Nobilis® IB H120

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Live Injectious Bronchitis virus strain H120 at least 3.0 log_{10} EID_{50}	The vaccine is recommended for active Immunization of chicken against Infectious Bronchitis	One dose per bird through drinking water, spray, intra-nasal / intra-ocular	1000 ds 2500 ds 5000 ds

Nobilis® MG 6/85

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Live Mycoplasma gallisepticum strain MG 6/85 minimum $10^{6.9}$ CFU	The vaccine is recommended for active immunization of chicken to reduce the clinical signs of Mycoplasma gallisepticum infection.	One dose per bird through intraocular	1000 ds

Cell Associated Vaccine

innovax™ ND-SB1

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each lyophilised ampoule per dose (1 ml) contains : Live Turkey Herpes virus strain HVT/NDV-F at least 1534 PFU/bird Marek's disease virus serotype 2 strain SB-1 at least 1514 PFU per bird dose	The vaccine is recommended for active immunization of chicken against Marek's Disease (MD) and Newcastle Disease (ND)	0.2 ml injection subcutaneously per chick in the neck	2000 ds 4000 ds

Inactivated Vaccine

Nobilis® MG inac

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated Mycoplasma gallisepticum strain MG 6/85 NLT 0.23 units	The vaccine is recommended for active immunization of chicken against infections caused by Mycoplasma gallisepticum.	0.5 ml S/C	500 ml (1000 ds)

Nobilis® E. coli inac

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each 0.5 ml dose contains : F11-antigen Suspension containing 100 µg F11-68.3 mg FT-antigen Suspension containing 100 µg FT-68.3 mg	The vaccine is recommended for passive immunization of broilers against colibacillosis by vaccination of broiler breeders	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® Salenvac T

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each ml contains, Formalin killed cells of Salmonella Enteritidis (phage type 4 strain 109) : 2 x 10^9 cells inducing $\geq$ 2 RP*, Formalin killed cells of Salmonella Typhimurium DT104 : 2 x 10^9 cells inducing $\geq$ 2 RP* (*relative potency)	The vaccine is recommended for active immunization of chickens against S. enteritidis and S. typhimurium and to give passive immunity against these agents in the progeny	0.1 ml for day-old chicks and 0.5 ml for older birds I/M	500 ml (1000 ds)

Nobilis® Newcavac

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each 0.5 ml dose contains: Inactivated ND virus (Clone 30) inducing $\geq$ 4 $\log_2$ HI Unit per 1/50th of a dose or $\geq$ 50 PD_{50} units/dose	The vaccine is recommended for booster vaccination of layers and breeding stock for protection against Newcastle Disease throughout the laying period	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® ND Broiler

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each 0.1 ml dose contains: Inactivated Newcastle Disease virus (Strain Clone 30) cantoning $\geq$ 20 PD_{50} units/dose or inducing $\geq$ 4 $\log_2$ HI Unit per 1/50 dose	The vaccine is recommended for the vaccination of Newcastle Disease in day-old chicks in areas where ND is endemic	0.1 ml S/C or I/M	200 ml (2000 ds)

Nobilis® Corvac

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each 0.5 ml dose contains: Inactivated Avibacterium paragallinarum Strain 083 (serotype A), at least 1 CPD_{70}*, Strain Spross (serotype B), at least 1 CPD_{70}, Strain H-18 (serotype C) at least 1 CPD_{70}. (*CPD_{70} : 70% chicken protective dose)	The vaccine is recommended for protection against Avibacterium paragallinarum infections in chicken	0.5 ml S/C	500 ml (1000 ds)

Nobilis® Coryza

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each 0.25 ml dose conrains : Inactivated Avibacterium paragallinarum Strain 083 (serotype A) at least 1 CPD_{70}, Strain Spross (serotype B) at least 1 CPD_{70}, Strain H-18 (serotype C) at least 1 CPD_{70}	The vaccine is recommended for protection against Avibacterium paragallinarum infections in chicken.	0.25 ml I/M or S/C	250 ml (1000 ds)

Nobilis® Reo inac

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated Reovirus strains 1733 and 2408, inducing $\geq$ 7.4 $\log_2$ ELISA units/dose per 1/50th dose	The vaccine is recommended for booster vaccination of breeding stock against Avian Reovirus to protect their offspring against Avian Reovirus infections	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® G + ND

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated infectious Bursal Disease virus (Strain D78) inducing $\geq$ 14.5 $\log_2$ VN units/dose, Inactivated Newcastle disease virus (Strain Clone 30) inducing $\geq$ 4 $\log_2$ HI units per 1/50th of a dose or containing $\geq$ 50 PD_{50} Units/dose	The vaccine is recommended for booster vaccination of future breeders to protect against Newcastle Disease throughout the laying period, and to induce high maternal antibody levels against infectious Bursal Disease in their offspring.	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® IB + ND

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains: Inactivated Infectious Bronchitis virus (strain M41) inducing $\geq$ 6.0 $\log_2$ HI units/dose, Inactivated Newcastle Disease Virus (Clone 30) inducing 4 $\log_2$ HI units per 1/50th of dose or $\geq$ 50 PD_{50} units/dose	The vaccine is recommended for the booster vaccination of layers and breeding stock for protection against Newcastle Disease and the Massachusetts type of Infectious Bronchitis.	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® IB multi + ND

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated Infectious Bronchitis virus (Strain M41) inducing $\geq$ 4.0 $\log_2$ VN units/dose, IB virus (Strain D249G) inducing $\geq$ 4.0 $\log_2$ VN units/dose, Inactivated Newcastle Disease virus (Strain Clone 30) inducing $\geq$ 4.0 $\log_2$ HI units per 1/50th dose or containing $\geq$ 50 PD_{50} units/dose	The vaccine is recommended for booster vaccination of layers and breeding stock for protection against the Massachusetts and D207/D274 (and related nephropathic) serotype of Infectious Bronchitis and Newcastle Disease.	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® IB + G + ND

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated Injections Bronchitis virus (strain M41) inducing $\geq$ 6.0 $\log_2$ HI units, Inactivated Injections Bursal Disease virus (Strain D78) inducing $\geq$ 14.5 $\log_2$ VN units, Inactivated Newcastle Disease Virus (Strain Clone 30) inducing $\geq$ 4 $\log_2$ HI units per 1/50th of a dose or Containing $\geq$ 50 PD_{50} units/dose	The vaccine is recommended for breeding stock: as a booster vaccination to protect against Newcastle Disease and the Massachusetts serotype of Infectious Bronchitis, and to induce high maternal antibody levels against Infectious Bursal Disease in their offspring	0.5 ml S/C or I/M	500 ml (1000 ds)

Nobilis® Reo + IB + G + ND

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated Injections Bronechitis virus (Strain M41) inducing > 6.0 log_2 HI units Inactivated Injections Bursal Disease virus (strain D78) inducing > 14.5 log_2 VN units Inactivated NDV (Strain Clone 30) > 4 log_2 HI units per 1/50th of dose containing > 50 PD_{50} units/dose Inactivated Reo virus (Strain 1733 & 2308) inducing > 7.4 log_2 ELISA.	For vaccine of Chicken against disease caused by Reo-virus, infectious Bronchitis virus of Massachusetts type Newcastle Disease virus & injections bursal disease virus.	0.5 ml S/C or I/M	500 ml (1000 ds)

Feed Supplement

Enradin®

CONTENTS PER KG	BENEFITS	INCLUSION RATE	PRESENTATION
Each 1 Kg of Enradin contains 80 gm of Enramycine HCL	Helps in ease the incidence of sub-clinical necrotic entritis in chicken	5-10 ppm (63-125 gm) per ton of feed	20 Kg Withdrawal period - 7 days Avoid use in laying hens

Amnovit®

CONTENTS PER KG	BENEFITS	INCLUSION RATE	PRESENTATION
Scientifically Balance formulation of vitamins and amino acids	Helps in relieving the stress conditions by supporting vitamins and minerals	Through water 1gm/lit for 5-7 days Through feed 500gm/ton for 5-7 days	1 Kg

Pharma Product

Floxidin™

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Enrofloxacin 10% oral solution	The product is recommended for treatment of the common infections caused by gram-positive, gram-negative, anaerobes and mycoplasma species	10 mg per kg BW for 3-5 days	5 Lt Withdrawal period - Meat - 8 days Eggs - Stop using 14 days before laying

VAC-SAFE®

CONTENTS	BENEFITS	INCLUSION RATE	PRESENTATION
An effervescent tablet that dilutes easily and neutralizes the chlorine in the water	Helps in improving the quality of drinking water during vaccination	1 tablet /100 Lt water	Box of 30 tablet

A trusted source for comprehensive animal health solutions

Today's Merck is a global healthcare leader working to help the world be well. MSD Animal Health, known as Merck Animal Health in the United States and Canada, is the global animal Health business unit of Merck. MSD Animal Health offers veterinarians, farmers, pet owners and Governments the widest range of veterinary pharmaceuticals, vaccines, health management solutions and services. MSD Animal Health is dedicated to preserving and improving the health, well being and performance of animals. It invests extensively in dynamic and comprehensive R & D resources and a modern, global supply chain. MSD Animal Health is present in more than 50 countries, while its products are available in some 150 markets.

For more information,

visit www.msd-animal-health.co.in

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