The Blue Cross Book

For the advancement of the veterinary profession

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The results/ conclusions drawn and recommendations made in the article (s) are of the author (s) and not necessarily of the Editorial Board.

From Editor's Desk

The Editorial Board of "Blue Cross Book" is happy to bring out the 32nd volume of this professional publication of MSD Animal Health. It has been the effort of this publication since its relaunch with its 25th volume, to enlighten its readers about the latest inventions, changed therapeutic approaches, new zoonotic threats and various happenings in the livestock sector. "Know the Prestigious Institute" page of the Blue Cross Book, which informs about the objectives and achievements of the national Animal Science Institutes working in India, has brought laurels to this publication. This volume also details out the activities of the "Central Institute for Research on Cattle", Meerut. We propose the continue such information in future also.

Emergence and re-emergence of zoonotic diseases is a perpetual problem in the developing countries like India, where livestock and human population live in very close contact without observing any hygienic limitations. Brucellosis through marine mammals is a new threat to the human population. Mass education of people regarding the diseases spread via animals and their prevention is the only visible solution to zoonosis. This is being followed in many advanced countries, who have eradicated diseases like salmonellosis and TB through this measure. The Veterinarians in India have to play a great role in this regard, as they are the true custodians of not only the livestock health, but also the human health.

The consumption of milk from exotic and crossbred animals has been raising a controversy during the recent past in India as the milk from these animals has been shown to introduce certain human health problems in countries like New Zealand and Australia due to the presence of A1 Beta-casein variant in the milk of Holstein and Jersey cows. Considering that milk production in India is futured primarily on crossbred cows, the Veterinarians must be aware about the A1 and A2 variants of Beta-casein in cow milk and their effects of human health. This volume of Blue Cross Book is publishing a very important and informative article on this topic and it is proposed to throw more light on this topic in the future volumes.

Dr. P. W. Borgaonkar, Technical Service Manager and Field Trial Co-ordinator, so also the Member on the Editorial Board of Blue Cross Book, is retiring in August 2015 and his valuable services to this publication would not be available from the next issue. The Editorial Board, very proudly and humbly, puts on record the yeomen efforts put in by Dr. Borgaonkar in relaunching this professional publication and making wide open the knowledge horizon for the Veterinary fraternity in general. On behalf of all the contributors and readers, the Blue Cross Book bids farewell to Dr. Borgaonkar, wishing him a happy life during the years to come.

Dr. Yash Goyal
Managing Director,
MSD Animal Health

Dear Professional Colleagues,

It gives me an immense pleasure and opportunity to present the 32nd edition of our technical journal, "The Blue Cross Book". With this, I wish to extend my sincere thanks to all the fellow professionals, who regularly contribute their knowledge and experience for updating this journal, dedicated to veterinary profession.

We, in MSD Animal Health, give utmost importance to clinical research that generates the field data. This we incorporate in "The Blue Cross book" to build confidence in Veterinary practitioners. We thus update a Veterinary practitioner, who uses this advanced technology as the key to his success in dealing with the problems related to livestock health and productivity.

The scientific innovations and advanced technologies today mean a combined effort of all stake holders including the field professionals and needy customers. We constantly partner with Veterinary Colleges and Universities, research institutions and Veterinary professionals for scientific update. Our close knit with field Veterinarians also helps us to strengthen our scientific base.

MSD-Animal Health has been in the Indian market with products of international repute in livestock, poultry and companion animal segments, offering hormones, anti-infectives, ecto and endo-parasitic controls, supportive medicines and biologicals, produced in our most sophisticated manufacturing sites, both locally and globally.

By virtue of having own manufacturing site for biologicals, supported by R&D and Service laboratory at Pune, MSD-Animal Health has been in a very strong position to cater the customer needs with its products at affordable prices, along with diagnostic support to combat the livestock and poultry diseases prevalent in India.

We sincerely believe that you would enjoy reading this compilation with professional interest and support MSD Animal Health further by providing your valuable feedback, which has helped us a long way in our mission to serve Veterinary profession and our customers in the best possible way, in the years to come.

Best Wishes,

Yash Goyal

POULTRY

Meeting consumer demand is the key to success for our poultry customers. Like the demand for impeccable food safety.

Salmonella is one of the most common food-borne, disease-causing bacteria, and can cause severe gastroenteritis in humans. Our Food Safety Platform combines vaccines like NOBILIS Salenvac T (the broadest spectrum Salmonella vaccine available) with services that ensure effective, timely intervention. These include the Pin-Point Monitoring Program for poultry operations, helping producers identify critical food safety hazard points.

As well as food safety we've also developed platforms for Intestinal Health, Respiratory Protection and Dynamic Immunity.

Good intestinal health is vital for birds to convert their feed properly, and gain sufficient weight. Our Intestinal Health Platform is made up of market-leading vaccines like COCCIVAC and PARACOX – offering protection against coccidiosis and necrotic enteritis.

The INNOVAX range is the cornerstone of our Respiratory Protection Platform. It includes live virus vector vaccines, offering protection against Marek's disease, Newcastle disease (ND) and infectious laryngotracheitis (ILT).

Our PROTECTOTYPE approach to vaccination is a uniquely effective way to tackle respiratory infections like bronchitis. Combining live vaccines such as NOBILIS IB Ma5, IB 4/91 and inactivated ones like NOBILIS IB Multi, gives optimal protection against the strain range. The vaccination schedule is tailored to local requirements, epidemiological situation and the type of production.

Strong pillars of our Dynamic Immunity Platform are our live infectious bursal disease virus (IBDV) vaccines, protecting birds against Gumboro disease. NOBILIS D78 and NOBILIS 228E live vaccines set the standard for IBD protection around the world.

CREATING COMPANIONSHIP THAT LASTS

SPOTLIGHT ON HOME AGAIN

5.2 MILLION PETS IN THE USA HAVE BEEN ENROLLED IN OUR HOME AGAIN MICROCHIP-BASED PET RECOVERY PROGRAM, RESULTING IN MORE THAN 1 MILLION SUCCESSFUL RECOVERIES SO FAR. IT'S JUST ONE WAY WE USE OUR PARTNER NETWORK TO HELP ANIMALS AROUND THE WORLD.

THE GREAT SHIFTS IN GLOBAL WEALTH MEAN MANY MORE PEOPLE NOW HAVE THE TIME AND MONEY THEY NEED TO KEEP PETS IN THE HOME. AND MERCK ANIMAL HEALTH IS HELPING THEM LOOK AFTER THEIR HEALTH AND WELL BEING.

Merck Animal Health is one of the few companies with the manufacturing capabilities to help veterinarians meet this rapid rise in demand for pet healthcare products.

Our vaccine portfolio for dogs and cats is led by the NOBIVAC range. The canine parvo virus (CPV) strain C154 in our NOBIVAC DHPPi dog vaccine means it is the first low-passage/high titer vaccine that can break through maternally-derived antibodies against CPV effectively.

In response to a call from the American Veterinary Medical Association (AVMA), we have also developed the first vaccine against canine influenza, NOBIVAC Canine Flu H3N8.

Cats are protected against feline panleukopenia virus, feline herpes virus and feline calicvirus by NOBIVAC Tricat Trio. Similarly, NOVIBAC Myxo-RHD is a live chimera rabbit vaccine that protects against myxomatosis and rabbit hemorrhagic disease (RHD) with a single injection.

As more people keep pets, it's important we do more to control the spread of parasites in the home. Along with PANAC UR/SAF E-GUARD (fenbendazole) and TRIHEART PLUS (ivermectin plus pyrantel), a canine heartworm preventative, the SCALIBOR Protector Band (deltamethrin)

protects dogs against Leishmaniasis. Leishmaniasis is one of the world's most deadly parasitic diseases and is linked to 60,000 human deaths annually.

As our bond with our pets becomes stronger, we want to make sure treatments are as gentle on them as they are effective. AC TIVYL (indoxacarb) and AC TIVYL TickPlus (indoxacarb plus permethrin) are new and unique flea products that use 'bioactivation'. AC TIVYL minimizes pet exposure to chemicals, because it only becomes active inside the flea.

We know too that pets are now living longer. Our portfolio has grown to match them. CANINSULIN (porcine insulin zinc suspension) helps dogs and cats with diabetes mellitus, and VIDALTA (carbimazole) is our prolonged-release tablet for easy oral treatment of feline hyperthyroidism.

Vol. 32

Contents

July 2015

Review Articles

- Marine Mammal Brucellosis and its Zoonotic Implications. 1
  - *A. Vijaya Kumar, P. Anusha, N. Krishnaiah and Sujatha Singh*
- Japanese Encephalitis: An Arboviral Zoonosis. 9
  - *Sunanda Pandey, Vandip D. Chauhan, B. C. Parmar and J. B. Nayak*
- Canine Transmissible Venereal Sarcoma (CTVS) - a review. 15
  - *Roshini S., Moregaonkar S. D. and Sawale G. K.*
- Kyasanur Forest Disease - a zoonotic threat. 20
  - *Gupta Payal., Pradhan Shashi., Roy Kabita and Shukla P. C.*

Research Articles

- Effect of ethonoloic leaf extracts of *Senna alata* and *Lantana camara* in the treatment of bovine Dermatophilosis. 25
  - *Siva Prasad. B, Raniprameela. D, Srinivasulu. D, Alpha Raj. M*
- Evaluation of different strategies for improving reproductive efficiency of Postpartum Anoestrus Jaffarabadi Buffaloes. 29
  - *Parikh S. S., Gajbhiye P. U. and Parmar K. H.*
- Clinico-hematobiochemical studies in Equines infected with *T. evansi.* 33
  - *Chavda Kirit J., Mandali G. C., Tank P. H.*
- Efficacy of Cloprostenol Sodium administration during early post partum period on uterine involution in cows. 39
  - *Raut M. V., Ingawale S. G., Deshmukh and Arbal R. W.*
- Incidence and Pathogenicity of Aeromonas Spp. in Chicken and Emu meat. 43
  - *D. Hareesh, Krishnaiah Nelapati, P. Anusha, Sujatha Singh*
- Antimicrobial susceptibility of *Clostridium perfringens* strains Isolated from various sources. 49
  - *Anusha Poluru and Krishnaiah Nelapati*
- Effect of Methanolic Extract of Caesalpinia sappan Lin on Lead induced oxidative stress in Rats. 54
  - *K. Hemalatha, P. Eswara Prasad, K. Padmaja, K. Adilaxmamma*
- Standardization of Polymerase Chain Reaction (PCR) for diagnosis of Dermatophilosis from Cattle & Buffalo in Andhra Pradesh. 58
  - *Siva Prasad. B, RaniPrameela. D, Sreenivasulu. D, Vijayalaxmi. S*

- Occurrence of Marek's disease associated with immunosuppression in unvaccinated commercial broilers. 61
  - *Reddy M. R. and Rama Rao S. V.*

- Efficacy of Tube Cystostomy for the treatment of obstructive urolithiasis in Buffalo calves. 64
  - *Sangeetha P., Prasoon Dubey, Aswathy Gopinathan and Kiranjeet Singh*

Clinical Reports

- Photosensitization due to ingestion of *Lantana camara* in Buffaloes. 68
  - *Priyanka. U Ravindra B. G, Vivek R. K and Vijay Kumar. M*

- Management of atonic bladder in a Bitch following an Ovariohysterectomy. 71
  - *Parrah Jalal-ud-Din, Ansari Muhammad Moin and Moulvi Bashir Ahmed*

- Therapeutic management of Downer Syndrom in a cow with supportive Physiotherapy. 75
  - *B. L. Kaswan*

- Ehrlichiosis in a Cow - A case report. 78
  - *H. Dhanalakshmi, Priyanka, Rakesh. R. L, P. M. Thimmareddy and M. Narayana Bhat*

- Mummification of foetus in a crossbred cow and its management. 81
  - *Desai B. V. and Parab P. R.*

- Therapeutic management of ulcerative mamillitis in buffaloes. 84
  - *C, Pavan Kumar, N. Syaama Sundar, G. Praveena*

- Congenital preputial orifice stenosis and Its surgical management in a Kid. 86
  - *Parrah Jalal-ud- Din, Ansari Muhammad Moin* and Moulvi Bashir Ahmed*

- Surgical management of Intussusception in a Calf. 89
  - *Divya mohan, Aswathy Gopinathan, Kiranjeet Singh, Deepesh Gautam and Shanti Jaylekshmi*

- Diagnosis and therapeutic management of Horses infected with *Trypanosoma evansi.* 91
  - *Chavda K. J., Mandali G. C., Tank P. H., Kanani A. N., Goel R. K.*

- Subacute Hemorrhagic Septicemia in a Cow - A Case Report. 95
  - *Priyanka, Dhabale R. B., Rashmi L. and Padalkar R. D.*

- Successful expulsion of Mummified Fetus in a Cow. 98
  - *Ingawale M. V.*

- A foreign body in a Cow at the floor of Oral Cavity and its management. 100
  - *Kaswan B. L. and Pandla Mahesh*
- Horn Cancer in a cow and its surgico-medical management. 103
  - *Kaswan B. L.*
- Sarcoptic Mange in a Persian Cat. 106
  - *P. Revathi, S. Bharathi, N. Syaama Sundar, L. Sivasudharsan*

General Articles

- Occurrence of Fowl Pox virus in Grey Jungle Fowls - A report. 108
  - *D. Raniprameela, , D. Srinivasulu, Eswara Prasad. P, Vijayalaxmi. S and Sudheer Babu. G*
- Beta-casein protein of bovine milk and its possible effects on human health. 111
  - *Dr. R K Patel*
- Studies on biochemical parameters in Leptospira infected cattle. 116
  - *D. Raniprameela, D. Srinivasulu, P. Eswara Prasad. and S. Vijayalaxmi*
- Changing pathogenecity of Adenovirus infections in commercial poultry farms. 118
  - *Suryawanshi D. S. and Pawar Pavan*
- Toxocariosis - Pet's enemy. 121
  - *A. Sangaran, S. T. Bino Sundar and B. R. Latha*
- Zoonotic Diseases of Sheep and Goat. 123
  - *Sahu Rajesh Kumar, Patil Mayura R. Bhong, C. D. and Deshmukh V. V.*
- Effect on Temperature, Respiration and Pulse Rate before and after administration of Bupivacaine epidurally in Camels. 127
  - *Lakhvinder Singh and Amit Kumar*

- **News** - National. 130
- **Know the Institute** - CIRC, Meerut Kantt. 131
- **Good Bye Dr. Borgaonkar.** 135

The Blue Cross Book July 2015 Vol. 32 : 1-8

Marine Mammal Brucellosis and its Zoonotic Implications

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Abstract

The present article focuses on the etiology, epidemiology and diagnosis of brucellosis in marine mammals and its zoonotic implications. The marine Brucella isolates may act as etiological agents for various reproductive disorders in sea mammals (cetaceans and pinnipeds) leading to abortion and stillbirth, and can be of concern for the existence of threatened marine mammal species. Marine Brucella strains represent a Zoonotic threat; though, the pathogenicity of these microorganisms to humans is yet to be clearly established. Sea mammals can also introduce brucellosis to new hosts and new areas.

Keywords: Brucellosis-Marine mammals-Human-Zoonotic importance

Introduction

The Genus Brucella, belonging to the class Alpha-proteobacteria and order Rhizobiales (Williams *et al.,* 2007), contains gram-negative, non-motile, facultative intracellular, small coccobacilliary bacteria. Classically, there were six species; *Brucella melitensis*,*B. abortus*, *B. suis*, *B. ovis*, *B. neotomae* and *B. canis* (Corbel and Brinley-Morgan 1984). Recently, four additional species have been added to this genus, which include two species of marine origin i.e., *Brucella ceti* and *Brucella pinnipedialis*, with cetaceans and seals as their preferred hosts, respectively (Foster *et al.,* 2007). Brucella spp. have zoonotic potential. The main source of human infection is production animals. The most frequently reported cause of zoonotic Brucella infection is *B. melitensis*. *B. suis* (biovar 1, 3 and 4) and *B. abortus* are also zoonotic. Much information is not available about the marine mammal brucellosis, so the present article focuses on the etiology, epidemiology and diagnosis of brucellosis in marine mammals and its zoonotic implications.

Etiology and epidemiology of marine brucellosis

Brucella spp. was isolated from marine mammals for the first time in 1994 from stranded common seals, harbour porpoises and dolphins in Scotland (Ross *et al.,* 1994) and it was also recovered from the aborted foetus of a bottlenose dolphin in California (Ewalt *et al.,* 1994). This new strain, initially designated as *Brucella maris,* was further divided into biovar 1 (isolated from seals and otters), biovar 2 (from cetaceans) and biovar 3 (from Californian bottlenose dolphin) (Jahans *et al.,* 1997). Marine mammalian *Brucella* isolates have a host preference for either the order Cetacea (whales, dolphins and porpoises) or Pinnipedia (seals, sea lions and walruses), with the exception of one isolate which was recovered from sea otter (family Mustilidae and order Carnivora). The

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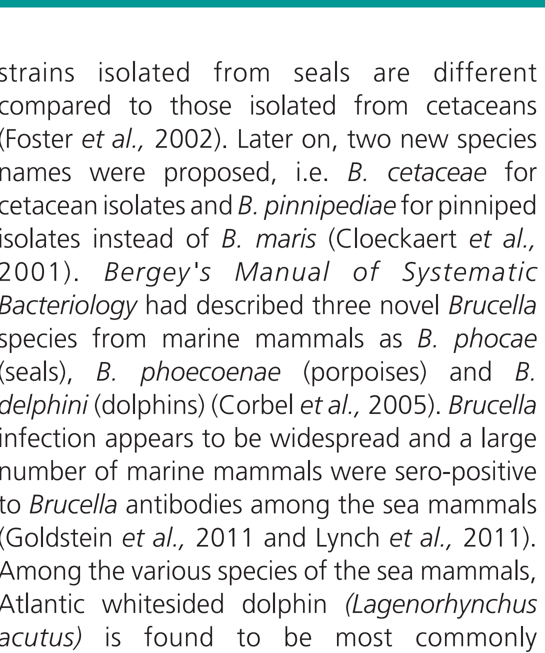

strains isolated from seals are different compared to those isolated from cetaceans (Foster *et al.,* 2002). Later on, two new species names were proposed, i.e. *B. cetaceae* for cetacean isolates and *B. pinnipediae* for pinniped isolates instead of *B. maris* (Cloeckaert *et al.,* 2001). *Bergey's Manual of Systematic Bacteriology* had described three novel *Brucella* species from marine mammals as *B. phocae* (seals), *B. phoecoenae* (porpoises) and *B. delphini* (dolphins) (Corbel *et al.,* 2005). *Brucella* infection appears to be widespread and a large number of marine mammals were sero-positive to *Brucella* antibodies among the sea mammals (Goldstein *et al.,* 2011 and Lynch *et al.,* 2011). Among the various species of the sea mammals, Atlantic whitesided dolphin *(Lagenorhynchus acutus)* is found to be most commonly associated with *Brucella* infections, whereas *Stenella coeruleoalba,* the striped dolphin, is reported to be a highly susceptible host and may act as a reservoir of *Brucella* infection (Hernández-Mora *et al.,* 2008). Recently, a high (57%) sero-prevalence has been reported among Australian fur seals *(Arctocephalus pusillus doriferus)* (Lynch *et al.,* 2011).

Transmission

The transmission of marine brucellosis is poorly understood. The route of infection and marine mammal reservoirs and animal-to-animal transmission remain uncertain. Gregarious nature of some of the sea mammals is believed to aid in the transmission of brucellosis among the sea mammals (Dagleish *et al.,* 2007). Brucellae have also been isolated from longstanding cestodes *(Phyllobothrium delphini)* from bottlenose dolphin. It may also be possible that species down the marine food chain may act as a common source of infection to different species of marine mammals (Tryland *et al.,* 1999). In marine mammals also, isolations of *Brucella* have been made from milk and mammary glands, reproductive organs, placenta, umbilical cord, foetal tissues, aborted foetus and secretions of pregnant sea mammals. Therefore, marine mammal *Brucella* isolates also have tropism for placenta and foetal tissues as in *Brucella*-infected terrestrial animals. The vertical transmission of the Brucella-infection has been recorded and possibility of the horizontal transmission among sea mammals cannot be denied. Further, isolation of the organism from the reproductive organs suggests the possible sexual transmission of the organism and/or sterility as sequelae to infection, similar to those reported in terrestrial animals (Davison *et al.,* 2011).

Disease caused by marine mammal Brucella isolates

Disease in marine mammals

Brucella spp. have been reported from both apparently healthy and symptomatic animals (Maquart *et al.,* 2009). The symptoms or clinical syndrome for brucellosis in the sea mammals are not clearly documented. Systematic brucellosis appears to be common in marine mammals, but it is rarely associated with pathological changes (Dagleish *et al.,* 2008). Brucellae have been isolated from a wide variety of tissues and from reproductive organs of both the sexes and also from the aborted foetuses and placentas. *Brucella* spp. in marine mammals have been associated with various pathological expressions such as subcutaneous lesions, abscesses, hyperplastic lymph nodes, congested mammary glands, splenic and hepatic necrosis, necrotizing thrombo embolic pneumonia or meningitis/meningoencephalitis and abnormal joints and testes, epididymitis and abortions (Jauniaux *et al.,* 2010). Placentitis and abortions are reported in the captive bottlenose dolphins and wild Atlantic white-sided dolphin (Muñoz *et al.,* 2006). Atlantic white-sided dolphins were found to have *Brucella* lesions mainly consisting of hepatic and splenic coagulative necrosis,

splenomegaly, congested lungs, lymphadenitis, mastitis and possible abortions. *Brucella* organisms in marine mammals were also found to be associated with oesophageal ulceration and necrosis. *Brucella* has also been reported as a secondary pathogen among stressed porpoises, seals and dolphin. The main pathological findings recorded in porpoise *(P. phocoena)* were blubber abscessesion, spinal discospondlitis and splenic necrosis. *Brucella* can also act as an opportunistic pathogen in marine mammals with poor state of nutrition or those suffering from some other disease or parasitism (Foster *et al.,* 2002). *Brucella* as a main etiological agent can cause death due to hepatic abscess, peritonitis and epididymitis in marine mammals. Nervous form of the disease resulting in meningoencephalitis is seen in striped dolphins only. Neurobrucellosis is evident by the inability to maintain buoyancy, ophisthtonus, tremors and seizures. Animals suffering from nervous form of the disease had hyperemic meninges, congested brains and altered cerebrospinal fluid. *Brucella* organisms were also isolated from adult female harbour porpoises with occluded bile duct and from the lungs and kidneys of malnourished pups of grey seal, *Halicherus grypus.* The pregnant animals can develop placental abscesses due to *Brucella* infection (Hernández-Mora *et al.,* 2008). Abnormal testes and caseated and calcified uterus were recorded as the main pathological findings among Brucella sero-positive common mink whales *(Balaenoptera acutorostrata)* and Bryde's whales *(Balaenoptera edeni)* (Ohishi *et al.,* 2004)

Disease in other animals

The *Brucella* organisms are known to cross the species barrier causing disease in animals other than their preferred host (Thakur *et al.,* 2002). Studies indicate that *Brucella* spp. isolated from marine mammals can also cause disease in terrestrial animals. The disease was induced in cattle, sheep and piglets through experimental inoculations with *Brucella* strains isolated from marine mammals. Marine *Brucella* species was re-isolated from the aborted cows showing histopathological changes and from various organs of unaborted animals with 100% sero-conversion (Rhyan *et al.,* 2001). Sheep inoculated with an isolate of seal origin developed a transient low level of anti-Brucella antibodies and the microorganism was also isolated from one of the aborted ewes and its foetus (Perrett *et al.,* 2004). The organisms were re-isolated from lymph nodes of experimentally infected pigs. Low and transient antibody titres were detected in culture-negative, experimentally infected pigs (Bingham *et al.,* 2008). Antibodies against *Brucella* have also been detected in polar bears *(Ursus maritimus)* from Svalbard and the Barents Sea. The ringed seals *(Phoca hispida),* an important prey species for the Svalbard polar bears and harp seals *(Phoca groenlandica)* from the same geographical areas were also sero-positive to *Brucella* antibodies, suggesting possible transmission of brucellosis from prey to predator (Tryland *et al.,* 2001) . All these studies suggest that the disease occurring in sea mammals can be transmitted to domestic animals and wildlife residing in the nearby coastal areas.

Pathology in association with *B. ceti* infection in cetaceans

Gross pathology in association with Brucella infection in marine mammals is seen exclusively in cetaceans. The infection may have several outcomes and a wide range of pathological changes have been reported. *B. ceti* has been associated with a range of pathological changes in cetaceans, including blubber abscesses, subcutaneous lesions, skin lesions, hepatic and splenic necrosis and inflammation, macrophage infiltration in the liver and spleen, pneumonia, peritonitis and lymph node inflammation and

necrosis. Pathologic changes, including spinal discospondylitis, meningoencephalitis, meningitis, choroiditis, altered cerebrospinal fluid and remodeling of the occipital condyles, often associated with neurologic symptoms, have been reported several times in cetaceans. *B. ceti* and *B. pinnipedialis* have been isolated from lungworms found in cetacean and pinniped lungs, respectively (Jauniaux *et al.,* 2010). B. ceti has been isolated from aborted foetuses and reproductive organs in captive bottlenose dolphins (Ewalt *et al.,* 1994) with placentitis (Jauniaux *et al.,* 2010), and from the reproductive organs, milk and foetus of stranded striped dolphins (Hernández-Mora et al., 2008). Bacteria have also been isolated from the uterus and a dead foetus of a stranded striped dolphin with placentitis (Gonzalez-Barrientos *et al.,* 2010). Immunohistochemical investigations with polyclonal antiserum and electron microscopy revealed *B. ceti* in a genital ulcer, uterus, mammary gland and milk from a stranded harbour porpoise with endometritis and signs of a recent pregnancy (Jauniaux *et al.,* 2010). *B. ceti* has also been isolated in association with mastitis and endometritis in cetaceans. Suppurative granulomatous lesions have been found in both female and male reproductive organs in seropositive baleen whales. *B. ceti* and *B. pinnipedialis* have also been isolated from the testes (Foster *et al.,* 1996), the uterus (Garcia-Yoldi *et al.,* 2006) and the mammary gland (Ross *et al.,* 1996) of cetaceans and pinnipeds without any apparent pathology.

Zoonotic potential of marine mammal strains of Brucella

It is important to note that the modes transmission of brucellosis from marine mammals to man is still questionable. But reports on isolation of marine mammal brucellae indicate that people eating raw or uncooked food and those involved in recreational activities such as swimming are at higher risk of acquiring the infection (Whatmore *et al.,* 2008).

There are indications that certain of the marine mammal Brucella spp. have zoonotic potential. A laboratory worker cultivating marine mammal Brucella strains developed bacteraemia, and the bacteria isolated from the blood matched one of the isolates she was working with, indicating a laboratory infection (Brew *et al.,* 1999). Two patients from Peru were presented with intracerebral granulomas and marine mammal brucellae were isolated from the lesions. Both had been at the coast and had eaten raw shellfish. Interestingly, to date four human cases with *Brucella* infections have been reported, presumably of marine mammal origin (Whatmore *et al.,* 2008). Three of these cases were acquired through natural infection by marine origin *Brucella* - one case of spinal osteomyelitis from a patient in New Zealand (McDonald *et al.,* 2006) and two cases of neurobrucellosis from Peruvian patients. Cases of Zoonotic marine brucellosis reported from Peru had serious central nervous system disease with intra-cerebral granuloma. In both cases, there was no direct contact with the marine mammals. The patients had history of consumption of queso fresco (soft cheese) and raw shell fish ceviche (citrus-marinated seafood) respectively. One had frequently swum in the Pacific Ocean, whereas, the other seldom visited the sea coast. However, the mode of transmission in these cases remains questionable because of the history of regular consumption of unpasteurized cheese (Sohn *et al.,* 2003). In New Zealand, marine mammal-type *Brucella* strain was isolated from a patient with no direct exposure to marine mammals, but who had a history of regular fishing, contact with uncooked bait and consumption of raw snapper. Isolates from Peruvian patients were similar to

B. pinnipidae (seal strain), whereas, the isolate reported from New Zealand was closely related to a Brucella sp. originating from a bottlenose dolphin *(T. truncatus)* in the United States and common seals *(P. vitulina)*. **All these cases can be seen as the early warning signs of an emerging zoonosis (Sohn et al., 2003 and McDonald *et al.*, 2006).**

Disease in Humans

Infection with marine *Brucella* strains causes a range of symptoms, including fever, rigours, headaches, lassitude, sinusitis and lumbar spinal tenderness (spinal osteomyelitis). Nervous symptoms include headaches, nausea, vomiting, periorbital pain, periodic generalized tonicclonic seizures and progressive deterioration in vision. The relative zoonotic potential of marine mammalian isolates is yet to be clearly established (Sohn *et al.,* 2003 and McDonald *et al.,* 2006).

Diagnosis

The symptomatic diagnosis of the brucellosis in marine mammals cannot be made as no clinical syndrome has been established in the marine mammals. *Brucella* organisms have been isolated from both normal as well as symptomatic/diseased animals. Brucellosis can be diagnosed by host preference, serological and molecular techniques, or isolation of the organisms from the affected animal.

Cultural methods

The majority of isolations of *Brucella* organism from sea mammals were made from dead animals. The organisms had been isolated from male and female reproductive organs, mammary glands, brain, spinal cord abscesses, diseased atlanto-occipital joint, lungs, spleen liver, kidneys, cerebrospinal fluid, joints, foetal tissues, milk, secretion of pregnant animals, purulent blubber abscesses and a variety of lymph nodes. Primary isolation of marine brucellae (Whatmore *et al.,* 2008) is almost similar to other brucella species. Majority of *Brucella* isolations of organisms from sea mammals are done on Farell's medium, followed by Columbia sheep blood agar, *Brucella* agar with Brucella selective supplement and 1.4% crystal violet and brain heart infusion agar with 5 g of yeast extract. Cetacean isolates generally become visible within 4 days of inoculation on this medium. However, isolates from seals may fail to grow or take 7/10 days to grow. The samples should also be simultaneously incubated on certain non-selective media such as serum dextrose agar or blood agar.

Marine mammal *Brucella* isolates have smooth colony appearance with entire margins and are raised, convex and shiny. These appear as honey coloured and translucent when examined by transmitted light. Sea mammal *Brucella* strains can be differentiated from the other six *Brucella* species of terrestrial origin through a substrate-specific tetrazolium reduction test and phenotypic characters (Broughton and Jahans, 1997). Similar procedures are applied to isolate marine Brucella strains from infected human beings (Brew *et al.,* 1999). The importance of direct isolation of the organism from the suspected human cases is stressed since prolonged or chronic illness, unknown host factors, symptom-based medication and low immunogenicity of the marine *Brucella* strains may result in low or absence of immune responses.

Serological methods

A number of serological tests are in use to detect *Brucella* antibodies or agglutinins in man and animals. Each test has its own advantages and limitations in terms of sensitivity and specificity. Serological tests, based upon *B. abortus* antigen,

used for marine mammal brucellosis diagnosis are similar to those being used to diagnose brucellosis in terrestrial animals. These include Rose Bengal plate test, serum tube agglutination antigen (STAT)/tube agglutination test, ethylenediaminetetraacetic acid modified STAT, complement fixation test, cardagglutination test, buffered acid plate antigens, rivanol test, enzyme-linked immunosorbent assays (ELISA), fluorescence polarization assays (FPA) and immunoblotting. Competitive ELISA (C-ELISA) and FPA were found appropriate as diagnostic screening tests for detection of Brucella antibodies in marine mammals (Nielsen *et al.,* 2001)

Molecular methods

Molecular or the genomic methods of diagnosis and differentiation of *Brucella* species are more useful than serology or culture isolations because of serological cross-reactions and fastidious nature of this zoonotic bacterium. Molecular analysis has confirmed the genetic distinctiveness of marine strains from the terrestrial strains. On the basis of ribotyping (HindIII rDNA restriction patterns), marine isolates were classified as a separate subgroup of the genus *Brucella*. Occurrence of an IS711 element downstream of the *bp26* gene is a feature specific to the marine mammal *Brucella* isolates. Infrequent restriction site polymerase chain reaction (IRS-PCR) targeting IS711 was able to identify *B. cetaceae* and *B. pinnipediae* separately (Meegan *et al.,* 2010).

Control and prevention of marine brucellosis

Brucellosis in marine mammals is an emerging zoonotic disease. Although marine Brucella strains were recognized recently, the studies conducted till date indicate that the disease is probably endemic in marine mammals. It has already been indicated that these strains might affect the reproductive activities in these animals, which is particularly a concern in threatened or naïve species.

References

Bingham, J., Taylor, T. K., Swingler, J. E., Meehan, G., Middleton, D. J., Mackereth, G. F., ... & Daniels, P. W. (2008). Infection trials in pigs with a human isolate of Brucella (isolate 02/611 'marine mammal type'). *New Zealand veterinary journal,* **56**, 10-14.

Brew, S. D., Perrett, L. L., Stack, J. A., MacMillan, A. P., & Staunton, N. J. (1999). Human exposure to Brucella recovered from a sea mammal. *The Veterinary Record*, **144**, 483-483.

Broughton, E. S., & Jahans, K. L. (1997). The differentiation of Brucella species by substrate specific tetrazolium reduction. *Veterinary Microbiology*, **57**, 253-271.

Cloeckaert, A., Verger, J. M., Grayon, M., Paquet, J. Y., Garin-Bastuji, B., Foster, G., & Godfroid, J. (2001). Classification of Brucella spp. isolated from marine mammals by DNA polymorphism at the omp2 locus. *Microbes and Infection,* **3**, 729-738.

Corbel, M. J., & Brinley-Morgan, W. J. (1984). Genus Brucella, p 370. Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, MD.

Corbel, M., & Banai, M. (2005). Brucella Meyer and Shaw 1920, 173 AL. Bergey's Manual® of Systematic Bacteriology, 370-386.

Dagleish, M. P., Barley, J., Finlayson, J., Reid, R. J., & Foster, G. (2008). Brucella ceti associated pathology in the testicle of a harbour porpoise (Phocoena phocoena). *Journal of comparative pathology*, **139**, 54-59.

Dagleish, M. P., Barley, J., Howie, F. E., Reid, R. J., Herman, J., & Foster, G. (2007). Isolation of Brucella species from a diseased atlanto-occipital joint of an Atlantic white-sided dolphin (Lagenorhynchus acutus). *Veterinary Record*, **160**, 876-877.

Davison, N. J., Perrett, L. L., Law, R. J., Dawson, C. E., Stubberfield, E. J., Monies, R. J., ... & Jepson, P. D.

(2011). Infection with Brucella ceti and high levels of polychlorinated biphenyls in bottlenose dolphins (Tursiops truncatus) stranded in south-west England. Veterinary Record-English Edition, **169**, 14.

Ewalt, D. R., Payeur, J. B., Martin, B. M., Cummins, D. R., & Miller, W. G. (1994). Characteristics of a Brucella species from a bottlenose dolphin (Tursiops truncatus). *Journal of Veterinary Diagnostic Investigation*, **6**, 448-452.

Foster, G., Jahans, K. L., Reid, R. J., & Ross, H. M. (1996). Isolation of Brucella species from cetaceans, seals and an otter. *Veterinary Record*, **138**, 583-586.

Foster, G., MacMillan, A. P., Godfroid, J., Howie, F., Ross, H. M., Cloeckaert, A., ... & Patterson, I. A. P. (2002). A review of Brucella sp. infection of sea mammals with particular emphasis on isolates from Scotland. *Veterinary Microbiology*, **90**, 563-580.

Foster, G., Osterman, B. S., Godfroid, J., Jacques, I., & Cloeckaert, A. (2007). Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 2688-2693.

García-Yoldi, D., Marín, C. M., de Miguel, M. J., Muñoz, P. M., Vizmanos, J. L., & López-Goñi, I. (2006). Multiplex PCR assay for the identification and differentiation of all Brucella species and the vaccine strains Brucella abortus S19 and RB51 and Brucella melitensis Rev1. *Clinical Chemistry*, **52**, 779-781.

Goldstein, T., Gill, V. A., Tuomi, P., Monson, D., Burdin, A., Conrad, P. A., ... & Doroff, A. M. (2011). Assessment of clinical pathology and pathogen exposure in sea otters (Enhydra lutris) bordering the threatened population in Alaska. *Journal of Wildlife Diseases*, **47**, 579-592.

González-Barrientos, R., Morales, J. A., Hernández-Mora, G., Barquero-Calvo, E., Guzmán-Verri, C., Chaves-Olarte, E., & Moreno, E. (2010). Pathology of striped dolphins (Stenella coeruleoalba) infected with Brucella ceti. *Journal of Comparative Pathology*, **142**, 347-352.

Hernández-Mora, G., González-Barrientos, R., Morales, J. A., Chaves-Olarte, E., Guzmán-Verri, C., Baquero-Calvo, E., & Moreno, E. (2008). Neurobrucellosis in stranded dolphins, Costa Rica. *Emerging Infectious Diseases*, **14**, 1430.

Jahans, K. L., Foster, G., & Broughton, E. S. (1997). The characterisation of Brucella strains isolated from marine mammals. *Veterinary Microbiology*, **57**, 373-382.

Jauniaux, T. P., Brenez, C., Fretin, D., Godfroid, J., Haelters, J., Jacques, T., ... & Coignoul, F. L. (2010). Brucella ceti infection in harbor porpoise (Phocoena phocoena). *Emerging Infectious Diseases*, **16**, 1966.

Lynch, M., Nielsen, O., Duignan, P. J., Kirkwood, R., Hoskins, A., & Arnould, J. P. (2011). Serologic survey for potential pathogens and assessment of disease risk in Australian fur seals. *Journal of Wildlife Diseases*, **47**, 555-565.

Maquart, M., Zygmunt, M. S., & Cloeckaert, A. (2009). Marine mammal Brucella isolates with different genomic characteristics display a differential response when infecting human macrophages in culture. *Microbes and Infection*, **11**, 361-366.

McDonald, W. L., Jamaludin, R., Mackereth, G., Hansen, M., Humphrey, S., Short, P., & Simmons, G. (2006). Characterization of a Brucella sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *Journal of Clinical Microbiology*, **44**, 4363-4370.

Meegan, J., Field, C., Sidor, I., Romano, T., Casinghino, S., Smith, C. R., ... & Dunn, J. L. (2010). Development, validation, and utilization of a competitive enzyme-linked immunosorbent assay for the detection of antibodies against Brucella species in marine mammals. *Journal of Veterinary Diagnostic Investigation*, **22**, 856-862.

Muñoz, P. M., García-Castrillo, G., López-García, P., González-Cueli, J. C., De Miguel, M. J., Marín, C. M., & Blasco, J. M. (2006). Isolation of Brucella species from a live-stranded striped dolphin (Stenella coeruleoalba) in Spain. *Veterinary Record*, **158**, 450.

Nielsen, O., Stewart, R. E., Nielsen, K., Measures, L., & Duignan, P. (2001). Serologic survey of Brucella spp. antibodies in some marine mammals of North America. *Journal of Wildlife Diseases*, **37**, 89-100.

Ohishi, K., Takishita, K., Kawato, M., Zenitani, R., Bando, T., Fujise, Y., & Maruyama, T. (2004). Molecular evidence of new variant Brucella in North Pacific common minke whales. *Microbes and Infection*, **6**, 1199-1204.

Perrett, L. L., Brew, S. D., Stack, J. A., MacMillan, A. P., & Bashiruddin, J. B. (2004). Experimental assessment of the pathogenicity of Brucella strains from marine mammals for pregnant sheep. *Small Ruminant Research*, **51**, 221-228.

Rhyan, J. C., Gidlewski, T., Ewalt, D. R., Hennager, S. G., Lambourne, D. M., & Olsen, S. C. (2001). Seroconversion and abortion in cattle experimentally infected with Brucella sp. isolated from a Pacific harbor seal (Phoca vitulina richardsi). *Journal of Veterinary Diagnostic Investigation*, **13**, 379-382.

Ross, H. M., Foster, G., Reid, R. J., Jahans, K. L., & MacMillan, A. P. (1994). Brucella species infection in sea-mammals. *Veterinary Record,* **134**, 359-359.

Ross, H. M., Jahans, K. L., MacMillan, A. P., Reid, R. J., Thompson, P. M., & Foster, G. (1996). Brucella species infection in North Sea seal and cetacean populations. *Veterinary Record*, **138**, 647-648.

Sohn, A. H., Probert, W. S., Glaser, C. A., Gupta, N., Bollen, A. W., Wong, J. D., ... & McDonald, W. C. (2003). Human neurobrucellosis with intracerebral granuloma caused by a marine mammal Brucella spp. *Emerging Infectious Diseases*, **9**, 485.

Thakur, S. D., Kumar, R., & Thapliyal, D. C. (2002). Human brucellosis: review of an under-diagnosed animal transmitted disease. *The Journal of Communicable Diseases*, **34**, 287-301.

Tryland, M., Kleivane, L., Alfredsson, A., Kjeld, M., Arnason, A., Stuen, S., & Godfroid, J. (1999). Evidence of Brucella infection in marine mammals in the North Atlantic Ocean. *The Veterinary Record*, **144**, 588-592.

Tryland, M., Derocher, A. E., Wiig, Ø., & Godfroid, J. (2001). Brucella sp. antibodies in polar bears from Svalbard and the Barents Sea. *Journal of Wildlife Diseases*, **37**, 523-531.

Which marine mammal species are affected by Brucella?
Morbillivirus antibodies have been detected in the following species of marine mammals:

Atlantic white-sided dolphin (Lagenorhynchus acutus)	Killer whale (Orcinus orca)
Bottlenose dolphin (Tursiops truncatus)	Minke whale (Balaenoptera acutorostrata)
Common dolphin (Delphinus Delphi)	Pilot whale (Globicephala spp.)
Harbor porpoise (Phocoena phocoena)	Sei whale (Balaenoptera borealis)
Fin whale (Balaenoptera physalus)	Striped dolphin (Stenella coeruleoalba)
California sea lion (Zalophus californianus)	Harp seal (Pagophilus groenlandicus)
Grey seal (Halichoerus grypus)	Hooded seal (Cystophora cristata)
Harbor seal (Phoca vitulina)	Ringed seal (Pusa hispida)

Source : Internet

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Japanese Encephalitis: An Arboviral Zoonosis

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Abstract

Japanese encephalitis (JE) is a re-emerging mosquito-borne zoonotic flaviviral disease. The virus is found in pigs and birds, and is passed to mosquitoes that bite the infected animals. It is more common in rural areas where pig farms and rice fields are common. The virus caused huge mortality in children in several Asian countries. The children below 15 year of age are highly susceptible and affected children suffer from neurological sequel. The virus causes abortion in sows, encephalitis in horses, however other species of animals remain asymptomatic. The increased paddy and rice cultivation, presence of pigs near human dwellings, change in climatic condition and lack of vaccination in human host contributes to the disease occurence. There is currently no cure for Japanese encephalitis other than symptomatic treatment. Diagnosis is based on virus isolation, molecular and serological approaches. Prevention and control measures include vaccination of susceptible population, control of vector, use of personal protection equipments, mass awareness and education, early diagnosis and treatment with continuous sero surveillance in animals.

Keywords : Flavivirus, Sero surveillance, Re-emerging zoonosis, sequel and encephalitis.

Introduction

Japanese encephalitis (previously known as Japanese B encephalitis) is an arboviral disease caused by the Japanese encephalitis virus (Solomon, 2006) belonging to the family Flaviviridae, which infects both human and animals. It is a disease of high public health significance as half of the world population is living in JE endemic areas (Ghosh and Basu, 2009). Disease is prevalent in Southeast Asia and East Asia. It is a leading cause of viral encephalitis and neurological infection in Asia with 70,000 clinical cases reported annually (Campbell *et al.*, 2011).

The disease was first recognized in India in 1955 when cases of encephalitis from Tamil nadu and Andhra Pradesh were admitted to Vellore and were serologically identified as JE. Virus was isolated from mosquitoes in the same year, followed by isolations from patient in the same area in 1958. Since 1972, it spread to new areas like West Bengal, UP, Assam, Manipur, Bihar, AP, Pondicherry, Karnataka, Kerala, Maharashtra and Goa. In south India, it occurs in children below 15 years and in all age groups in north India, its occurrence is higher in males than females.

Lifecycle of the virus involves birds (especially herons and egrets) as reservoirs or natural hosts (Acha and Szyfres, 2003; Pant, 2006; Ghosh and Basu, 2009), mosquitoes as vector, pigs as amplifying host (Guerin and Pozzi, 2005) and

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human being and horses as incidental and dead-end hosts (Acha and Szyfres, 2003; Monath and Heinz, 1996; Weaver and Barrett, 2004; Solomon, 2006).). Among the vectors, *Culex tritaeniorhynchus* and *Culex vishnui* are the most common. In November 2011, the Japanese encephalitis virus was reported in *Culex bitaeniorhynchus* in the Republic of Korea (Kim *et al.,* 2011). Virus is normally present in humans, especially in children, as an in apparent infection, but may cause febrile response and sometimes encephalitis. It may cause fatal encephalitis in horses (OIE, 2004) and infection in swine is asymptomatic, except in pregnant sows, where abortion and fetal abnormalities are common sequelae.

Etiology

The Japanese encephalitis virus is closely related to the West Nile virus (WNV), Murray Valley encephalitis virus (MVEV) and St. Louis encephalitis virus (SLEV) (Mackenzie *et al.,* 2004). Japanese encephalitis virus is an enveloped virus of the genus flavivirus having positive sense single-stranded RNA genome packaged in the capsid formed by the capsid protein, surrounded by a lipid bilayer composed of the two membrane glycoproteins, E (50 kDa) and prM (22kDa) which later being replaced by a shorter M protein (8 kDa) in case of extracellular virions.

Based on the envelope gene, there are five genotypes (IV). The Muar strain, isolated from a patient in Malaya in 1952, is the prototype strain of genotype V (Uchil and Satchidanandam, 2001). Genotype IV appears to be the ancestral strain, and the virus appears to have evolved in the Indonesian Malayasian region. The genotype III is the most widely distributed genotype with the prototype Nakayama strain belonging to it (Mackenzie *et al.,* 2004). It is widely distributed in Asian countries, including India. However, during the past decade, genotype I has been introduced into South Korea, Thailand and China and has replaced the genotype III strains that had been circulating in Japan and Vietnam during the mid-1990s (Nga *et al.,* 2004). In India, till 2007, all the JEV strains isolated belonged to genotype III (Uchil and Satchidanandam, 2001; Mackenzie *et al.,* 2004), but later genotype I has also emerged and strains belonging to both genotypes were isolated during an outbreak in Gorakhpur in 2009 (Fulmali *et al.,* 2011).

Epidemiology

The virus is maintained in nature by a complex natural cycle involving vertebrate species like pigs, ardeid birds and mosquitoes, which act as reservoir and amplifier hosts. Human is the dead end host due to low viremia. Human to human transmission is not reported till date.

Vectors

JEV has been isolated from 16 species of mosquitoes *(Culex-10, Anopheles-3 and Mansonia-3)* in India. Mosquitoes belonging to *C. vishnui* group, which includes *C. tritaeniorhynchus, C. vishnui* and *C. pseudovishnui,* are the most common vectors of JEV in India. The virus is mainly transmitted by *C. tritaeniorhynchus* mosquito which is prolific in rural areas where their larvae breed in ground pools and especially in flooded rice fields, paddy fields and drainage ditches, thus making the disease a major concern in rice growing areas. The average life period of mosquito is about 21 days and mosquito remain infected throughout the life. *Culex* mosquito can fly for a distance of 1-3 km or even more and transmit virus to other susceptible host. Transovarial transmission is an important mechanism for maintenance of virus in nature in culicine mosquitoes.

Transmission

Female mosquitoes become infected after feeding on domestic pigs and wild birds infected with the Japanese encephalitis virus. Infected mosquitoes then transmit the Japanese encephalitis virus to humans and animals after an extrinsic incubation period of 9 to 12 days. The Japanese encephalitis virus is amplified in the blood systems of domestic pigs and wild birds, chiefly Ardeid (wading) birds. All elements of the transmission cycle are prevalent in rural areas of Asia, and human infections occur principally in this setting. Because vertebrate-amplifying hosts and agricultural activities may be situated within and at the periphery of cities, cases of Japanese encephalitis are occasionally reported from urban locations. The virus tends to spill over into human populations when infected mosquito populations build up explosively and the human biting rate increases (these culicines are normally zoophilic, i.e. they prefer to take blood meals from animals). Transplacental transmission resulting in the abortion of embryo has been reported in human (Chaturvedi et al., 1980). Boar semen is also responsible for the transmission of JE virus with infected boars showing high level of viraemia (Guerin and Pozzi, 2005). Laboratory acquired infections have also been reported (Steffen, 1987).

Japanese encephalitis virus is transmitted seasonally. In temperate regions, it is transmitted during the summer and early fall, approximately from May to September. In subtropical and tropical areas, seasonal patterns of viral transmission are correlated with the abundance of vector mosquitoes and of vertebrate-amplifying hosts. These, in turn, fluctuate with rainfall, with the rainy season, and with migratory patterns of avian-amplifying hosts. In some tropical locations, however, irrigation associated with agricultural practices is a more important factor affecting vector abundance, and transmission may occur year-round.

Signs and Symptoms

Japanese encephalitis has an incubation period of 5 to 15 days and the vast majority of infections are asymptomatic. Only 1 in 250 infections develop into encephalitis. Mild infections occur without apparent symptoms other than fever with headache. More severe infection is marked by quick onset, headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, occasional convulsions (especially in infants) and spastic paralysis. Mental retardation developed from this disease usually leads to coma. Life-long neurological defects such as deafness, emotional liability and hemiparesis may occur in those who have had central nervous system involvement. In known cases, some effects also include nausea, headache, fever, vomiting and sometimes swelling of the testicles. The case fatality rate can be as high as 60% among those with disease symptoms; 30% of those who survive from lasting damage to the central nervous system. In areas where the JE virus is common, encephalitis occurs mainly in young children because older children and adults have already been infected and are immune.

Subclinical infection with no clinical signs were reported in cattle, sheep, goat, dog, cat and chicken (Pant *et al.,* 2006). Birds and pigs are effective viraemic hosts and help in perpetuation of the disease in nature. In pigs, viraemia occurs with a high titre and lasts for 2-4 days without displaying any overt clinical signs except for abortion and still birth in pregnant sows (Acha and Szyfres, 2003; Guerin and Pozzi, 2005). In equines, the infection is in apparent though some cases may show encephalitis, pyrexia, depression, photophobia, muscle tremors and ataxia (Burke and Monath, 2001; Acha and Szyfres, 2003). The affected horse may collapse, fall in a coma and eventually die. Mortality rate due to JE in equines range between 5 percent and 30 per cent (Gulati *et al.,* 2011).

Diagnosis

Diagnosis is based on virus isolation, molecular and serological approaches specifically with little emphasis on disease history, virus exposure and clinical features. The JEV can be isolated using cell culture system, intra-cerebral inoculation of suckling mice and mosquito. Suitable clinical specimens for virus isolation are blood, serum, CSF, brain and spinal cord in equines, while blood and aborted foetuses in case of pigs are useful. JE is most commonly diagnosed by detection of specific antibodies in serum or CSF. Different serological tests viz., plaque reduction neutralization test, virus neutralization test, haemagglutination inhibition test, enzyme linked immunosorbent assay and latex agglutination assay can be employed for diagnosis of JE in animals (OIE, 2010). Definitive early diagnosis has led to the development of nucleic acid based assays viz., reverse transcription PCR (RT-PCR), real time RT-PCR, reverse transcription loop mediated isothermal amplification (RT-LAMP). RT-PCR has been used for detection of JEV from blood, brain and CSF (Lian et al., 2002).

Treatment

There is currently no cure for Japanese encephalitis. Antibiotics are not effective against viruses, and no effective anti-viral drugs have been discovered. The treatment is targeted at symptomatic relief (Solomon, 2000) which involves supporting the functions of the body as it tries to fight off the infection with fluid therapy, oxygenation and medication to treat any symptoms. Up to one in every three people who develop more serious symptoms die, and many of those who survive are left with permanent brain damage.

Prevention and Control

The prevention and control strategies must be designed to cover several important factors viz., mosquito control and intensive vaccination of humans, control of amplifying host, regular veterinary surveillance and public awareness to prevent human to get infected. Infection with Japanese Encephalitis confers lifelong immunity. The neutralizing antibody persists in the circulation for at least two to three years, and perhaps longer (Gambel et al., 1995; Kurane and Takashi, 2000). The total duration of protection through vaccination is unknown, but there is no firm evidence for protection beyond three years, so boosters are recommended every three years for people who remain at risk. There are currently three vaccines available: SA14-14-2, IC51 and ChimeriVax-JE (Schioler et al., 2007) based on the genotype III virus. A formalin-inactivated mouse-brain derived vaccine was first produced in Japan in the 1930s but its high cost due to use of live mice made it unaffordable and unavailable as part of a routine immunization program (Solomon, 2006). In September 2012 an Indian firm Biological E Limited has launched an Inactivated Cell culture derived vaccine based on SA 14-14-2 strain.

Mosquito control aims at reducing mosquito density through residual sprays, fogging and biological control by larvivorous fishes, environmental sanitation and proper irrigation management of paddy fields and use of personal protective measures (using repellents and/or mosquito nets) to avoid mosquito breeding and subsequent risk of mosquito bite. Certain water management measures (alternate wetting and drying) may be applied that reduce vector populations. Control of amplifying host involves slaughtering and vaccination of pigs to prevent pigs getting infected, decreases amplification of virus and reduces risk of abortion. Continuous vector surveillance and seromonitoring in pigs are very essential in determining the virus activity in endemic areas (NVBDCP, 2014). Increased vector activity coupled with detection/isolation of virus in mosquitoes and or sudden appearance

of antibodies in pigs against JEV can be used to forecast JE outbreak in humans (Dhanze et al., 2014). Public health awareness and mass education are necessary to make aware people regarding importance of vaccination, sanitation and personnel protection to prevent and combat JE successfully.

References

Solomon T. (2005) Control of Japanese encephalitis - within our grasp. *New England Journal of Medicine*; **355**: 869871.

Ghosh D, Basu A. (2009) Japanese Encephalitis A Pathological and Clinical Perspective. *PLoS Neglected Tropical Diseases* ; **3**: 437-441.

Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, et al. (2011) "Estimmated global incidence of Japanese encephalitis: a systematic review". Bull World Health Organ; **89**: 766774. doi:10.2471/BLT.10.085233.

Acha PN, Szyferz B. (2003) Japanese encephalitis. In: Zoonoses and Communicable Diseases Common to Man and Animals. *Vol. III, Chlamydioses, Rickettsioses and Viroses, 3rd edn, PAHO, NY, Washington DC, USA.* ; 172-176.

Pant GR. (2006) A serological survey of Pigs, Horses, and Ducks in Nepal for evidence of infection with Japanese encephalitis virus. *Annals of New York Academy of Sciences* ; 1081: 124-129.

Guerin B, Pozzi N. (2005) Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. *Theriogenology* ; **63**: 556-572.

Monath TP, Heinz FX. (1996) Flaviviruses. *In: Fields Virology, 3rd edn, Lippincott-Raven Publishers, Philadelphia* ; 961-1034.

Weaver SC, Barrett ADT. (2004) Transmission cycles, host range, evolution and emergence of arboviral disease. *Nature Reviews Microbiology* ; **2**: 789-801.

Kim, Heung Chul, Terry AK, (2011) Ratree Takhampunya, Brian PE, Sirima M, Ampornpan K, *et al*. Japanese Encephalitis Virus in Culicine Mosquitoes (Diptera: Culicidae) Collected at Daeseongdong, a Village in the Demilitarized Zone of the Republic of Korea. *Journal of Medical Entomology* ; **48** : 12501256.

OIE. (2004) Japanese encephalitis. *In: Manual of diagnostic tests and vaccines for terrestrial animals,* Part II, Chapter 2.5.14.; 1-8.

Mackenzie JS, Gubler DJ, Petersen LR. (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and Dengue viruses. *Nature Medicine* ; **10**: S98- S109.

Sumiyoshi H, Mori C, Fuke I, Morita K, Kuhara S, Kondou J. et al. (1987) Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. *Virology* ; **161**: 497-510.

Solomon T, Ni H, Beasley DW, Ekkelenkamp M, Cardosa MJ and Barrett AD. (2003) Origin and evolution of Japanese encephalitis virus in Southeast Asia. *Journal of Virology* ; **77**: 3091-3098.

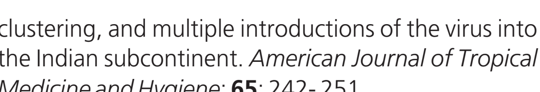
Chambers TJ, Hahn CS, Galler R and Rice CM. (1990) Flavivirus genome organization, expression, and replication. *Annual Review of Microbiology* ; **44**: 649-688.

Wallis TP, Huang CY, Nimkar SB, Young PR and Gorman JJ. (2004) Determination of the disulfide bond arrangement of dengue virus NS1 protein. *Journal of Biological Chemistry* ; **279**: 20729-20741.

Yusof R, Clum S, Wetzel M, Krishna Murthy HM and Padmanabhan R. (2000) Purified NS2B/NS3 serine protease of dengue virus type 2 exhibits cofactor NS2B dependence for cleavage of substrates with dibasic amino acids in vitro. *Journal of Biological Chemistry* ; **275**: 9963-9969.

Matusan A, Pryor M, Davidson A and Wright P. (2001) Mutagenesis of the Dengue virus type 2 NS3 protein within and outside helicase motifs. *Journal of Virology* ; **75**: 9633-9643.

Uchil PD, Satchidanandam V. (2001) Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic



clustering, and multiple introductions of the virus into the Indian subcontinent. *American Journal of Tropical Medicine and Hygiene*; **65**: 242- 251.

Nga PT, Del Carmen Parquet M, Cuong VD, Ma SP, Hasebe F et al. (2004) Early isolates of Japanese encephalitis virus in Vietnam: implications for frequent introductions of JEV from Southeast Asia to East. *Asian Journal of General Virology* ; **85**: 1625-1631.

Fulmali PV, Sapkal GN, Athawale S, Gore MM, Mishra AC and Bondre VP. (2011) Introduction of Japanese Encephalitis Virus Genotype I, India. *Emerging Infectious Disease* ; **17**: 319-321.

WHO. (2006) Guidelines for prevention and control of Japanese encephalitis. Published by NICD, Delhi. ; 1-18.

Chaturvedi UC, Mathur A, Chandra A, Das SK, Tandon HO, Singh UK. (1980) Transplacental infection with Japanese encephalitis virus. *Journal of Infectious Diseases* ; **141**: 712-715.

Steffen R. (1987) Vaccinating against Japanese encephalitis. *Lancet* ; **2**: 511.

Burke DS, Monath TP (2001) Flavivirus. In: Fields Virology, 4th Edn. Lippincott Williams and Wilkins, Philadelphia ; 1043-1125.

Gulati BR, Singha H, Singh BK, Virmani N, Khurana SK, Singh RK. (2011) Serosurveillance for Japanese encephalitis virus infection among equines in India. *Journal of Veterinary Science* ; **12**: 341-345.

OIE. (2010) Japanese encephalitis. *In: Manual of diagnostic tests and vaccines for terrestrial animals,* Part II, Chapter 2.1.7 ; 1-11.

Lian WC, Liau MY, Mao CL. (2002) Diagnosis and genetic analysis of Japanese encephalitis virus infected in horses. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health* ; **49**: 361-365.

Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT. (2000) Japanese encephalitis. *Journal of Neurology Neurosurgery and Psychiatry* ; **68**: 405415.

Gambel JM, DeFraites R, Hoke C. (1995) Japanese encephalitis vaccine: persistence of antibody up to 3 years after a three-dose primary series. *J Infect Dis* ; **171**: 1074.

Kurane I, Takashi T. (2000) Immunogenicity and protective efficacy of the current inactivated Japanese encephalitis vaccine against different Japanese encephalitis virus strains" . *Vaccine* ; **18**: 3335.

Schiøler KL, Samuel M, Wai KL. (2007) Vaccines for preventing Japanese encephalitis". *Cochrane Database Syst Rev* ;CD004263.

NVBDCP. (2014) Operational Guidelines National Programme for Prevention and Control of Japanese Encephalitis/ Acute Encephalitis Syndrome; 1-114.

Dhanze Himani, H.B. Chethan Kumar, K.N. Bhilegaonkar, A. Kumar. (2014) Japanese Encephalitis: A Veterinary Perspective. *Journal of Foodborne and Zoonotic Diseases* ; **2**: 59-67.

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Canine Transmissible Venereal Sarcoma (CTVS) - a review

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Abstract :

CTVS is a sexually transmitted sarcoma, usually found in the external genitalia of dogs. The extra-genital locations of the tumor have also been reported. The transmission, susceptibility, clinico-pathological features, cytogenetics and immunological aspects of CTVS are reviewed.

Introduction

Canine transmissible venereal sarcoma (CTVS) is usually a sexually transmitted neoplasm of the external genitalia of dogs. This tumour is unique in oncology, because it was the first tumour to be transmitted experimentally, this being achieved by the Russian veterinarian Nowinsky in 1876. This stimulated a lively interest among scientists and became a new starting point for the study of oncology. CTVS has been studied and reported extensively since the early days of nineteenth century, but a recent, widely circulated review of this condition has not been published. This review is designed to specify the epidemiological, pathological and immunological concepts related to CTVS.

Distribution

CTVS has been reported from many regions of the world The disease is mostly seen in free-roaming, sexually active dogs in tropical and subtropical countries, particularly in the cities and rural parts of the Southern United States, Central and South America, South-East Europe, Ireland, Japan, China, the Far East, Middle-East and parts of Africa. In Japan, it is the most common tumour in dogs. It is common in South-west France, where it appears in an enzootic form in some areas but it is not seen in Sweden and is rare in Denmark and Great Britain.

In India, it is the most common tumour of dogs owing to uncontrolled breeding practices. The prevalence of CTVS in Punjab was 23.5% to 28.6% of the total number of tumours in canine patients. The ignorance of the dog owners and lack of stringent legislation for monitoring the sexual health of dogs are the main causes of the widespread distribution of this disease throughout the country. While working in the different parts of India, the authors have come across clinical cases of CTVS in the desert of Rajasthan; arid zones of Haryana, Madhya Pradesh, Bihar and W. Bengal; sub-Himalayan Uttar Pradesh and Bengal; the Ganges basin of Uttar Pradesh, Bihar and W. Bengal; the East and West coasts, Chotanagpur and Gondowana plateau; and in the Himalayan cities of Nainital, Musouri and Darjeeling. This suggests a homogeneous distribution of the neoplasm, irrespective of the diversified altitudes and climates. In rural areas it is more predominant, possibly because of the sylvan habitat and lack of adequate veterinary services.

Transmission and Aetiology

In 1876, Nowinsky succeeded in transplanting the tumour from one dog to another by rubbing the excised tumour on the scarified genital

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mucosa of a susceptible dog. The violent exertions associated with coitus in dogs render both sexes prone to genital injury and increased susceptibility to transplantation of the tumour cells. The growth generally appears within 2 to 6 months of first mating. The tumour may also be transmitted when a susceptible dog first licks the genitals of an affected dog and then its own or those of another susceptible dog. Dass and colleagues (1986) described this tumour as a `naturally occurring allograft'. Experimentally, Karlson and Mann (1952) succeeded in passaging the tumour through 40 generations of dogs over a period of 17 years. Of the 564 dogs involved, 68% developed tumours and there were no changes in the histopathology of tumour during the passage. Transmission of the venereal tumour occurs only by transplantation of viable tumour cells and not by a virus that transforms cells in a susceptible host (Rust, 1949). Oncogenic viral particles have never been seen in the tumour cells with the electron microscope (Moulton, 1990). However, some authors have claimed transmission with cell-free filtrates and C-type particles have been reported to be associated with this tumour which would suggest that the agent may be a type C retrovirus.

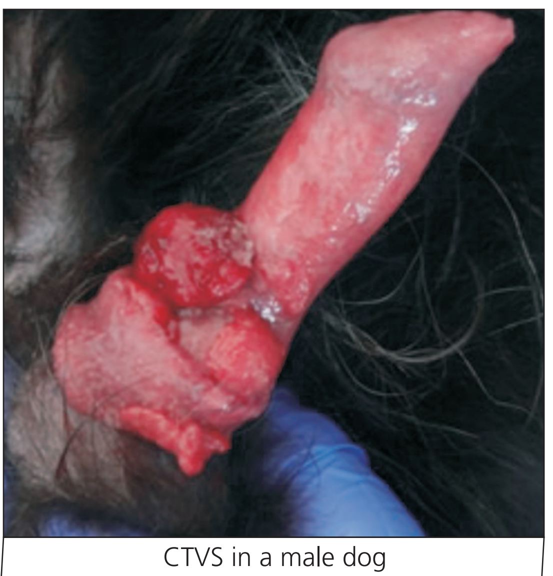
Season, Age, Breed and Sex Susceptibility

Venereal tumours are most common during the period of maximum sexual activity in dogs and the animals are particularly at highest risk when females exhibit the signs of oestrus. Dogs of any breed, age or sex are susceptible (Betamuzi, 1992). Although dogs over one year of age are at high risk in endemic areas (Bashford et al., 1905), CTVS is most common in dogs 2 to 5 years old (Higgins, 1966). The mean age of the affected dogs was 4.2 years with a range of 3.9-4.5 years. On the other hand, vulvar or vaginal tumours in dogs other than CTVS showed a higher prevalence in the age group of 10-11 years. The tumour is never found in virginal females. Moulton (1990) found females to be more susceptible than males. Naturally occurring disease may be more common in females because one infected male often mates with numerous females, both in kennels and in free range. Metastasis is mostly observed in adult male dogs. The tumour is reported to be strictly host specific for the dog and fox (Rust, 1949). However, the occurrence of this disease in related canidae may not have been noticed. There is no heritable breed-related prevalence of this tumour (Betamuzi, 1992).

Clinico-Pathological Features

Primary genital tumours

In the male dog, the tumour is usually located on the caudal part of the penis, from the crura to bulbis glandis or the area of the glans penis, and occasionally on the prepuce. In the bitch, the neoplasm is usually found in the posterior part of the vagina, often at the junction of the vestibule and the vagina. It sometimes surrounds the urethral orifice and, if it is just within the vagina, it may protrude from the vulva (Moulton, 1990).



CTVS in a male dog

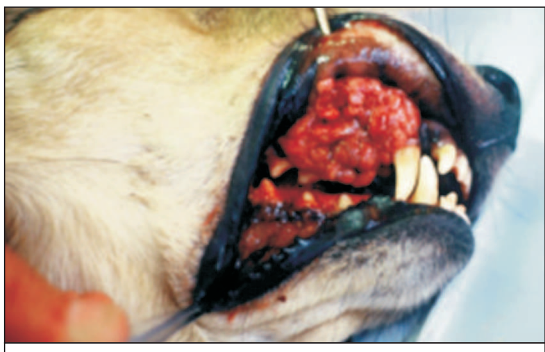
MSD
Animal Health

Tumours on the external genitalia of both sexes appear initially as small hyperaemic papules that later progress to nodular, papillary multilobulated, cauliflower-like or pedunculated proliferations measuring up to 15 cm in diameter. The mass is firm but friable, and the superficial part is commonly ulcerated and inflamed.

During rapid tumour growth, the colour is bright red owing to extensive vascularization. The tumour often oozes a serosanguinous or simple hemorrhagic fluid and eventually becomes ulcerated, with a necrotic appearance. The continuous discharge from the external genitalia, soiling the floor, carpet and even clothes, is a great nuisance for the owner. The bloody discharges may be confused with oestrus, urethritis or cystitis and, in the male, with prostatitis. In older dogs, the differential diagnosis must also include urinary bladder and urethral neoplasms. Phimosis or paraphimosis may complicate the case in the male. There are few cases on record where this neoplasm has caused actual mechanical obstruction to the flow of urine, or has produced dystocia in whelping females.

Extragenital lesions without any genital involvement

CTVS may also develop at extragenital sites, even when there are no genital lesions, e.g. on the skin or in and around the mouth. Higgins (1966) suggested that many of the cutaneous sites where these tumours are found, represent lesions caused by biting and scratching, common in stray dogs, which predispose the skin to implantation of the tumour. He observed scars in the skin above subcutaneous tumours, suggestive of previous wounds. Skin tumours were found on the back, flank, neck, head and limbs of dogs; they were usually up to 6 cm in diameter, raised above the surface, often ulcerated and bleeding.



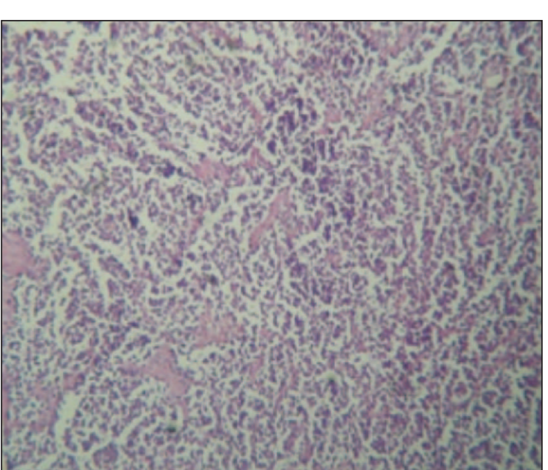
Extragenital CTVS in the oral cavity

Metastatic lesions

Metastasis occasionally occurs to the inguinal lymph nodes, the external iliac lymph nodes and cutaneous sites. Metastatic growth of this tumour has also been recognized in the tonsils, orbit brain, adenohypophysis, maxillary bone of nose, kidney and other sites. Tumours on the lips are similar to the lesions on the genitalia, but those in the mouth and on the tonsil appear more diffuse and bright red in colour. Orbital growth of the tumour may cause blindness. Metastasis develops in less than 5% of reported cases. Dass and Sahay (1989) found metastasis in about 7% of dogs with CTVS. Many of the reported cases of metastasis are actually mechanical extension of the growth or either auto- or hetero-transplantation to the skin, cervix, uterus and fallopian tubes from the tumour on the external genitalia.

Histological Features

These were studied in considerable detail early in the twentieth century, firstly by Bashford and colleagues (1905), who came to the conclusion that CTVS was not a sarcoma, but an infective granuloma, and the following year by Sticker (1906), who erroneously called it `contagious lymphoma'. Rust (1949) described this neoplasm as a lymphosarcoma; Jackson (1944) described it as a tumour of the neuro-ectodermal cells or an aortic body tumour; Mulligan (1949) described it


H & E stained section showing typical appearance of tumor cells

as a histocytoma; and Nanta and colleagues (1949) described it as an infective granuloma. According to Moulton (1990), this tumour was not morphologically similar to a histiocytoma, a mastocytoma, an aortic body tumour or a seminoma. Following electron-microscopic studies, Hernandez-Jauregui and colleagues (1973) have suggested that it is a tumour of reticuloendothelial origin. Moulton (1990) described it as a round-cell sarcoma.

Cytogenetic Features

There is a remarkable aberration in the numbers and morphology of the chromosomes of the constituent cells of CTVS. The normal number of chromosomes in the somatic cells of dogs is 78, of which all but two are acrocentric chromosomes. In CTVS, there are usually 58-59 chromosomes with 13-17 metacentric and 42 acrocentric chromosomes. These abnormal features of the tumour cells are consistent and unique, in that they have always been observed in tumours of dogs examined in different countries and different continents. The similarities between the cytogenetic features of the primary neoplasm in the genitalia and the metastatic tumour strengthen the evidence for the consistency of the cytogenetic abnormalities in the cells of CTVS. The same chromosomal pattern is also maintained in cell culture.

IMMUNITY

Specific circulating antibodies to antigens from the venereal tumour have been demonstrated in dogs bearing the tumour and these antibodies are believed to be associated with the mechanism of natural regression that commonly occurs with this tumour. Complete regression is accompanied by the development of resistance to further successful implantation and growth of the tumour cells. Spontaneous regression is due to the formation of IgG in the sera of dogs after a period (40 days) of tumour growth (Cohen, 1985).

The antibody can be demonstrated on the cell surface membrane by direct immunofluorescence, complement fixation and erythrocyte rosette formation. Spontaneous regression of experimentally transplanted CTVS is well documented and spontaneous regression of naturally occurring tumours has been alluded to by Higgins (1966). However, spontaneous regression is not recorded in most reports of naturally occurring CTVS (Rust, 1949). It is experienced that any spontaneous regression usually starts within 3 months after the implantation of the tumour and that the chance of self-regression is remote in naturally occurring CTVS if the age of the tumour is over 9 months. It seems reasonable to assume that failure on the part of the host to produce a sufficient amount of antibody may predispose to widespread mestastasis. Newborn puppies from `immune' dams (mothers with antibody to the tumour) show a longer latent period for tumour development and the neoplasms in these puppies are smaller and show more rapid spontaneous regression (Moulton, 1990).

References

Bashford, E.F., Murray, J.A. and Cramer,W., 1905. Comparison between the transmission of an infective granuloma of the dog and carcinoma of the mouse. Scientific Report, Imperial Cancer Research Fund, **2**,33-37.

Betamuzi, E.K., 1992. Risk factors associated with canine TVT in Tanzania. *Preventive Veterinary Medicine*, **13**, 13-17.

Cohen, D., 1985. The canine transmissible venereal tumour: a unique result of tumour progression. *Advances in Cancer Research*, **43**, 75-112.

Cotchin, E., 1954. Neoplasia in the dog. *Journal of Veterinary Science*, **66**, 879-888

Dass, L.L., Sayay, P.N., Khan, A.A. and Jha, G.J., 1986. Malignant transmissible venereal tumour. *Canine Practice*, **13**, 15-18.

Higgins, D.A., 1966. Observations on the canine transmissible venereal tumour as seen in the Bahamas. *Veterinary Record*, **79**, 67-71.

Karlson, A.G. and Mann, F.C., 1952. The transmissible venereal tumour of dogs: observation of forty generations of experimental transfer. *Annals of the New York Academy of Sciences*, **54**, 1197-1213

Moulton, J.E., 1990. Tumours of Domestic Animals, 3rd edn, revised), (University of California Press, Berkeley and Los Angeles), **10**, 498-502.

Mulligan, R.M., 1949. Neoplasms of the Dog, (Williams and Wilkins, Baltimore). Rust, J.H., 1949. Transmissible lymphosarcoma in the dog. *Journal of American Veterinary Medical Association*, **114**, 10-14.

Sticker, A., 1906. Transplantables rundzellensarkom des Hundes. Ein Beitrag Zur Lehre der Krebsuber-tragbarkeit. Z. Krebsforsch., 4, 227. (Cited in Cohen, D., 1972. Detection of humoral antibody to the transmissible venereal tumour of the dog. *International Journal of Cancer*, **10**, 207-212.

Congratulation

Dr. S. Abdul Rahman, former Dean, College of Veterinary Sciences, Bangalore (Karnataka) and presently the President of Commonwealth Veterinary Association and Chairman, OIE Animal Welfare Working Group, has been endowed with Meritorious Award (2015) of World Organization of Animal Health (OIE) for his contribution to the Veterinary profession through his work on Animal welfare, zoonosis with special reference to control of Rabies and for his work on Veterinary education.

The award was presented to Dr. Abdul Rahman on 24/5/2015 during the 83rd Session of OIE World Assembly of Delegates, in Paris, France.

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Blue Cross Book

The Blue Cross Book July 2015 Vol. 32 : 20-24

Kyasanur Forest Disease - a zoonotic threat

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Abstract :

Kyasanur Forest Disease (KND) is a disease caused by a virus of Flaviviridae family and known as Kysanur Forest Disease Virus (KFSV). The disease was first reported in monkeys from Kysanur forest in Karnataka, killing several of them during 1957. It is a tick transmitted disease and the host range includes rodents, bats, squirrels, monkeys and humans. The domestic animals (cattle, buffaloes, sheep and goats) are less affected. In humans, the disease characterized by high fever, frontal headache and bleeding from nose, throat and gastrointestinal tract. The morbidity in humans is reported to the extent of 1 to 2% and fatality to 3-5%. Vaccine has been made available by the National Institute of Virology, Pune/Bangaore.

Key words : Kyasanur Forest disease, zoonosis, monkeys

Introduction

The disease was first reported from Kyasanur Forest of Karnataka in India in March 1957. The disease first manifested as an epizootic outbreak among monkeys killing several of them in the year. Hence, the disease is also locally known as Monkey Disease or Monkey Fever (NichterandMark,1987).The similarity with Russian Spring-summer encephalitis was noted and the possibility of migratory birds carrying the disease was raised.(Work *et al.*,1959) Studies began to look for the possible species that acted as reservoirs for the virus and the agents responsible for transmission. The virus was found to be quite distinctive and not closely related to the Russian virus strains. Antigenic relatedness is, however, close to many other strains including the Omsk hemorrhagic fever (OHF) and birds from Siberia have been found to show an antigenic response to KFD virus. Sequence based studies, however, note the distinctiveness of OHF(Lin *et al.,* 2003). Early studies in India were conducted in collaboration with the US Army Medical Research Unit and this led to controversy and conspiracy theories (Lewisand Michael, 2002). This study also found using immune response tests that birds and humans in the region appeared to have been exposed to the virus. Another study has suggested that the virus is recent in origin, dating the nearest common ancestor of it and related viruses to around 1942, based on the estimated rate of sequence substitutions. The study also raises the possibility of bird involvement in long-distance transfer. It appears that these viruses diverged 700 years ago.

Etiology

Kyasanur Forest disease (KFD) is caused by Kyasanur Forest disease virus (KFDV), a member of the virus family Flaviviridae. KFDV was identified in 1957 when it was isolated from a sick monkey from the Kyasanur Forest in Karnataka (formerly Mysore) State, India. Since then, 400-500 humans cases per year have been reported. Hard ticks *(Hemaphysalis spinigera)* are

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the reservoir of KFD virus and once infected, remain so for life. Rodents, shrews and monkeys are common hosts for KFDV after being bitten by an infected tick. KFDV can cause epizootics with high fatality in primates

Risk Factor-

KFD has historically been limited to the Western and Central districts of Karnataka State, India. However, in November 2012, samples from humans and monkeys tested positive for KFDV in the southernmost district of the State which neighbors Tamil Nadu State and Kerala State, indicating the possibility of wider distribution of KFDV. People with recreational or occupational exposure to rural or outdoor settings (e.g., hunters, herders, forest workers and farmers) within Karnataka State are potentially at risk for infection by contact with infected ticks. Seasonality is another important risk factor as more cases are reported during the dry season, from November through June.

Epidemiology

KFDV was first recognized in 1957 when it was isolated from sick and dying monkeys in the Kyasanur Forest of the Shimoga district, Karnataka State in India. Veterinary scientists investigating the sick monkeys, as well as local people utilizing the forest, were bitten by KFDV infected ticks and developed a haemorrhagic disease. During the initial outbreak, there were 466 human cases and 181 more the following year. KFDV is common in young adults exposed during the dry season in the forest and has caused epidemic outbreaks of haemorrhagic fever affecting 100 to 500 people per year since then, with a case fatality rate that is estimated between 2 and 10%(GouldandSolomon, 2008).

Host Range

Humans, a variety of tick species, rodents (shrews, forest rats), monkeys (grey langur, black-faced langur, bonnet macaque), bats, squirrels, Indian crested porcupines, and, to a lesser extent, domestic animals i.e. goats, cows, sheep) (Burk and Monath, 2001) are indicated in the possible host range..

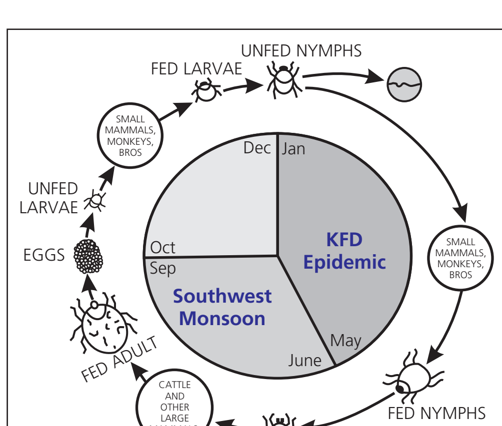
Transmission

There are a variety of animals thought to be reservoir hosts for the disease, including porcupines, rats, squirrels, mice and shrews. The vector for disease transmission is *Haemaphysalis spinigera,* a forest tick. Humans contract infection from the bite of nymphs of the tick.

LIFE-CYCLE

The KFD virus belongs to Russian Spring Summer Encephalitis group, a member of family Flaviviridae. Virionsare spherical particles, 45 nm in Ticks have a definite stage-wise seasonal activity. The adults become active after a few monsoon rains in June. The adult population reaches its peak during July and August and gradually declines in September. Each fed female lays large number of eggs. Larvae preferably feed on small animals like rodents, shrews, etc. Larval population builds up in the monsoon months but remains dormant under the forest litter and becomes suddenly active when the litter dries up during the post monsoon months - October to December.

Nymphal activity is high from January to May. Epidemics coincide with nymphalactivity; hence nymphs are considered as the most important stage for human transmission. Adult ticks feed on large animals like cattle, monkeys, etc. These large animals are good hosts for proliferation of ticks, but are not significant for virus dissemination due to insignificant viremia in them.



Pathogenicity/Toxicity

Clinical or post-mortem biopsies of organs have found that KFD infection occurs in four stages, each lasting around a week in length.

- The initial prodromal stage is brought on by a sudden onset of fever and severe headache, hypotension and hepatomegaly, sore throat, diarrhoea and vomiting, anorexia, insomnia, severe pain in the lower and upper extremities, and prostration (Brown *et al.*, 2005). Bradycardia and inflammation of the conjunctiva are also commonly observed, along with acute lymphopenia and eosinopenia which can occur within the first or second week of infection.
- The next stage is characterized by haemorrhagic complications such as intermittent epistaxis, hematemesis, melena, and frank blood in stool; neurological manifestations such as mental confusion, tremors, and abnormal reflexes; and bronchopneumonia or development of coma which may occur in some cases prior to death (Achaand Szyfres, 2003).
- A stage of recovery may be observed next, followed by a last stage of fever in certain cases. Other pathologic manifestations of KFD in human patients include parenchymal degeneration of the liver and kidney, haemorrhagic pneumonitis, and a moderate to marked prominence of the reticuloendothelial elements in the liver and spleen with marked erythrophagocytosis (Pattnaik,2006).

Incubation Period

Usually 3 to 8 days (Prabha,1993)

Communicability

No evidence for human-to-human transmission

Sign and Symptoms

The disease has a morbidity rate of 2-10%, and affects 100-500 people annually (Gould and Solomon, 2008).The symptoms of the disease include a high fever with frontal headache, followed by haemorrhagic symptoms, such as bleeding from the nasal cavity, throat, and gums, as well as gastrointestinal bleeding. (Gerhard and Dobler, 2010). An affected person may recover in two weeks time, but the convalescent period is typically very long, lasting for several

months. There may be muscle aches and weakness during this period and the affected person is unable to engage in physical activities.

After an incubation period of 3-8 days, the symptoms of KFD begin suddenly with chills, fever, and headache. Severe muscle pain with vomiting, gastrointestinal symptoms and bleeding problems may occur 3-4 days after onset of initial symptom. Patients may experience abnormally low blood pressure, and low platelet, red blood cell, and white blood cell counts.

After 1-2 weeks of symptoms, some patients recover without complication. However, the illness is biphasic for a subset of patients (10-20%) who experience a second wave of symptoms at the beginning of the third week. These symptoms include fever and signs of neurological manifestations, such as severe headache, mental disturbances, tremors, and defects in the vision. The estimated case-fatality rate is from 3 to 5% for KFD.

Treatment and Control

Although the transmission cycle of KFD virus is well documented, its control remains challenging. Measures to minimize the human tick interface are less likely to succeed considering the forest ecosystem and the dependence of local villagers on it. Control of ticks in the forest is far from easy, but health authorities need to continue educating villagers about using tick repellent before visiting the forest, especially during spring and summer, and ensure distribution of tick repellents to them. Health authorities must ensure that vaccination campaigns are initiated on time and completed before November every year. More epidemiologic studies are needed to evaluate the long-term protection offered by booster doses of vaccine. Molecular studies also are needed to understand the phylogenetic relationships of the past and contemporary strains of the virus and to identify possible sources and origins of outbreak strains.

Vaccines

National Institute of Virology (Pune / Bangaore) has developed an inactivated chick embryo tissue culture vaccine against KFD. This vaccine evokes neutralizing antibodies response in about 70% of the vaccinated persons. The technology has been transferred to the Karnataka Public Health Department for production of vaccine and vaccination.

Prevention and Control

A timely supportive therapy reduces mortality in humans. One or 2 treatments of forest floor with the insecticide Lindane was highly effective in killing ticks. This was particularly useful to clear infection following detection of monkey deaths. Tick repellents such as DEET, DMP, DBP provide 90-100% protection against tick bite. Vaccination of villagers and forest workers is effective.

Biosafety Concern

Internationally, KFD virus is ranked as one of the highest risk categories of pathogens belonging to Bio Safety Level-4. During investigations, over 100 laboratory persons got infected and suffered with the disease. Majority of the infections occurred in field during investigations on etiological agent,

References:

Acha, P. N., and Szyfres, B. (2003). Zoonoses and Communicable Diseases Common to Man and Animals (3rd ed.). Washington, D.C.: Pan American Health Organization.

AdhikariPrabha, M. R., Prabhu, M. G., Raghuveer, C. V., Bai, M., and Mala, M. A. (1993). Clinical study of 100 cases of Kyasanur Forest disease with

clinicopathological correlation. *Indian Journal of Medical Sciences,* **47**, 124-130.

Brown, R. N., Lane, R. S., and Dennis, D. T. (2005). Geographic Distributions of Tick-Borne Diseases and Their Vectors. In J. L. Goodman, D. T. Dennis and D. E. sonenshine (Eds.), Tick-Borne Diseases of Humans (1st ed., pp. 363-391). Washington, DC: ASM press.

Burke, D.S., and Monath, T.P. (2001). Flaviviruses . In D. M. Knipe, and P. M. Howley (Eds.), Fields Virology (4th ed., pp. 1046-1109). Philadelphia, PA.: Lippencott-Raven.

EA Gould; T Solomon (February 9, 2008). "Pathogenic flaviviruses". The Lancet 371 (961): 500509. Gerhard Dobler (27 January 2010). "Zoonotic tick-borne flaviviruses". Veterinary Microbiology **140** , *Zoonoses: Advances and Perspectives*): 221-228.

Lewis, Michael (2002). "Scientists or Spies? Ecology in a Climate of Cold War Suspicion". *Economic and Political Weekly* **37**: 2324-2332.

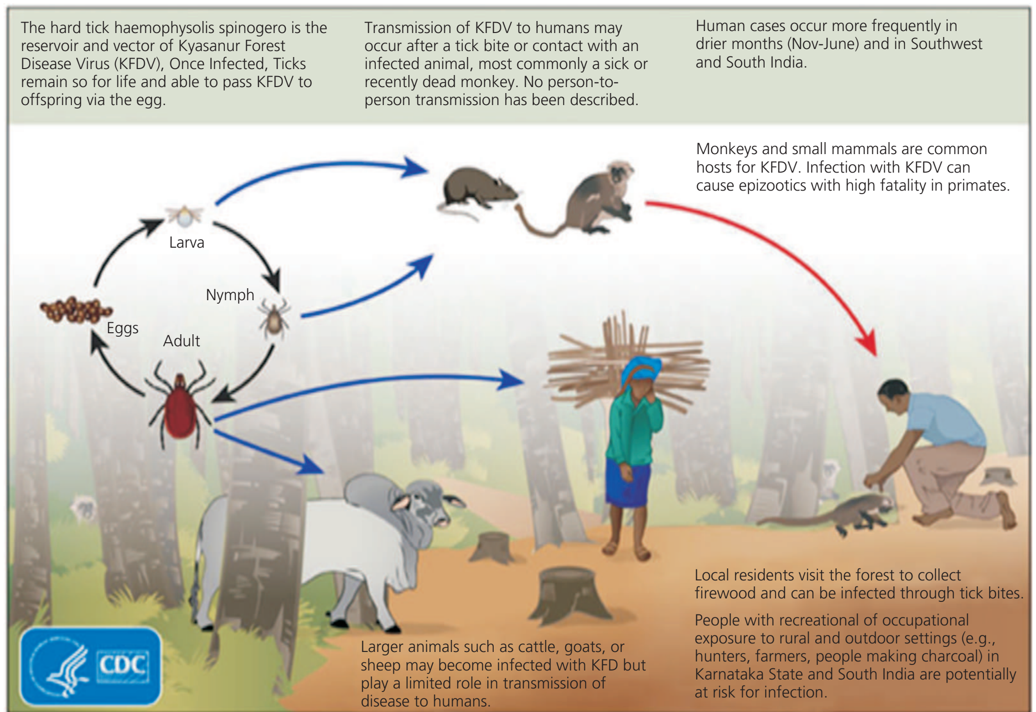
Lin D, Li L, Dick D, Shope RE, Feldmann H, Barrett AD, Holbrook MR (2003). "Analysis of the complete genome of the tick-borne flavivirus Omsk hemorrhagic fever virus". *Virology* **313**: 81-90.

Nichter, Mark (1987). "Kyasanur Forest Disease: An Ethnography of a Disease of Development". *Medical Anthropology Quarterly*, New Series **1**: 406-423.

Pattnaik, P. (2006). Kyasanur forest disease: An epidemiological view in India. *Reviews in Medical Virology*, **16**, 151-165.

Work, Telford H. Roderiguez, FR; Bhatt, PN (1959). "Virological Epidemiology of the 1958 Epidemic of Kyasanur Forest Disease". *American Journal of Public Health* **49**: 869-874.

Kyasanur Forest Disease (KFD) Virus Ecology



The Blue Cross Book July 2015 Vol. 32 : 25-28

Effect of ethanolic leaf extracts of *Senna alata* and *Lantana camara* in the treatment of bovine Dermatophilosis

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Abstract

The therapeutic efficacy of ointments prepared from ethanolic extracts of *Senna alata* and *Lantana camara* were studied on Bovine Dermatophilosis. A total of 15 infected animals were treated using ointments prepared from ethanolic extracts of leaves of Senna alata and Lantana camara, as topical application. The ointments were applied twice daily for 10–14 days. The crusts developed during the course of disease became loose and were falling off after 5-6 days of treatment. The healing without scarring and with the re-growth of hair was observed within 4-5 weeks post treatment. Follow up of 24 months of treatment revealed that animals were in good health without any recurrence of the disease. The results of the study indicated that the ointments were found to be more effective compared to parental antibiotic treatment with Strepto-Penicillin and long acting Terramycin (TLA), which failed to prevent the recurrence of the disease.

Keywords: Bovine; Dermatophilosis; Senna alata syn. Cassia alata; *Lantana camara*;

Introduction

Dermatophilosis is an economically important contagious zoonotic skin disease of livestock caused by Gram positive bacterium *Dermatophilus congolensis.* The disease has a wide host range from domestic to wild and aquatic animals (Zaria, 1993). The disease is characterized by acute and chronic, local and progressive and sometimes fatal exudative epidermatitis with serous exudation and drying to form characteristic matting of hair and scab formation (Zaria, 1993; Ambrose et al., 1999; Abdullahi 2001 and Loria et al., 2005). The severity of the disease results in gradual loss of condition, decrease in milk and meat production, reduced working ability in draft animals, failure of reproduction, deprived hide values and loss of body condition. Since the parental antibiotic treatment is ineffective against *Dermatophilosis* and further recurrence of the disease was observed in treated animals (ogwn *et al.,* 1981), an alternative to the antibiotic treatment, was tried by studying the efficacy of *Senna alata* and *Lantana camara* in bovine *Dermatophilosis.*

Material and methods

Studies were conducted in bovines infected with dermatophilosis in Chittor and Kadapa districts of Andhra Pradesh, where the high incidence of the disease was reported. The scabs from the infected animals were collected, processed and diagnosed as dermatophilosis based on direct microscopic giemsa smear examination, cultural, biochemical and by PCR methods. Fifteen cases of both cattle and buffaloes showing moist hard

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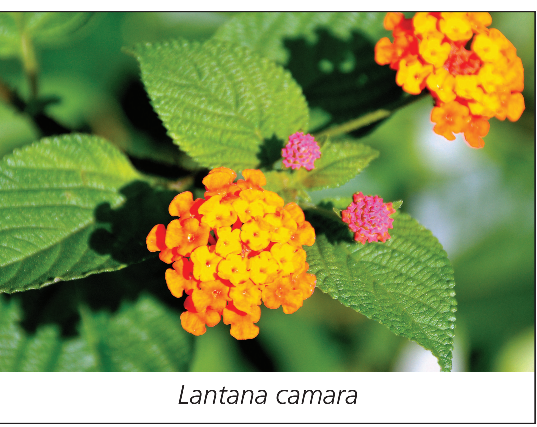
Table-1 Treatment schedule for naturally infected animals with ointments of plant extracts

S. No	Animal Reference	Cattle/ Buffalo	Lesions observed before treatment	Plant extract used	Number of applications	Start of crusts falling off
1	KDP1	C	Oozing crusty lesions on the perineum and scrotal	*Senna alata*	Twice a day for 14 days	6th day onwards
2	KDP2	B	Confluent, Mosoic crust on four limbs	*Senna alata*	Twice a day for 12 days	5th day onwards
3	KDP3	C	Hard and oozing crusty lesions on perineum	*Senna alata*	Twice a day for 13 days	6th day onwards
4	KDP4	B	Confluent, Mosoic crust on four limbs	*Senna alata*	Twice a day for 10 days	5th day onwards
5	KDP5	B	Confluent, Mosoic crust on four limbs	*Senna alata*	Twice a day for 14 days	6th day onwards
6	KDP6	B	Confluent, Mosoic crust on four limbs	*Senna alata*	Twice a day for 10 days	5th day onwards
7	KDP7	B	Moist lesions on back and rump	*Senna alata*	Twice a day for 11 days	5th day onwards
8	KDP8	C	Ventral oozing lesions	*Senna alata*	Twice a day for 14 days	6th day onwards
9	KDP9	C	Oozing crusty lesions on the perineum and scrotal	*Lantana camara*	Twice a day for 14 days	6th day onwards
10	KDP10	C	Hard crust on face and ears	*Lantana camara*	Twice a day for 10 days	5th day onwards
11	KDP11	B	Confluent, Mosoic crust on four limbs	*Lantana camara*	Twice a day for 10 days	5th day onwards
12	KDP12	B	Confluent, Mosoic crust on four limbs	*Lantana camara*	Twice a day for 14 days	5th day onwards
13	KDP13	B	Moist lesions on back and rump	*Lantana camara*	Twice a day for 14 days	5th day onwards
14	KDP14	B	Confluent, Mosoic crust on four limbs	*Lantana camara*	Twice a day for 12 days	5th day onwards
15	KDP15	C	Hard and oozing crusty lesions on perineum	*Lantana camara*	Twice a day for 14 days	6th day onwards

Senna alata


Lantana camara

crust skin lesions were selected for treatment study. The animals were treated twice a day with topical application of each ointment for 10-14 days- (Table-I).

Plant material

Sena alata (L.), synonym Cassia alata, (Family: Fabaceae) and Lantana camara (Family: Verbenaceae) were collected from the areas of Talakona forest and in and around areas of College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, AndhraPradesh. The plants were identified by the Department of Botany, Sri Venkateswara University, Tirupati. The plants were shade dried for seven days followed by oven drying at 50°C for 24 hours and powdered using a grinder (European Pharmacopeia, 2002).

Preparation of ointments with alcoholic extracts of plants :

For each plant, 500 grams of macerated powder dissolved in 4 L of ethanol (Merck) and kept under constant shaking for 72 hours. The mixture was filtered and concentrated at reduced pressure at 50°C. The yields of extraction were, 28.08% for S. alata and 15.22% for L. camara. Ointments were prepared by mixing three parts of paraffin and one part of ethanolic extract of respective extract. The resulting ointments were stored at room temperature until further use.

Diagnosis of Dermatophilosis

Dermatophilosis in cattle and buffaloes was preliminarily diagnosed by examining skin scab impression stained with Giemsa staining. The skin scab material was collected and confirmatory diagnosis was performed using polymerase chain reaction (PCR). The sequences of oligonucleotide primers specific for *D. congolensis* were employed. Forward primer:

5'-ACATGCAAGTCGAACGATGA-3'; Reverse primer : 5'-ACGCTCGCACCCTACGTATT-3'. The target amplification of 500 bp product of 16s ribosomal RNA gene was carried out as described by Shaibu et al., (2010).

Treatment Schedule

Fifteen cases of acute or chronically infected cattle and buffaloes showing moist, hard crusty lesions with confirmed diagnosis were chosen for the study. Both cattle and buffaloes were treated with topical application of the each ointment for 10-14 days as indicated in Table 1. The lesions were located on the lower parts of limbs and ventral parts of the body such as the mammary glands, scrotum and perineum area. The ointments were applied twice a day to allow prolonged contact between the ointment and wounds in order to avoid crust reformation.

Results and Discussion

Among the parental and topical treatments, topical treatment is considered as effective way of limiting the spread of the skin lesion of Dermatophilosis. During the present study, the topical application of ethanolic extracts of *Senna alata* and *Lantana camara* ointments, was found to initiate softening and dislodging the hard crusts after sixth day of application, there by inhibiting the growth of *D. congolensis*. Similar findings were reported by Wilson and Amakiri (1989) in their treatment studies by fifth or sixth day. Progressive healing and regrowth of hair was observed after 10 days of treatment during the present study. Complete healing without any scar was observed between 4-5 weeks post treatment probably due to long contact of the ointment with the skin. The results of the present investigation indicated that non recurrence of the disease over the follow period of 24th months of treatment. These observations were in accordance with Lioyd et al (1990) and Damodaran and Venkata Ramanan (1994). The study also showed that the ointments can also act as good fly repellents and reduce the risk of secondary infections as supported by the study of Ali-Emmanuel (2003). Further, the ointments were shown to cure ordinary wounds caused by thorny bushes and trauma within 4 weeks during the period of study.

Conclusion:

In conclusion, ethanolic extracts of Senna alata and Lantana camara could successfully be used to treat and limit the spread of dermatophilosis without any risk of overdose and toxicity. Further, the usage of these ointments are less expensive , reduce the cost of treatment, easy to prepare and prevent the reccurrence of the disease.

References

Abdullahi, U.S., 2001. Chemotherapeutic and chemo prophylactic control of bovine dermatophilosis. PhD Thesis Ahmadu Bello University, Zaria, Nigeria, pp: 66-68.

Ali-Emmanuel, N., Moudachirou, M., Akakpo, J.A., Quetin-Leclercq, J., 2003. Treatment of bovine dermatophilosis with Senna alata, Lantana camara and Mitracarpus scaber leaf extracts, *Journal of Ethno Pharmacology*, **86**:167-171.

Ambrose, N., Lloyd, D., Mailard, J.C., 1999. Immune responses to D. congolensis infection. *Parasitol Today*, **15**, 295-300.

Damodaran, S., Venkataraman, S., 1994. A study on the therapeutic efficacy of Cassia alata, Linn., leaf extract against Pityriasis versicolor. *Journal of Ethnopharmacology* **42**, 19–23.

European Pharmacopeia, (2002). European Council, Strasbourg.

Lloyd, D.H., Noble W.C., 1982. Interaction between antibiotic producing bacteria and D. congolensis: a potential therapeutic tool. In: Woodbine, M. (Ed.), Antimicrobial Agents and Agriculture. Butterworths, London, pp. 277–283.

Lloyd, D.H., Hawkins, J.P., Pragnell, J., 1990. Efficacy of long acting oxytetracycline in the treatment and control of bovine dermatophilosis. *Veterinary Dermatology* **1**, 79–84.

Loria, G.R., Babera, E la., Monteverde, V., Piraino, C., Caracappa, S. 2005. Dermatophilosis in goats in Sicily. *Veterinary Record*, vol. 156 No. 4pp. 120-121.

Mannan, M.A., Khan, M.S.R., Rahman, M.M., Begum, F., Uddin, M.Z., 2009. Isolation and identification of Dermatophilus Bacteria from skin lesions of cattle, *Bangl. J. Vet. Med*. **7**: 342 – 347.

Ogwu, D., Alhaji, S., Osori, D.I.K., 1981. Effectiveness of long action terramycin injectable solution in the treatment of streptothricosis in cattle. *British Veterinary Journal* **137**, 585–589.

Wilson, O.B., Amakiri, S.F., 1989. Chemotherapy of dermatophilosis in a herd of Fulani cattle. *Bulletin of Animal Health and Production in Africa* **37**, 315–317.

Zaria, L.T., 1993. Dermatophilus congolensis infection (Dermatophilosis) in animals and man, an update. *Comp. Immunol. Infect. Dis*., **16**: 197-222.

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Evaluation of different strategies for improving reproductive efficiency of Postpartum Anoestrus Jaffarabadi Buffaloes

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Abstract:

Thirty postpartum anoestrus buffaloes were examined gynaeco-clinically, dewormed with Fenbendazole 3g orally and were randomly divided into three groups, viz., A) Inj. GnRH (Receptal) 5.0 ml, im once (n=10), B) Inj. inorganic phosphorus 15 ml, Inj. vitamin AD3E 10 ml, im, OID and Herbal heat inducer bolus Heat plus OID (n=10) and C) untreated control group of buffaloes (n=10). The higher (80.00 %) estrus induction response was observed in buffaloes of group-A followed by group-B (60.00 %),) and group-C (only 30 %). The highest conception rate (87.50%) and highest reproductive efficiency (70.00 %) was observed for group-A buffaloes. The corresponding values for group-B were 83.33 per cent, and 49.99 per cent, only 30.00 per cent (3/10) buffaloes of group-C exhibited oestrus and two of them conceived with the reduced reproductive efficiency (20.00 %). It was concluded that in buffaloes, injection GnRH had more beneficial effect on reproductive performance, better oestrus induction response with improved conception rate than other treated and control groups.

Key words: Postpartum anoestrus buffaloes, oestrus induction response, conception rate.

Introduction

In India, the incidence of postpartum anoestrus in buffalo herds ranges from 20 to 80%, with the greatest incidence during hot summers (Nanda *et al*., 2003). The period of postpartum anoestrus or anoestrus is usually longer in buffalo than in cattle under comparative management conditions (Jainudeen and Hafez, 1993). Under optimal conditions, buffalo resume anoestrus by 30–90 days, but factors such as poor nutrition and body condition (Baruselli *et al.,* 2001), suckling management, climate (Nanda *et al.,* 2003) and nutrition (through feed quality, availability) can also delay this considerably.

Postpartum anoestrus in buffaloes is a major cause of infertility, resulting in economic loss to buffalo breeders in many countries. In Egypt, India and Pakistan only 34–49% of animals showed oestrus during the first 90 days after calving, while 31–40% remained anoestrous for more than 150 days (El-Wishy, 2007). Thus, an attempt has been made to find a more effective regimen to induce oestrus in postpartum Jaffrabadi buffaloes for improving reproductive efficiency.

Materials and Methods

The present study was conducted on postpartum anoestrus buffaloes having completed 90 days after calving at Cattle Breeding Farm JAU,

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Treatment groups	No. of animals treated	Oestrus induction response		Conception rate				Sub-mission rate (%)	Reprodu-ctive efficiency (%)
		No.	(%)	No. of animals conceived	Ist service (%)	Iist service (%)	*Overall (%)		
A (Receptal-GnRH)	10	08	80.00[a]	07	06	01	87.50[a]	80.00	70.00
B (Tonophos-phan, Vetade & Heat Plus)	10	06	60.00[a]	05	04	01	83.33[a]	60.00	49.99
C (Control)	10	03	30.00[a]	02	01	01	66.67[a]	30.00	20.00

Values within columns with different superscripts are significantly different ($P<0.01$).

Junagadh in Gujarat during the months of December, 2013 to April, 2014. The buffaloes covered under the study were divided into three groups. They were monitored for two to three months from 90th day of postpartum period. The effect of treatment on induction of oestrus, improvement in conception, submission rate and reproductive efficiency was carried out for all the treated as well as untreated control groups of buffaloes. The reproductive efficiency of buffaloes was calculated by following formulae.

$$\text{Submission Rate (\%)} = \frac{\text{No. of buffaloes served those are listed}}{\text{No. of buffaloes those are listed}} \times 100$$

$$\text{Reproductive Efficiency (RE \%)} = \frac{\text{Submission rate X overall pregnancy rate}}{100}$$

Anoestrus buffaloes of Group-A (n=10) were treated with single Injection of GnRH, 5 ml, i/m (Buserelin acetate 0.0042 mg/ml equivalent to 0.004 mg Buserelin/ ml "Receptal", Intervet International GmbH, Germany), In Group-B the anoestrus buffaloes (n=10) were treated from day 90 postpartum with intramuscular injection of Tonophosphan (Sodium salt of 4-dimethylamino-2-methylphenyl phosphoriic acid 0.2g/ml, Intervet) 15ml im and Vetade (Vitamin AD3E, Zydus) 10ml i/m per animal OID and herbal heat inducer (Heat Plus) One bolus to each animal orally for induction of oestrus. The animals treated were observed for detection of estrus and served by Artificial Insemination. The non-returned buffaloes were examined for pregnancy per-rectum 60 days post-AI. Group-C (n=10) untreated control buffaloes were followed for natural exhibition of oestrus and served by AI, if required.

Results and Discussion

In group-A, 80 (08/10) percent treated buffaloes exhibited oestrus and among those seven buffaloes conceived. The higher oestrus induction response and conception rate in GnRH treated group was attributed to resumption of

ovarian activity and exhibition of early oestrus in this group of buffaloes. The present findings of 80.00 per cent oestrus induction response and 87.50 per cent conception rate after GnRH treatment, respectively, were quite appreciable as against only 30 per cent exhibited oestrus and 66.67 per cent conception rate found in untreated control group. This finding is in agreement with the study of , Sheshpppa *et al*. (2002) and Khasatiya *et al*. (2004), and it partly collaborated with those of Dugwekar *et al*. (2006), wherein, either oestrus induction response or conception rate were comparable with the present findings.

On the contrary, Dhoble and Gupta (1986) found oestrus response only in 12 of 53 (22.64 per cent) anoestrus buffaloes within 15 days of GnRH (21 μg) treatment while, Reddy *et al*. (1994) found lower oestrus induction response (50 per cent) with only 40 per cent conception using 5 ml Receptal. Zaghloul *et al*. (1993) recorded only 60 per cent oestrus response within 25 days of GnRH (500 μg Fertagyl) treatment, but found 100 per cent fertility in postpartum acyclic buffaloes. Little lower rate of oestrus induction and conception as noticed in their study might be due to the lower dose of GnRH, nutritional status and the effect of season.

The findings of oestrus induction response and conception rate clearly indicated that resumption of ovarian cyclicity with ovulatory oestrus can be effectively induced with GnRH treatment in anoestrus buffaloes, thereby achieving the goal of augmenting reproductive efficiency for better economic return.

In group-B, treated buffaloes showed 60.00 (06/10) per cent oestrus induction response. Buffaloes responded were inseminated at induced oestrus. The overall conception rate was 83.33 (5/6). Whereas, submission rate and reproductive efficiency were 60.00 per cent and 49.99 per cent, respectively. The present findings of oestrus induction response and conception rate colloborated well with the reports of Akhtar *et al*. (2004) and Srivastava (2008) who recorded oestrus induction response and conception rate as 50 and 80 per cent within 10 and 30 days following Tono-Prepaline therapy for 2 weeks. However, the oestrus induction response and conception rate were reported to be very low (23 per cent) with simple oral supplementation of macro-micro minerals for 30 days in anoestrus buffaloes during summer. On the contrary, Randhawa *et al*. (2004) reported higher induction of oestrus (90.30 per cent). They injected 150 mg copper glycinate sc in 30 anoestrus buffaloes. Butani *et al*. (2008) also reported higher oestrus induction (82.08 per cent) and conception rate (69.10 per cent) than that of the present study.

In the present study, Tonophosphan plus Vetade i/m treatment had beneficial effect on reproductive performance as compared to untreated control group. The mean oestrus induction response, conception rate and reproductive efficiency of Tonophosphan plus Vetade + Heat Plus group vs control group was 60.00 vs 30.00 per cent, 83.00 vs 66.67 per cent, and 49.99 vs 20.00 per cent.

References:

Akhtar, M.H., Samantaray, S., Ankesh Kumar., . and Singh, A.P. (2004). Effect of deworming and vitamin-mineral-feed supplementation in combating anoestrus in crossbred heifers. *Intas-Polivet.,* **5**: 43-45.

Baruselli, P.S., Barnabe, V.H., Barnabe, R.C., Visintin, J.A., Molero-Filho, J.R., Porto, R. (2001). Effect of body condition score at calving on postpartum reproductive performance in buffalo. *Buffalo J.* **17**, 53-65.

Butani, M.G. (2008). Studies on major infertility problems, their diagnosis and therapeutic management in buffaloes under field conditions. M.V.Sc. thesis AAU, Anand, India.

Dhoble, R.L. and Gupta, S.K. (1986). Serum calcium and inorganic phosphorus during postpartum anoestrus in buffaloes. *Indian J. Anim. Health,* **25**: 123-126.

Dugwekar, Y.G., Sarvaiya, N.P., Suthar, B.N., Shah, R.M., Chaudhary, C.F., Brahmkshtri, K.G. and Derashri, H.J. (2006). Influence of GnRH treatment on service period in buffaloes. *Indian J. Anim. Reprod.,* **27**: 86-88.

El-Wishy, A.B. (2007). The postpartum buffalo II. Acyclicity and anestrus. *Anim. Reprod. Sci.* **97**, 216-236.

Jainudeen, M.R., Hafez, E.S.E. (1993). Cattle and buffalo. In: Hafez, E.S.E. (Ed.), Reproduction in Farm Animals, 6th ed. Lea and Febiger, Philadelphia, USA, pp. 315-329.

Khasatiya, C.T., Desai, P.M., Dhami, A.J., Dugwekar, Y.G., Panchal, M.T. and Kavani, F.S. (2004). Effect of GnRH and PGF2 alpha on productive performance and progesterone profile in postpartum true anoestrus and suboestrus Surti buffaloes. *Indian J. Dairy Sci.,* **57**: 324-328.

Nanda, A.S., Brar, P.S., Prabhakar, S. (2003). Enhancing reproductive performance in dairy buffalo: major constraints and achievements. Reproduction 61 (Suppl.), 27-36.

Randhawa, C.S., Randhawa, S.S., Singh, Narinder and Sidhu, S.S. (2004). Copper supplementation and fertility response in anoestrus buffaloes- A clinical trial. *Indian J. Anim. Reprod.,* **25**: 41-42.

Reddy, K.R.C., Rao, A.S., Reddy, V.S.C., Yadagiri, B., Sharma, G.P., Rami Reddy, M. and Eswar Reddy, C. (1994). Efficacy of certain non-hormonal and hormonal drugs on oestrus induction in postpartum anoestrus buffaloes. *Indian J. Anim. Reprod.,* **15**(2): 127-130.

Sheshappa, H., Honnappagol, S.H., Dhabale, R.B., Tandle, M.K. (2002). Efficacy of GnRH and PGF2 alpha in augmenting reproductive performance of postpartum dairy cows. *Indian J. Anim. Reprod.,* **23**: 75-76.

Srivastava, S.K. (2008). Effect of mineral supplement on oestrus induction and conception in anoestrus crossbred heifers. *Indian J. Anim. Sci.,* **78**: 275-276.

Zaghloul, A.H., Daghash, H., Megahed, G.A. and Shehata, S.H. (1993). The use of GnRH analogue for treatment of ovarian inactivity in cows and buffaloes. *Assiut Vet. Med. J.,* **29**: 235-240.

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The Blue Cross Book July 2015 Vol. 32 : 33-38

Clinico-hematobiochemical studies in Equines infected with *T. evansi*

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ABSTRACT

Trypanosomiasis, an arthropod borne blood protozoan disease commonly known as Surra is caused by Trypanosoma evansi and transmitted by Tabanids and Stomoxys which act as mechanical vectors. In the present study a total of seventeen (n=17) equines tested positive for presence of *T.evansi* were evaluated for clinical and haematobiochemical alteration. The main clinical signs observed in clinically infected equines (n=17) include anorexia, general weakness, dullness and depression. The other signs were inermittent fever, inappetance, oedema of legs or abdomen, pale mucous membrane, serous nasal discharge, congested mucous membrane of eye, lacrimation, incoordination of legs, paraplegia and two horses showed falling down. The infected equines showed marked anaemic tendency as revealed by significant decrease in Hb, PCV and RBC levels. The infected equines showed significant neutropenia and lymphocytosis. The total serum protein was significantly ($P<0.01$) decreased in infected horses with significant ($P<0.01$) increase in BUN level.

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

Introduction

Equines hold a special position in livestock both for civil and military purpose in view of its multifaceted utility. In northen India, particularly in the states of Jammu and Kashmir, Haryana, Punjab, Uttar Pradesh, Rajasthan and Gujarat, donkeys and horses are extensively used as a mode of transportation and for draught purposes especially in hilly, arid and semi-arid zones where motorable roads are inadequate or not feasible. Trypanosomiasis, an arthropod borne blood protozoan disease, commonly known as Surra is caused by *Trypanosoma evansi.* Several species of haematophagous flies, including Tabanids and Stomoxys are implicated in transferring infection from host to host, acting as mechanical vectors. This was the first trypanosome identified in mammals by Sir Griffith Evans (1880) in Dera Ismil Khan (Now in Pakistan). It is widely prevalent in livestock of Africa, Asia and South America (Hunter, 1990) and is endemic in China, the Indian sub continent, Northern America, The Philippines, Bulgaria, parts of the former U.S.S.R. and parts of Indonesia (O.I.E., 2008). The "Office international des epizootics" categoriesed the disease Surra under 'B' disease of significance (O.I.E., 2004). Zoonotic potential of the disease has been documented through a report of *Trypanosoma evansi* infection in a person from Maharashtra, India (Power, 2006).

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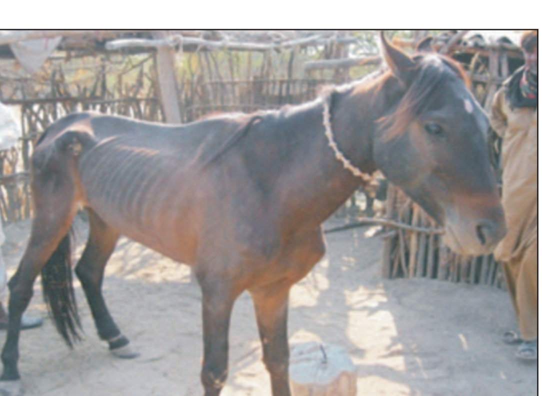

Plate - 1 (a):General weakness, emaciation, dullness and depression

Plate -1 (b):Oedema of hind legs

Materials and Methods

The present study was conducted in different districts of Gujarat state. The equines were screened randomly for presence of *T. evansi* by three methods i.e. based on clinical signs, thin blood smear with Geimsa stain and Indirect ELISA test. A total of seventeen (17) equines were tested for presence of *T. evansi.* The positive equines were further evaluated for clinical and haematobiochemical alterations. The statistical procedure used for analysis of data generated from the present study was Student's 't' test as per the method described by Snedecor and Cochran (1994).

Each animal was observed clinically for respiration, pulse and body temperature, presence or absence of congested mucous membranes, oedema of dependent parts, presence or absence of nasal and ocular discharge and for any neurological signs like incoordination of legs, paraplegia, falling down.

Hematological analysis of samples were performed on whole blood to estimate Hb, TEC, TLC, DLC, PCV, Platelet count, MCV and MCH, by autoanalyzer (Abacus Junior Vet.5).

For biochemical studies, serum was separated from blood by centrifugation at 3000 rpm for 15 min and was used for estimation of total protein, blood urea nitrogen, creatinine, calcium and phosphorus, using commercial available kits of Merck Specialities Pvt. Ltd. India with the help of clinical chemistry analyser (Junior Selectra, Vital Scientific, Netherland).

Results and Discussion

Clinical signs

The main clinical signs observed in infected equines (n=17) included anorexia, general weakness, dullness and depression. The other signs were intermittent fever, inappetance, oedema of legs or abdomen, pale mucous membrane, serous nasal discharge, congested mucous membrane of eye and lacrimation. Apart from these clinical observations, three horses showed nervous signs like incoordination of legs, paraplegia and two horses showed falling down. The rectal temperature, heart rates per minute and respiration rates per minute were also increased in affected horses. (**Table-1)**.

Presence of fever in trypanosomiasis is due to the toxin liberated by the parasites, irrespective of

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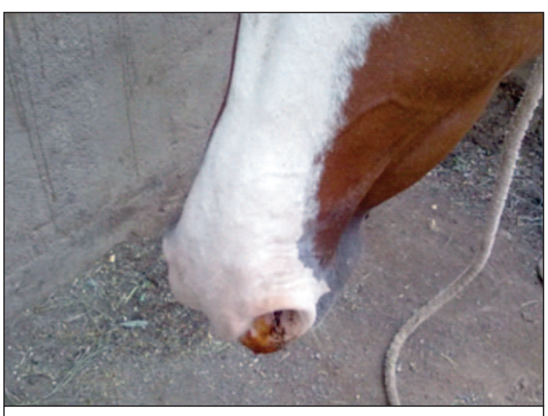
Plate - 2 (a) : Serous nasal discharge

Plate - 2 (b) : Incoordination of legs

Table 1: Clinical findings in equines suffering with trypanosomiasis

Sr. No.	Clinical signs	No. of cases (17)	%
1	Anorexia	8	47.05
2	Inappetance	7	41.17
3	Lacrimation	3	17.64
4	Oedema of hind legs	12	70.58
5	Emaciation	3	17.64
6	Congested mucus membrane of eye	11	64.70
7	Dull and depressed	15	88.23
8	Stiffness	3	17.64
9	Serous nasal discharge	5	29.41
10	Nervous signs like incordination, paraplegia	7	41.17

concentration of the parasite. This leads to the change in the body temperature set point in the hypothalamus under the influence of pyrogenic stimuli released during infection (Singh *et al.* 2011). However, Pyrexia may or may not reflect the degree of parasitaemia as it is due to direct contact of monocytes and macrophages with trypanosoma antigen producing pyrogen or antigen-antibody complex, which stimulates for pyrogen release. Nervous symptoms like incoordination, paraplegia and falling down as observed in three horses may be due to long standing chronic infection resulting in invasion of brain by *Trypanosoma evansi*. Similar findings were reported by Yadav and Kumar (2010) in a horse at NRCE farm, which was heavily infested with *Trypanosoma evansi* and died subsequently.

Haematological studies

The results of haematological investigation in control and infected equines are given in **Table-2**. Red blood cells count, packed cell volume and haemoglobin concentration values of infected

Table -2: Mean±SE haematological changes in *Trypanosoma evansi* infection

Parameter (units)	Infected group (n=17)	Control healthy (n=14)
RBC ×10 6/µl	5.26**±0.13	9.15±0.12
PCV (%)	25.81**±0.20	37.14±0.25
HGB (g/dl)	7.35**±0.15	13.18±0.16
WBC ×103/ µl	7.38**±0.17	8.27±0.07
LYMPHOCYTES %	42.73**±0.22	38.21±0.33
NEUTROPHILS %	56.15**±0.27	62.22±0.41
PLT ×10 3/µl	183.78±7.61	164.49±2.41
MCV (fl)	50.28**±0.96	40.24±0.30
MCH (pg)	15.69±0.68	13.94±0.12
MCHC (g/dl)	33.86±1.33	36.21±1.12

Means in different columns differ significantly (*=P<0.05) or (**P<0.01)

horses decreased significantly (P<0.01) than those control group of horses. Significant (P<0.01) increase in the MCV was observed in the infected horses. The alteration in hematological indices observed during the infection are consistent with the findings of previous workers (Jani and Jani, 1993, Silva *et al*. 1995, Varshney *et al*. 1999, Laha *et al*. 2004, Laha and Sasmal 2008, Singh *et al*. 2011). The anaemia may be caused due to inhibition of the haemopoietic system by the toxin liberated by the parasites resulting in failure in production of the red blood cells. The lowered RBCs counts in infected group can be attributed to the rapid multiplication of trypanosomes leading to physical damage, destruction of RBCs either mechanically or chemically due to the release of certain toxic metabolites by the parasites and inefficient erythropoetic mechanism.

Significant (P<0.01) neutropenia and lymphocytosis were found in the affected horses. Neutropenia may be due to the predominant effect of toxins liberated by parasites on bone marrow rather than on lymphoid organs. Neutropenia and lymphocytosis were observed in *Trypanosoma* infected horses by Jani and Jani, (1993), Silva *et al*. (1995) and Singh *et al*. (2011). Moreover, Aline *et al* (2005) observed leucocytosis due to lymphocytosis associated to large atypical lymphocytes in affected horses. However, Marques *et al*. (2000) reported leukocytosis with neutrophilia and relative lymphopenia in some horses in experimentally induced equine trypanosomosis. These finding reveal that there is not a defined trend for leukocytes changes in horses infected with *Trypanosoma evansi*.

Increased value of MCV in infected horses might have been due to decrease in number of RBCs and the decreased in Hb value. Consequently, ratio of Hb and RBCs is more and each red cell contains more amount of Hb. Type of anemia observed was Macrocytic Normochromic.

Despite being a significant feature of the disease, the origin of the anaemia in Trypanosomiasis is not completely elucidated. Evidences suggested that its etiology is multifactorial and haemolysis, hemodilution and disorder and /or noncompensatory erythropoiesis are some of the mechanisms proposed.

Biochemical studies

The biochemical observations in control and infected animals are given in **Table 3.** The non significant difference in serum calcium, phosphorus, creatinine level was observed in infected horses compared to control group of horses.

The total serum protein was significantly (P<0.01) decreased in infected horses. Similar findings were also observed by Sazmand *et al*. (2011). However, Jani and Jani (1993) observed no change in total protein in affected horses. It is believed that hypoalbuminemia occurs due to increased vascular permeability and extravasation of albumin into the interstitium or it may be due to chronic stage of the disease, leading to hypoproteinemic oedema. There was significant (P<0.01) increase in BUN level in infected horses. Similar findings were also observed by Jani and Jani (1993) and Singh *et al.* (2011). This change in BUN level may be due to either altered nitrogen metabolism or reduction in renal circulation leading to kidney damage (Jani and Jani, 1993).

Table -3: Mean±SE biochemical changes in Trypanosoma evansi infection

Parameter	Infected group (n=17)	Control healthy (n=14)
Ca (mg/dl)	11.87±0.13	12.20±0.19
P (mg/dl)	4.25±0.10	4.42±0.15
TP (g/dl)	4.65**±0.09	6.89±0.17
BUN (mg/dl)	17.85**± 0.57	12.00 ± 0.22
Creatinine (mg/dl)	1.53± 0.04	1.25±0.06

Means in different columns differ significantly (*=P<0.05) or (*=P<0.01)

References

Aline, R., Rafael, A., Tatiana, M., Ana, L. S., Mauro, P. and Claudio, S.L. (2005). Outbreaks of trypanosomiasis in horses by *Trypanosoma evansi* in the state of Rio Grande do Sul, Brazil: epidemiological, clinical, hematological and pathological aspects. *Pesq. Vet. Bras*. **25**:4.

Evans, G. (1880). Report on 'surra' disease in the Dera Ismail Khan District. Punjab Govt Milit Dept. 493: 446.

Hunter, A. G. and Luckins (1990). Trypanosomiasis. Handbook on Animal Diseases in the Tropics. 4th ed.(Sewell, M. M. H., D. W. Brocklesby, Eds.). pp 204-226.

Jani, R.G. and Jani, B.M. (1993). Haematological and biochemical changes in clinical cases of equine surra. *Journal of the Remount Veterinary Corps,* XXXII **(2)**: 91-94.

Laha, R. and Sasmal, N.K. (2008). Endemic status of *Trypanosoma evansi* infection in a horse stable of eastern region of India – a field investigation. *Trop Anim Health Prod.* **40**:357-361.

Laha, R., Bera, A. K., Panja, P. and Sikdar, A. (2004).Clinical and haemato-biochemical studies in natural trypanosomosis of equines. *Indian Journal of Animal Sciences.* **5**:125-127.

Marques, L. C., Machado, R. Z., Alessi, A. C., Aquino, L. P. C. T. and Preeira, G. T. (2000). Experimental infection with *Trypanosoma evansi* in horse: clinical and haematological observation. Brazil. *J. Vet. Parasitol,* **9**:11-15.

O.I.E. (2004) Surra *(Trypanosoma evansi)*. In: "Manual of tandards for diagnositic tests and vaccines". Office International des Epizooties. 5th ed. World Health Organization for Animal Health, Paris. pp 891-900.

O.I.E. (2008) Manual of tandards for diagnositic tests and vaccines for terrestrial animals 2008". Ch 2.1.17, Trypanosoma evansi infections (including surra), pp. 352-360.

Power, R. M. (2006) *Indian J. of Medical Microbiol.* **24**:72-4.

Radostits, O. M., Gray, C. C., Hinchcliff, K. W. (2009). Veterinary Medicine: A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th Ed., W.B.Sounders Company, London, UK, Protozoan parasites in horses. Pp: 1537-1538.

Sazmand, A., Rasooli, A., Nouri, M., Hamidinejat, H. and Hekmatimoghaddam, S. (2011). Serobiochemical alterations in subclinically affected dromedary camels with *Trypanosoma evansi* in Iran. *Pak. Vet. J.* **31**: 223-226.

Silva, R.A.M.S., Arosemenab, N.A.E., Herrerac, H.M., Sahibd, C.A. and Ferreira, M.S.J. (1995).Outbreak of trypanosomosis due to *Trypanosoma evansi* in horses of Pantanal Mato-grossense, Brazil. *Veterinary Parasitology*. **60**: 167-171

Singh, R., Gupta, S. K., Upadhyay, S. and Koul, A. L. (2011). Clinicohaematobiochemical studies in equines infected with *T. evansi. Veterinary Practitioner.* **12** : 142-147.

Snedecor, G. W. and Cochran, W. G. (1994) Statistical Methods. 6th ed., Oxford and IBH Publishing Company, Calcutta.

Varshney, J.P., Varshney, V.P. and Dwivedi, S.K.(1999). Endocrine dysfunction in clinical Trypanosomosis in horses. *J. Vet. Parasitol.* **13** : 33-35.

Yadav, S.C. and Kumar, R. (2010). *Immunological Diagnosis of Trypanosomiasis in Equines*. Compedium Equine Health and Production Management, National Research Centre on Equines, Hisar, Haryana. Pp. 51-53.

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Efficacy of Cloprostenol Sodium administration during early post partum period on uterine involution in cows.

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Abstract

The present research study was planned to study the efficacy of cloprostenol sodium during early post-partum period on uterine involution in cows. Sixteen normally calved cows of two to seven lactations were randomly selected immediately after calving and divided into two group. Cows in Group-I (n=8) were treated with 500 g chloprostenol sodium intramuscularly immediately after calving as well as on day 5th and 10th post partum intramuscularly. The cows from Group-II (n=8) were treated with two ml NSS (normal saline solution) immediately after calving as well as on day 5th and 10th of post-partum intramuscularly. The time required for expulsion of placenta, starting of lochial discharge, cessation of lochial discharge, the diameters of left and right horns of the uterus and the average time required for involution of uterus have been reported .

Introduction

The suboptimal reproductive efficiency of dairy animal increases the calving interval, reduces the life time milk production and calf crop, leading to economic losses to the animal owner. Acceptable reproductive efficiency requires each cow to calve per year to maximize economic output of milk production. Early resumption of ovarian and uterine activities after parturition is one of the ways for economic management during postpartum period. Minimum time for uterus to regain its original non pregnant state reduces the days open. Use of PGF2α is common during the early postpartum period to improve uterine involution (Lindell *et al.* 1983) and fertility in dairy cattle (Archbald *et al.* 1994). PGF2α agonist is also used for early expulsion of placenta (Tiwari *et al.* 2004) in cows (Khatri *et al.* 2013) and in buffaloes to reduce the chances of placental retention (Sinha *et al.* 2002). The present research work was planned to study efficacy of cloprostenol sodium during early post partum period on uterine involution in cows.

Materials and Methods

The present research work was undertaken at "Dairy Farm" of Department of Animal Husbandry and Dairy Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and Department of Animal Reproduction, Gynecology and Obstetrics PGIVAS, Akola. The selected cows were maintained under ideal farm conditions with similar management practices. The cows were also fed green fodder like Yashwant, Maize and dry fodder like kadba-kutti (Jawar or Chunni) as per the body weight and milk production. The cows were fed concentrate and mineral mixture according to the milk yield. All the experimental cows had free access to good quality drinking water thrice a day. The

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cows were milked twice daily by hand method in the morning and in the evening. The normally calved cows of two to seven lactations were randomly selected (n=16) immediately after calving and divided into two groups i.e treatment group (Group-I) and control group (Group-II) comprising eight cows(n=8) in each group. The selected cows in Group-I (n=8) were treated with 500 g cloprostenol sodium (Inj. Estrumate) immediately after calving as well as on day 5 and 10 post partum intramuscularly. The selected cows in Group-II (n=8) were treated with two ml NSS (normal saline solution) immediately after calving as well as on day 5 and 10 of post partum intramuscularly. The time required for expulsion of placenta from calving were recorded in hours in both the experimental groups. The time required for starting the expulsion of normal lochial discharge were noted by visual observations twice a day i.e morning and evening by observing discharge on the floor where the experimental cows were housed. The cessation of lochial discharge was considered when the discharge was not observed on the floor, where the cows were housed. The uterine diameter was recorded with transrectal ultrasonography using 7.5 MHz probe on day 21,28 and 35 post partum in both the experimental groups. The diameter of uterine horns was measured at the point of bifurcation in both the uterine horns.

Result and Discussion

The time required for expulsion of placenta was 2.53±0.287 and 5.45±0.503 hr in treatment and control group, respectively. The result of the present study for expulsion of placenta after PGF2α treatment immediately after calving is in agreement with Khatri *et al*. (2013) who have reported 3.1±0.3 hr as the time required for expulsion of placenta after cloprostenol treatment in buffaloes while 4.45±0.5 and 6.3±0.6 hr in 100 IU oxytocin and control buffaloes, respectively. Tiwari *et al.* (2004) have reported 5.50±0.68 and 7.80± 0.90 hr required for expulsion of placenta in treatment and control group, respectively.

The earlier expulsion of fetal membrane in the treatment group may be due to prolonged uterine contraction induced by PGF2α. The prolonged uterine contraction after PGF2α administration have been reported by Edquist *et al.* (1978) in sheep and in vitro studies on ovine myometrium by Gautam *et al.* (2002). The spasmogenic effect of PGF2α on the uterine musculature has been established (Eiler et al. 1981) and this appears to be the reason for early expulsion of placenta after calving in the present experiment. The variation in the result may be due to breed, dose and route of administration of PGF2α , season and nature of PGF2α.

The time required for starting of lochial discharge was 6.63±0.32 and 7.63±0.26 days in treatment and control group, respectively. From the above result, it is observed that the expelling of lochial discharge was started earlier in treatment group than the control group, which may be due to administration of PGF2α immediately after calving and on day fifth postpartum which might be attributed for prolonged uterine contraction induced by PGF2α on myometrium. Toppozada *et al.* (1974) and Lindell and Kindahl (1983) observed a positive effect of PGF2α on uterine muscular tone. The time required for cessation of lochial discharge was 9.75±0.25 and 13.25±0.620 days in treatment and control group, respectively . In the present study, cessation of lochial discharge is observed earlier in treatment group. The result of present study for the cessation of lochial discharge is in agreement with the result reported by Tiwari *et al.* (2004) who have recorded 9.50±0.628 days required for clearance of genital discharge in Dinoprost

MSD Animal Health

tromethamine treated buffaloes while 13.9±0.68 days in untreated buffaloes. However, Thomson *et al.* (1987) have reported that PGF2α is not uterotonic in the puerperal cows and its therapeutic use may be avoided.

The diameter of right horn of uterus was 3.15±0.237 and 3.31±0.251, 2.63±0.154 and 2.72±0.213 and 2.39±0.085 and 2.47±0.208 cm in treatment and control group on day 21, 28 and 35, respectively. The diameter of left horn of uterus was 3.11±0.225 and 2.92±0.109, 2.66±0.168 and 2.59±0.193, 2.38±0.084 and 2.46±0.190 cm in treatment and control group on day 21,28 and 35, respectively.

Sinha *et al.* (2002) observed 6.28±0.18, 4.78±0.14, 2.70±0.10, 2.28±0. and 6.67±0.14, 4.92±0.12, 3.08±0.18, 2.54±0.11 cm in Dinoprost treated and control group on day 10, 20, 30 and 40, respectively. Melendez *et al.* (2004) reported 4.92 and 5.65 cm uterine diameter of previous gravid horn in Dinoprost tromethamine treated and control Holstein cows, respectively. with acute puerperal metritis on day 12 post partum. In the present study, the uterine diameter of left and right horn are slightly less in treated cows than control which might be due to effect of PGF2*α* on uterine musculature and starting speedy process of involution of uterus.

The experimental cows were examined transrectally with 7.5 MHz probe on day 21, 28 and 35 post partum to measure the diameter of uterine horns. Uterine involution was considered when the diameter of both the horns become same which were monitored by ultrasonographically. The average time required for uterine involution was 29.75±1.00 and 33.25±1.43 days in treatment and control group, respectively. The result of the present experiment for the uterine involution is in agreement with Iqbal *et al.* (2003) who have reported the complete uterine involution in 28.90±1.79 days in PGF2**α** treated buffaloes and 35.40±3.9 days in control group. Khatri et al. (2013) also reported the 29.4±1.74 days required for uterine involution which were treated with 0.150 mg cloprostenol intramuscularly immediately after starting of labor pain while 37.8±1.54 days in untreated control buffaloes. Pandey et al. (2009) reported duration of uterine involution as 36.47±1.62 and 29.17±1.0 days in cloprostenol sodium (500µg) treated cows on day 4 post partum and cloprostenol sodium (500µg) treated on day 4 post partum and 15 post partum i/m while 43.11±1.44 days in control group, respectively.

The earlier uterine involution in treated cows in the present study might be due to repeated dose of PGF2**α** in early postpartum period. Lindell *et al.*(1980) reported that uterine involution is dependent upon the concentration of PG metabolites at parturition while Lindell and Kindahl (1983) reported that PGF2α had a positive effect on uterine muscular tone and cows with a short period of high PG metabolites required relatively longer period for complete uterine involution. The variation in the days required for uterine involution may be due to difference in the breed, dose, time and frequency of administration, climate and managemental practices.

References :

Archbald, L. F., S. Constant, T.Tran, C. Risco, E. Klapstein and J. Elliot (1994) Effect of sequential treatment with prostaglandin F2 alpha and/or oxytocin on estrus and pregnancy rate of lactating dairy cows. *Theriogenology* **42**:773-80.

Eiler H., J. Oden, R.Schaub and M. Sima (1981). Refractoriness of both uterus and mammary gland of the cow to PGF2**α** administration: Clinical implication. *American Journal of Veterinary Research,* **42**: 312-317.

Gautam, R. P., Tiwari R. P andHore S. K. (2002) Effect of oxytocin, prostaglandin and some herbal preparation on goat uterus in-vitro. *The Indian Journal of Animal Science.* **68**:669-676.

Iqbal, S., M. Aleem and M. A. Saeed (2003) Role of single injection of prostaglandin F2 alpha on breeding efficiency of postpartum buffaloes. *Pakistan Vet. J.,* **23**:4-8.

Khatri, P., S. A. Tunio., I. Kaka, M. U. Samo, B. Bhutto and M. R. Memon (2013) Effect of Exogenous PGF2α and Oxytocin on Postpartum Anestrus and Uterine Involution in Kundhi Buffaloes. *Journal of Anim. Prod. Adv.,* **3**:158-163.

Lindell J. O., H. Kindahl and L.E. Edquist (1980) Uterine involution in relation to Post portum release of PGF2α in cows. IN 9th International Congress of Animal Reproduction and Artificial Insemination 16th - 20th June 1980, III Symposia.

Lindell J. O. and H. Kindahl (1983) Exogenous PGF2α promotes uterine involution in cow. *Acta Vet. Scand.* **24**:269-74.

Melendez, P., J. McHale, J Bartolome, LF. Archbald and G. A. Donovan (2004) Uterine Involution and Fertility of Holstein Cows Subsequent to Early Postpartum PGF2α Treatment for Acute Puerperal Metritis. http:// www. Journalofdairyscience. Org/ article/ PIIS 0022030204734608.

Pandey Prabhat, A. Pandey A. K. Sinha, B. and Singh (2009) Effect of Cloprostenol Sodium on Postpartum Reproductive Efficiency of Dairy Cattle.http://www.thedairysite.com/articles/2110/effect-of-cloprostenol sodium-on-postpartum-reproductive-efficiency-of-dairy-cattle#sthash.NFfUnCJW.dpuf.

Thompson, F. N., R. D. Page, C. B. Cook and A. B. Caudle (1987) PGF2α metabolite levelsin normal and uterine infected postpartum cows. *Veterinary Research Communications* **11**: 503-507.

Tiwari, R.P., S. Jogi, S. K. Sahu (2004) Effect of prostaglandin administration after calving in buffaloes on postpartum reproductive performance. *Buffalo Bulletin* **23**:53-57.

Toppozada, M., A. Gaafar and S. Shaala(1974) In-vivo inhibition of the human nonpregnant uterus by prostaglandin E2. *Prostaglandins,* **8**:401-410.

Toppozada, M., A. Gaafar, S. Shaala and M. Osman (1975) The relaxant property of local prostaglandin F2 on the nonpregnant uterus: A cyclic triphasic response. *Prostaglandins,* **9**: 475-486.

Sinha, V. K., Balraj Singh and A. K. Sinha (2002) Management of postpartum reproduction in crossbred cows with Dinoprost. *The Indian journal of Animal Reproduction.* **23**:21-24.

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INDICATIONS

Induction of luteolysis in dairy cattle and horses-
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- Luteal Cyst,
- Pyometra,
- Persistant Corpus Luteum (PCL),
- Chronic Endometritis,
- Expulsion of Mummified Foetus,
- Termination of Pregnancy,
- Induction of parturition
- Synchronization of Estrous

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Cattle - 2.0 ml by IM route Ponies - 0.5-1.0 ml by IM route
Thoroughbreds, hunters and heavy horses 1.0-2.0 ml by IM route

MSD Animal Health

The Blue Cross Book July 2015 Vol. 32 : 43-48

Incidence and Pathogenicity of Aeromonas Spp. in Chicken and Emu meat

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Abstract

Aeromonas spp. is considered as an emerging pathogen, due to increasing incidence in various foods and growing reports of various human infections since last decade. Present study was envisaged on the incidence of *Aeromonas spp.* in chicken and emu meat. Out of 50 samples, each of chicken and emu meat, 18(36%) and 10(20%) were positive by cultural method, whereas PCR assay revealed 22(44%) and 14(28%) positives, respectively. Aerolysin toxin was detected in 27.27% of chicken meat and 14.28% of emu meat whereas thermostable cytotonic enterotoxin was detected in 36.36% of chicken meat and 14.28% of emu meat among the samples positive for *Aeromonas spp.*

Keywords: *Aeromonas*-chicken and emu meat-Cultural method-PCR.

Introduction

The genus *Aeromonas spp.* are Gram negative, motile (except *A.salmonicida*), rod shaped, catalase and oxidase positive bacteria, which are ubiquitous and widely distributed in aquatic environments (Daskalov, 2006). Aeromonads are listed under the family *Aeromonadaceae*, which include *Oceanimonas* and *Tolumonas* along with *Aeromonas* (Martin carnahan and Joseph, 2005). Motile Aeromonads are considered as emerging pathogens and neoenteropathogens (Igbinosa, 2012), not only because of their ability to produce virulent factors at optimum temperature, but also at refrigeration temperature. Although disease caused by *Aeromonas spp.* is multifactorial, the exact role of the virulence factors is still not fully elucidated. Disease manifestations caused by *Aeromonas spp.* range from gastro enteritis and septicemia to a variety of extra intestinal infections like endocarditis, wound infections, acute suppurative cholangitis, cellulitis, septic arthritis, osteomyelitis and meningitis (Janda and Abbott, 2010; Mukhopadhyay *et. al.,* 2008). *Aeromonas spp.* are able to utilize variety of nutrients and adopt to diverse environmental conditions, which answer their wide distribution in untreated and chlorinated drinking water, fish, sea food, milk, vegetables and several meats like chicken, pork, beef and mutton (Kingombe, 2004; Ottaviani *et. al.,* 2006; Isonhood and Drake, 2002; Arora *et. al.,* 2006). There are many reports of food borne outbreaks caused by *Aeromonas spp.,* due to consumption of meat products, land gang, a Swedish food aqua foods, meat and offals, fish (Hansman, 2000) etc., which lead to their designation as "new" foodborne pathogens by FDA (Tsai and Chen, 1996).

Chicken meat holds the top position among the various meats consumed in India. Use of untreated water & improper hygiene is common while processing and preparation. Changing lifestyles and consumer preference towards

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packed and refrigerated meat products improve the chances of contamination of *Aeromonas spp.* Hence, a study was undertaken to investigate the incidence of *Aeromonas spp*. and its toxins among chicken meat & emu meat samples in order to understand the epidemiology & pathogenicity of *Aeromonas spp.*

Materials And Methods

100 samples, 50 each from chicken meat and emu meat were collected randomly from various retail outlets, Sunday markets and supermarkets in and around Greater Hyderabad Municipal Corporation. All the samples were collected aseptically in sterile polythene bags and transferred on ice to the laboratory at the earliest possible for further analysis.

10 g of each sample was homogenized in mortar and pestle and enriched into alkaline peptone water with ampicillin 10 mg/L (APW-A) obtained from Himedia labs. APW-A was the most promising enrichment broth for the isolation of *Aeromonas spp.* (Perales, 2003). The sample was incubated at 37°C for 18 hours. A loopful of inoculum form enrichment broth was streaked on to Ampicillin dextrin agar (ADA) and *Aeromonas* Isolation medium obtained from Himedia labs. Streaked media plates were incubated at 37°C for 24-48 hours .Large, convex yellow colonies on ADA and dark green, opaque colonies with dark centre on *Aeromonas* isolation medium were presumed as *Aeromonas spp*. and sub streaked on nutrient agar for further confirmation by standard biochemical tests (Rose and Okrend, 1998; Yucel *et. al.,* 2005).

PCR assay:

Bacterial DNA was obtained from APW-A broth inoculated with sample by boiling and snap chilling (Arora *et. al.,* 2006). 2 µl of the snap chilled bacterial lysate was taken as template. The primers used for the detection of *Aeromonas spp.* and its toxins were custom synthesized by SR life science solutions, Hyderabad (Table I). Master mix was prepared by using 2 µl of the bacterial lysate, 2 µl of 10x Taq polymerase buffer, 1.2 µl of Mgcl2, 1 µl of Taq

Table I: Primers used in this study

Primer	Target gene	Length		Primer sequence	Amplification product (bp)	Reference
16S rRNA	16S rRNA	21	5'F	TCA TGG CTC AGA TTG AAC GCT	599	Graf (1999); Arora et. al. (2006)
		24	5'R	CGG GGC TTT CAC ATC TAA CTT ATC		
Aerolysin	Aer	18	5'F	GCA GAA CCC ATC TAT CCA	252	Santos (1996), Porteen et. al. (2006)
		20	5'R	TTT CTC CGG TAA CAG GAT TG		
Cytotonic enterotoxin	Ast	21	5'F	TCT CCA TGC TTC CCT T CC ACT	331	Sen and Rodgers (2004)
		21	5'R	GTG TAG GGA TTG AAG AAG CCG		

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DNA polymerase (1U/µl), 0.8 µl of 10 mM dNTP mix and 2 µl each of forward and reverse primer (10 pmol/µl), which was made up to 20 µl using molecular grade water. Routinely, master mix was set up and 18 µl, each was distributed to the PCR tubes, to which 2 µl of the template was added. The cyclic conditions for various primers listed in table II. The samples were analysed in 1.5% agarose gel electrophoresis with ethidium bromide.

Two *Aeromonas spp.* viz. *A.hydrophila* (MTCC 1739) and *A.sobria* (MTCC 3613) were obtained from MTCC (Microbial Type Culture Collection, Chandigarh) and were run in parallel as standards for cultural and PCR assay to confirm the presence of *Aeromonas spp*. in the samples under investigation.

Table II: Cycling conditions standardized for various primers

S.No.	Step	16S rRNA	aer	ast
1	Initial denaturation	94 °C/5min	94 °C/5min	95 °C/5min
2	Denaturation	94 °C/1min	94 °C/1min	95 °C/30sec
3	Annealing	55 °C/1min	51°C/1min	54°C/30sec
4	Initial extension	72 °C/1min	72 °C/1min	72 °C/1min
5	Cycles	30 cycles	30 cycles	30 cycles
	Final extension	72 °C/5min	72 °C/5min	70 °C/5min
6	Hold	4 °C	4 °C	4 °C

Results and Discussion

A total of fifty chicken samples were screened for Aeromonas spp. and it was found that 36% and 44% were positive by cultural method and PCR (Fig 1 and Table III) assay, respectively. The results observed were in agreement with overall incidence of 32% reported by Dallal et. al. (2012) in various fresh and frozen meats. On contrary, lower results were obtained by other workers viz., 4% by Porteen et. al. (2006), 6 % by Arora et. al. (2006) by PCR assay and 14 % by Das et. al. (2012) by cultural method. Higher incidences by cultural method were reported i.e. 58% by Turgay and Cifci (2011), 86.9 % by Yucel and Erdem (2004), 90.5 % by Akan et. al. (1998). Igbinosa et. al. (2006) isolated Aeromonas spp. in 80% of poultry, 54% of meat and 80% from meat products. Dallal et. al. (2012) isolated Aeromonads from fresh chicken (70%), frozen chicken (14%), fresh minced meat (21%), frozen minced meat (9%) and frozen processed minced meat (22%). Even though chicken meat holds top position in India among the various meats consumed, most of it is processed from small shops, roadside vendors and importance given to hygiene is comparatively negligible, leading to high incidence of Aeromonas spp. among chicken meat samples in this study via contaminated water, utensils and through the hands of butcher (Akan et. al., 1998).

Frequency of Aeromonas spp. in emu meat (20% by cultural method and 28% by PCR assay) is lower than chicken meat in this study and comparable to slightly lower incidence of 16.7% by Elmanama and Ferwana (2011) in chicken meat and18.4% in pork (Sharma and Kumar, 2011) by cultural assay. Higher incidence of 28.65% by Nagar et. al. (2011) and 31.5% by Sharma and Kumar (2011) in chicken meat;

Table III: Results of chicken and emu meat samples by cultural and PCR methods

Type of sample	No. of samples	Positive result for Aeromonas spp.					Distribution of Toxins among isolates positive by PCR			
		Cultural method		PCR assay		% of cultural method compared to PCR	aer		ast	
		No	%	No	%		No	%	No	%
Chicken	50	18	36	22	44	81.8	6	27.27	8	36.36
Emu meat	50	10	20	14	28	71.42	2	14.28	2	14.28
Total	100	28	28	36	36	77.8	8	22.2	10	27.8

28.6% (Elmanama and Ferwana, 2011), 53.75 % (Koca and Sarimehmetoglu, 2009) in turkey meat were also reported. Lower incidence of Aeromonads in emu meat might be due to the level of hygienic conditions followed in slaughter, processing and preservation in retail outlets, which play a key role in the microbiological quality of meat (Gracey, 1999). The emu meat being rare and costly meat, normally very high hygienic practices are followed, which might have led to reduced incidence in the present study. Variation among the findings of various scientists might be due to level of hygiene, type of water used in slaughtering and geographical variation.

The incidence of 27.27 % of aerolysin (aer) in chicken meat observed in this study (Fig 2) was comparable with findings of Nagar et. al. (2011), who reported 22.7 % in retail foods. Osman et. al. (2012) reported 17.64% in various meats, which is nearer to the findings of emu meat in this study. On contrary zero per cent was reported by Pinto et. al. (2012) in ready to eat foods and Kore et. al. (2014) in chicken meat, whereas higher incidence of 35% (Das et. al., 2012) in various food products, 66.6% (Onuk et. al., 2013) and 80.64% (Aguilera-Arreola et. al., 2005) in fish samples and 88.93% (Yousr et. al., 2007) in shellfish samples were also reported.

Thermostable cytotonic enterotoxin (ast) was a heat stable (56^{0}c for 10 to 20 min) enterotoxin elaborated by *Aeromonas spp.*, caused accumulation of fluid in rabbit ligated ileal loops and suspected to have a role in causing diarrhea and gastroenteritis in humans (Sha et. al., 2002). The gene ast was detected in 36.36% and 14.28% of chicken and emu meat samples, respectively (Fig 3). The findings of chicken meat are nearer to the incidence of 30% reported by Albert et. al. (2000) and Sen and Rodgers (2004) in various samples. On contrary, very low incidence (1.9%) was reported by Pinto et. al. (2012) in ready to eat foods whereas higher levels of 39 %, 96.7 % and 97.6 % were reported by Aravena-Román et. al. (2014), Aguilera-Arreola et. al. (2005) and Balsalobre et. al. (2009) in variety of food and environmental samples, respectively. High degree of variation among the pathogenic genes among the various authors may be due to changes in the expression of putative virulence associated factors by various environmental conditions (Ottaviani et. al., 2011).

Conclusion:

From this study, it is concluded that Aeromonas spp. were prevalent among chicken and emu meat and presence of toxins revealed the pathogenic potential of isolates, suggesting the need to include Aeromonads among regular bacterial flora screened in livestock foods as a preventive public health measures.

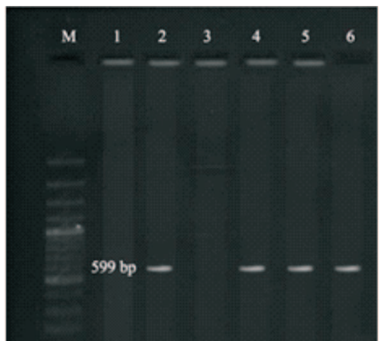

Fig 1: Result of Chicken and Emu meat samples for *Aeromonas spp.*
Lane M: 100bp DNA ladder
Lane 2 & 4: Samples from Chicken meat showing positive results
Lane 5 & 6: Samples from Emu meat showing positive samples

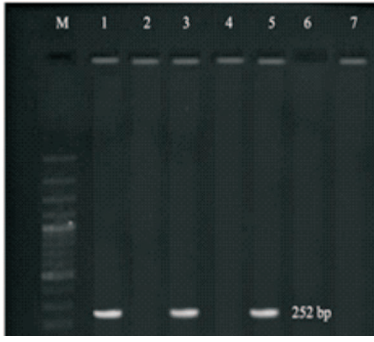

Fig 2: Result of Chicken and Emu meat for *aer*
Lane M: 100bp DNA ladder
Lane 1& 3: Samples from Chicken showing positive results
Lane 5: Samples from Emu meat showing positive results

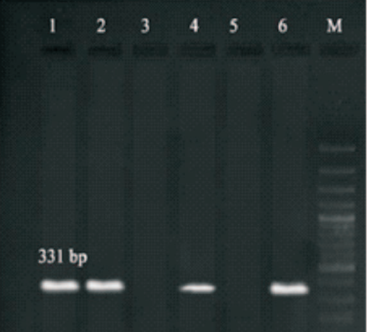

Fig 3: Result of Chicken and Emu meat samples for ast
Lane M: 100bp DNA ladder
Lane 1, 2& 4: Samples from Chicken meat showing positive results
Lane 6: Samples from Emu meat showing positive results

References:

Aguilera-Arreola, M., Hernández Rodríguez, C., Zúñiga, G., Figueras, M. J. and Castro Escarpulli, G. (2005). Aeromonas hydrophila clinical and environmental ecotypes as revealed by genetic diversity and virulence genes. FEMS Microbiol. Lett.., **242**: 231-240.

Akan, M., Eyigor, A. and Dikeri, K. S. (1998). Motile aeromonads in the feces and carcasses of broiler chickens in Turkey. J. Food Protect., **61**: 113-115.

Albert, M. J., Ansaruzzaman, M., Talukder, K. A., Chopra, A. K., Kuhn, I., Rahman, M., Faruque, A. S.,Islam, M. S.,Sack, R. & Mollby, R. (2000). Prevalence of enterotoxin genes in Aeromonas spp. isolated from children with diarrhea, healthy controls, and the environment. J. Clinic. Microbiol., **3**: 3785-3790.

Aravena-Román, M., Inglis, T. J. J., Riley, T. V. and Chang, B. J. (2014). Distribution of 13 virulence genes among clinical and environmental Aeromonas spp. in Western Australia. European J. Clinic. Microbiol. Inf. Dis., 1-7.

Arora, S., Agarwal, R. K. and Bist, B. (2006). Comparison of ELISA and PCR vis-à-vis cultural methods for detecting Aeromonasspp. in foods of animal origin. Int. J.Food Microbiol., **106**: 177-183.

Balsalobre, L., Dropa, M., Matte, G. and Matte, M. (2009). Molecular detection of enterotoxins in environmental strains of Aeromonashydrophila and Aeromonas jandaei. J. Water and Health., **7**: 685-691.

Bin Kingombe, C. I., Huys, G., Howald, D., Luthi, E., Swings, J., and Jemmi, T. (2004). The usefulness of molecular techniques to assess the presence of Aeromonas spp. harboring virulence markers in foods. International journal of food microbiology, **94**: 113-121.

Dallal, M. S., Yazdi, M. S., and Avadisians, S. (2012). Study of prevalence and antibiotic resistance in Aeromonas species isolated from minced meat and chicken samples in Iran. African Journal of Microbiology Research, **6**: 460-464.

Das, A., Vinayasree, V., Santhosh, C. R. and SreeHari, S. (2012). Surveillance of Aeromonas sobria and Aeromonas hydrophila from commercial food stuffs and environmental sources. J. Experim. Sci., **3**: 36-42.

Daskalov, H. (2006). The importance of Aeromonas hydrophila in food safety. Food Control., **17**: 474-483.

Elmanama, A. A. and Ferwana, N. (2011). Yersinia enterocolitica and Aeromonas hydrophila in Clinical, Food and Environmental Samples in Gaza Strip. J. Al Azhar University-Gaza. (13): 69-82.

Gracey, J. F., Collins, D. S. and Huey, R. J. (1999). Meat hygiene- 10th edition. Saunders Ltd.

Hansmann, Y., Harf-Monteil, C., Monteil, H., and Christmann, D. (2000). Collective food poisoning due to Aeromonas caviae. Medecineet Maladies Infectieuses, **30**: 534-535.

Igbinosa, I. H., Igumbor, E.U., Aghdasi, F., Tom, M. and Okoh, A.I. (2012). "Emerging Aeromonas Species Infections and Their Significance in Public Health," The Scientific World Journal, vol. 2012, Article ID 625023.

Igbinosa, O. E., Odjardare, E. E., Akpor, O. B., Aiyegoro,

O. A. and Ogunmwonyi, I. H. (2006). "Incidence and prevalence of Aeromonas species from retail food: public health implications," Science Focus, 12(2): 19–22. cit. Igbinosa, I. H., Igumbor, E.U., Aghdasi, F., Tom, M. and Okoh, A.I. (2012). "Emerging Aeromonas Species Infections and Their Significance in Public Health," The Scientific World Journal, vol. 2012, Article ID 625023.

Isonhood, J. H. and Drake, M. (2002). Aeromonas species in foods. J. Food protect., **65**: 575-582.

Janda, J. M. and Abbott, S. L. (2010). The genus Aeromonas: taxonomy, pathogenicity, and infection. Clinic. Microbiol. Reviews., **23** : 35-73.

Koca, C. and Sarimehmetoglu, B. (2009). Isolation and identification of motile Aeromonas spp. in turkey meat. Ankara Üniversitesi Veteriner Fakültesi Dergisi, **56**: 95-98.

Kore, P. U., Kolhe, R. P., Deshpande, P. D., Bhong, C. D., Jadhav, S. N., Nagargoje, A. B., Jagtap, U. V., Dhandore, C. V., Tumlam, U. M., Pawar, P. D., and Khasnis, M. W. (2014). Prevalence of antimicrobial resistant Aeromonas in chicken and fish washings. African Journal of Microbiology Research, **8**: 2609-2615.

Martin-Carnahan, A. and Joseph, S. W. (2005). Aeromonadales ord. nov. In Bergey's Manual® of Systematic Bacteriology (pp. 556-587). Springer US.

Mukhopadhyay, C., Chawla, K., Sharma, Y. and Bairy, I. (2008). Emerging extra-intestinal infections with Aeromonashydrophila in coastal region of southern Karnataka. J. postgraduate medic., **54**: 199.

Nagar, V., Shashidhar, R. and Bandekar, J. R. (2011). Prevalence, characterization, and antimicrobiol resistance of Aeromonas strains from various retail food products in Mumbai, India. J. Food sci., **76**: 486-492.

Osman, K., Aly, M., Kheader, A. and Mabrok, K. (2012). Molecular detection of the Aeromonas virulence aerolysin gene in retail meats from different animal sources in Egypt. World J. Microbiol. Biotech., **28** : 1863-1870.

Ottaviani, D., Parlani, C., Citterio, B., Masini, L., Leoni, F., Canonico, C., Sabatini, L., Bruscolini, F. and Pianetti, A. (2011). Putative virulence properties of Aeromonas strains isolated from food, environmental and clinical sources in Italy: A comparative study. Int. J. Food Microbiol., **144**: 538-545.

Ottaviani, D., Santarelli, S., Bacchiocchi, S., Masini, L., Ghittino, C., and Bacchiocchi, I. (2006). Occurrence and characterization of Aeromonas spp. in mussels from the Adriatic Sea. Food microbiology, **23**: 418-422.

Perales, I. (2003). Culture media for Aeromonasspp. and Plesiomonasshigelloides. Prog.Industr. Microbiol., 37: 317-344.

Pinto, A. D., Terio, V., Pinto, P. D. and Tantillo, G. (2012). Detection of potentially pathogenic *Aeromonas* isolates from ready to eat seafood products by PCR analysis. *Int. J. Food Sci. Tech*., **47**: 269-273.

Porteen, K., Agarwal, R. K. and Bhilegaonkar, K. N. (2006). PCR-based detection of *Aeromonas* from milk samples. *J. Food Tech*., **4**: 111-115.

Rose, E.B. and Okrend, GJ.A. (1998). Isolation and identification of *Aeromonas* species from meat and poultry products. USDAIFSIS Microbiology Laboratory Guide Book 3,d Edition

Sen, K. and Rodgers, M. (2004). Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. *J. Appl. Microbiol*., **97**: 1077-1086.

Sha, J., Kozlova, E. V., and Chopra, A. K. (2002). Role of various enterotoxins in *Aeromonas* hydrophila-induced gastroenteritis: generation of enterotoxin gene-deficient mutants and evaluation of their enterotoxic activity. *Infection and immunity*, **70**: 1924-1935.

Sharma, I. and Kumar, A. (2011). Occurrence of enterotoxigenic *Aeromonas* species in foods of animal origin in North East India. *European review for medic. Pharma. Sci*., **15**: 883-887.

Tsai, G. J. and Chen, T. H. (1996). Incidence and toxigenicity of *Aeromonas hydrophila* in seafood. *Int. J. Food Microbiol*., **31**: 121-131.

Turgay, O. and Cifci, E. (2011). Isolation, identification and antibiotic resistance of motile *Aeromonas* species in chicken meat. Erciyes Univ. *J. Institute. Sci. Technol*., **27**: 252-259.

Yousr, A. H., Napis, S., Ali, R., Rusul, G. and Radu, S. (2007). Detection of aerolysin and hemolysin genes in *Aeromonas spp*. isolated from environmental and shellfish sources by polymerase chain reaction. *ASEAN Food J*., **14**: 115-122.

Yucel, N. and Erdem, B. (2004). The isolation and identification of motile *Aeromonas spp*. from meats in Ankara, *Turkey. Indian Vet J*., **81**, 967-970.

Yucel, N., Erdem, B. and Kaya, D. (2005). Some virulence properties and characterization of motile *Aeromonas* species from milk and white cheese. *Int. J. Dairy Tech*., **58**:106-110.

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Antimicrobial susceptibility of *Clostridium perfringens* strains Isolated from various sources

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Abstract

Antimicrobial susceptibility of 100 isolates of *Clostridium perfringens* was done by disc diffusion assay with Muller Hinton agar using 11 selected anitibiotics. Isolates were obtained from various livestock foods (chicken meat, mutton, chevon, beef, canned meat, pork, fish, crabs, shrimps and milk) and environmental samples (water, cattle and poultry fecal samples), which were confirmed by both cultural and PCR assay. C. perfringens was sensitive to Enrofloxocin and Penicillin (88%), followed by Vancomycin (84%), Amoxicillin and Chloramphenicol (80%), Ciprofloxocin (72%), Tetracyclin (68%), Clindamycin (60%) and zero per cent sensitive to Gentamicin and resistant to Gentamicin (92%), followed by Streptomycin (88%), Clindamycin (24%), Ciprofloxocin and Erythromycin (20%) and Amoxocillin (16%) zero per cent resistance to tetracycline. This organism was intermediately resistant to Erythromycin (72%) followed by Tetracycline (32%), Clindamycin (16%), Enrofloxocin, Vancomycin, Chloramphenicol, Ciprofloxocin, Streptomycin and Gentamicin (8%).

Key Words: Clostridium perfringens, antibiotic, antibiotic sensitivity, antibiotic resistance

Introduction

Clostridium perfringens, a spore-forming anaerobic bacillus, is widely distributed in the environment (soil, sewage, food, dust) and in the intestinal tracts of human and domestic animals (Garcia-Alvarado et al., 1992; Hathaway and Wohnson, 1998). The carriers remain a constant source of infection and can contaminate the environment and foods (Peterson et al., 1988). Although the presence of this organism has been reported in different foods, it is mainly a concern for the meat industry because of its predilection for amino acids in meats (Boyd et al., 1948). In the U.S.A., *C. perfringens* ranks third in bacterial food poisoning cases (Mead et al., 1999). In the U.K., Germany and Canada, *C. perfringens* has been reported to be one of the leading causes of human food poisoning cases (Shandera et al., 1983; Mahony et al., 1992). In India, *C. perfringens* had been implicated in food poisoning/diarrheic cases (Kulshrestha et al., 1982). It has been recognized as one of the most important and common organism causing a broad spectrum of human and veterinary diseases. In human, it is responsible for a number of clinical conditions ranging from relatively mild food poisoning to the potentially life-threatening gas gangrene (Rood and Cole 1991); in domestic livestock, it causes a wide range of enteric diseases (Songer, 1996).

Recent attention has focused on the role of widespread use of antimicrobials in growth promotion and therapy of infections in food-producing animals as a potential transfer route of antimicrobial-resistant bacteria or the genes encoding antimicrobial resistance into the

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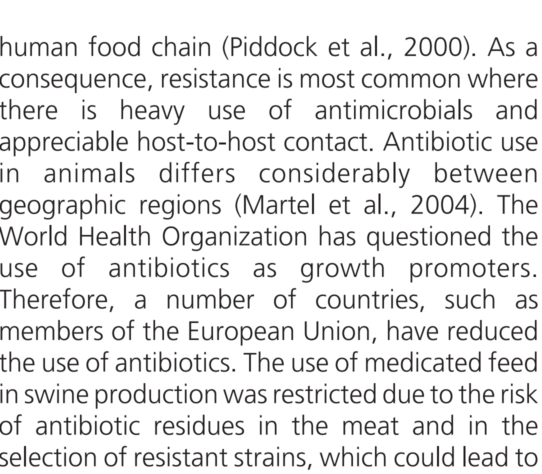

human food chain (Piddock et al., 2000). As a consequence, resistance is most common where there is heavy use of antimicrobials and appreciable host-to-host contact. Antibiotic use in animals differs considerably between geographic regions (Martel et al., 2004). The World Health Organization has questioned the use of antibiotics as growth promoters. Therefore, a number of countries, such as members of the European Union, have reduced the use of antibiotics. The use of medicated feed in swine production was restricted due to the risk of antibiotic residues in the meat and in the selection of resistant strains, which could lead to human infections with resistant bacteria (Burch, 2005; Vaz, 2009)

Large numbers of bacteria found in the large intestine, are exposed to antimicrobials, exchange genetic material with other bacteria and, on excretion, contaminate the environment or colonize other animals and humans resulting in varied antibiotic resistance/susceptibility (Van et al., 2000). As sufficient information is not available with regard to antibiotic susceptibility against *C. perfringens,* the present study was undertaken to explore the susceptibility/ resistance against 11 selected antibiotics.

Material and Methods

Isolation and Identification

About 10 g of each livestock foods (chicken meat, mutton, chevon, beef, canned meat, pork, fish, crabs, shrimps and milk) and environmental samples (water, cattle and poultry fecal samples) were inoculated into 90 ml fluid thioglycollate broth (FTG) in individual sterile polythene bags homogenized thoroughly in a stomacher for 3 to 5 min and incubated at 37°C for 24 h under anaerobiosis in McIntosh and Fields jars. The enriched inoculum from the broths was streaked onto Tryptose Sulphite Cycloserine (TSC) selective media agar plates and incubated at 37°C for 24 h under anaerobiosis. The presumptive colonies of *C. perfringens* were picked up and subjected to biochemical tests. The positive isolates by cultural method were confirmed by PCR assay using boiling and snap chilling method for DNA extraction targeting 16S rRNA. Antimicrobial susceptibility of 100 selected isolates was established by the disc diffusion assay with Muller-Hinton (MH) agar as described by Bauer et al. (1981). The antibiotic sensitivity of *C. perfringens* was tested for antibiotics like Amoxycillin (25µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Clindamycin (10µg), Erythromycin (10µg), Gentamicin (10µg), Penicillin G (10µg), Streptomycin (10µg), Tetracycline (30µg), Vancomycin (30µg) and Enrofloxacin (10µg).

MH broth was inoculated with five colonies from the sample and tubes were incubated at 37°C for 2-8 h 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 well in order to remove any excess liquid, and then seeded on the surface of a petridish containing MH agar, rotating at least twice. After the lid was placed, the dish was left at rest for five minutes to absorb any excessive humidity. Using sterile forceps, seven discs (sensifar) impregnated with antimicrobials were placed at equal distances from each other on the surface of each dish. Subsequently the dish was inverted and incubated at 37°C in anaerobiosis. Dish readings were performed 18 h after incubation and the diameter of inhibition halos was measured with the aid of a ruler. The interpretation was made as per the zone size interpretation chart provided by manufacturer of discs.

Results and Discussion

The results of antibiotic sensitivity of *C. perfringens* with 11 selected antibiotics are presented in Table 1.

C. perfringens was highly sensitive to Amoxicillin (80%), resistance (16%) and intermediate (4%). High sensitivity was reported by Lanckriet et al.

Table 1: Antibiotic sensitivity of C. perfringens

S.No	ANTIBIOTIC (µg)	ANTIMICROBIAL RESISTANCE-No. positive (%)		
		Sensitive	Intermediate	Resistant
1.	Amoxycillin (25 µg)	20(80%)	1(4%)	4(16%)
2.	Chloramphenicol (30 µg)	20(80%)	2(8%)	3(12%)
3.	Ciprofloxacin (5 µg)	18(72%)	2(8%)	5(20%)
4.	Clindamycin (10 µg)	15(60%)	4(16%)	6(24%)
5.	Erythromycin (10 µg)	2(8%)	18(72%)	5(20%)
6.	Gentamicin (10 µg)	-	2(8%)	23(92%)
7.	Penicillin G (10 µg)	22(88%)	1(4%)	2(8%)
8.	Streptomycin (10 µg)	1(4%)	2(8%)	22(88%)
9.	Tetracycline (30 µg)	17(68%)	8(32%)	-
10.	Vancomycin (30 µg)	21(84%)	2(8%)	2(8%)
11.	Enrofloxacin (10 µg)	22(88%)	2(8%)	1(4%)

(2010) whereas low sensitivity (40%) and high resistance (60%) was reported by Rahaman et al. (2013) and least resistance (7%) was reported by Osman and Elhariri (2013). A sensitivity of 80 per cent, 12 per cent resistance and 8 per cent intermediate to Chloramphenicol were observed in this study. High sensitivity was reported by Sapico et al. (1972) and Stevens et al. (1987) whereas 100 per cent resistance was reported by Rahaman et al. (2013) and 46 and 3 per cent resistance were reported by Osman and Elhariri (2013) and Tansuphasiri et al. (2005) respectively.

A sensitivity of 72 per cent, 20 per cent resistance and 8 per cent intermediate to Ciprofloxacin were observed in this study. Very high sensitivity (96.9%) and zero per cent resistance was reported by Singh et al. (2005) and Ibrahim et al. (2001) whereas moderate levels of sensitivity (42%) and resistance (58%) were reported by Osman and Elhariri (2013). For Clindamycin, a sensitivity (60%), resistance (24%) and intermediate (16%) was observed in this study. Very high sensitivities than the present study were reported by Sapico et al.(1972) and Stevens et al. (1987).

A sensitivity of 8 per cent, 20 per cent resistance and 72 per cent intermediate to Erythromycin were observed in this study. Almost similar results were reported by Sapico et al. (1972). Osman and Elhariri (2013) observed 100 per cent resistance and high levels of resistance was observed by Rood et al. (1978), Johansson et al. (2004) and Martel et al. em (2004). Very high sensitivity (92%) and 8 per cent intermediate to Gentamicin was observed in this study and almost similar high resistance was reported by Osman and Elhariri (2013).

For Penicillin G, sensitivity (88%), resistance (8%) and intermediate (4%) was observed in this study. Cent per cent sensitivity was reported by Sapico et al. (1972), Stevens et al. (1987), Silva et al. (2009), Osman and Elhariri (2013) and Rahaman et al. (2013). Zero and 9 per cent resistance were reported by Rahaman et al. (2013) and Tansuphasiri et al. (2005). Very high resistance (88%), 8 per cent intermediate and 4

per cent sensitivity to Streptomycin was observed in this study. Cent per cent resistance was reported by Secasiu and Pastarnac (1993), Ibrahim et al. (2001), Singh et al. (2005), Osman and Elhariri (2013)and Rahaman et al. (2013).

A sensitivity of 68 per cent and 32 per cent intermediate to Tetracyclin was observed in this study. Almost similar results were reported by Sapico et al. (1972) and Singh et al. (2005). Low level of sensitivity (40%), high resistance (41.8%) than the present study were reported by Silva et al. (2009). Very high level of sensitivity was reported by Stevens et al. (1987) and high resistance was reported by Rood et al. (1978), Johansson et al. (2004), Martel et al. (2004) and Tansuphasiri et al. (2005). Brefort et al. (1977) and Das et al. (1997) reported highly variable results. High sensitivity (84%), 8 per cent intermediate and 4 per cent resistance to Vancomycin was observed in this study. Almost similar results were reported by Sapico et al. (1972), whereas very high resistance (94.5%) was reported by Tansuphasiri et al. (2005).

A sensitivity of 88 per cent, 4 per cent resistance and 8 per cent intermediate to Enrofloxacin were observed in this study. Almost similar results were reported by Ibrahim et al. (2001), whereas high resistance (82%) was reported by Osman and Elhariri (2013). Low sensitivity (40%) and high resistance (60%) than the present study was reported by Rahaman et al. (2013).

Conclusion

C. perfringens isolates were sensitive to Penicillin G and Enrofloxacin (88%), Vancomycin (84%), Amoxycillin and Chloramphenicol (80%), Ciprofloxacin (72%), Tetracycline (68%) and Clindamycin (60%), whereas resistance to Gentamicin (92%), Streptomycin (88%), Clindamycin (24%), Ciprofloxacin and Erythromycin (20%).

References

Bauer, F.T., Carpenter, J.A. and Reagan, J.O. (1981). Prevalence of *Clostridium perfringens* in pork during processing. *J. Food. Prot.,* **44**: 279-283.

Boyd, M.J., Logan, M.A. and Tytell, A.A. (1948). The growth requirements of *C. perfringens* BP6K. *J. Biol. Chem.,* **174**:1013-1025.

Brefort, G., Magot, M., Ionesco, H. and Sebald, M. (1977). Characterization and transferability of *Clostridium perfringens* plasmids. *Plasm.,* **1**: 52-66.

Burch D. (2005): Problems of antibiotics resistence in pig in the UK. *In Practice*. **27**: 37-43.

Das, B.C., Dutta, G.N., Phukan, A. and Mukit, A. (1997). Necrotic enteritis in chickens due to field isolate of *C. perfringens* type A. *Indian J. Vet. Path.,* **21**: 27-29.

Garcia-Alvarado J.S., Labbe R.G. and Rodriguez M.A. (1992). Sporulation and enterotoxin production by *C. perfringens* type A at 37 and 43C. *Appl. Environ. Microbiol.,* **58**:1411-1414.

Hathaway C.L. and Wohnson E.A. (1998). Clostridium: The spore-bearing anaerobes. In Systematic Bacteriology, Topley and Wilson's Microbiology and Microbiol Infections. Vol 2 (A. Balows and B.I. Duerden, eds.):731-82.

Ibrahim R.S., Ibtihal M.M. and Soluman A.M. (2001). Clostridial infection in chickens studying the pathogenicity and evaluation of the effect of some growth promoter on broiler performance. *Assiut. Vet. Med. J.* **45**:253-267.

Johansson A., Greko C., Engstrom B.E. and Karlsson M.(2004). Antimicrobial susceptibility of Swedish, Norwegian, and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance genes. *Vet. Microbiol.* **99** :251-257.

Kulshrestha S.B., Kapoor K.N., Verma J.C., Garg R.K., Bass P.K., Singh B. and Jaipuriar (1982). Studies on food borne infections and intoxication in human patients. *IVRI, Ann. Sci. Re.*

Lanckriet A., Timbermont L., De Gussem M., Marien M., Vancraeynest D., Haesebrouck F., Ducatelle R. and

Van Immerseel F.(2010). The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model. *Avian Pathol.* **39**:63-68.

Mahony D.E., Ahmed R. and Jackson S.G. (1992). Multiple typing techniques applied to a *C. perfringens* food poisoning outbreak. *J. Appl. Bacteriol.* **72**:309-314.

Martel A., Devriese L., Cauwerts K., De Gussem K., Decostere A. and Haesebrouck F. (2004). Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials. *Avian pathology,* **33**: 3-7.

Mead P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., Griffin P.M. and Tauxe R.V. (1999). Food-related illness and death in the United States. *Emer. Inf. Dis.* **5**: 607-625.

Osman K.M. and Elhariri M. (2013). Antibiotic resistance of *Clostridium perfringens* isolates from broiler chickens in Egypt. *Rev. sci. tech. Off. Int. Epiz.* **32**:3.

Peterson L.R., Mshar R., Cooper Jr, G.H., Bruee A.R. and Hadler J.L. (1988). A large *Clostridium perfringens* foodborne outbreak with an unusual attack rate pattern. *Am. J. Exp.* **127**:605-11.

Piddock L.J.V., Ricci V., Stanley K. and Jones K. (2000). Activity of antibiotics used in human medicine for *Campylobacter jejuni* isolated from farm animals and their environment in Lancashire, UK. *J. Antimicrob. Chemother.* **46**:303-6.

Rahaman M.S., Akter M.R., Abdullah M., Sayed Khan M.A., Jahan M.S., Ziaul Haque A.K.M. and Kabir S.M. (2013). Isolation, identification and characterization of *Clostridium perfringens* from lamb dysentery in Dinajpur district of Bangladesh. *Sci. J. Microbiol.* **2**:83-88.

Rood J.I. and Cole S.T. (1991). Molecular genetics and pathogenesis of *Clostridium perfringens. Microbiol. Rev.* **55**:621-648.

Rood J.I., Maher E.A., Somers E.B., Compos E. and Duncan C.L. (1978). Isolation and characterization of multiply antibiotic-resistant *Clostridium perfringens* strains from porcine feces. *Antimicrob. Agents. Chemother.,* **13**:871-880.

Sapico F.L., Kwok Y.Y., Sutter V.L. and Finegold S.M. (1972). Standardized antimicrobial disc susceptibility testing of anaerobic bacteria: in vitro susceptibility of *Clostridium perfringens* to nine antibiotics. *Antimicrob. ag. Chemother.,* **2**:320-325.

Secasiu V. and Pastarnac N. (1993). Chemosensitivity of *C. perfringens* strains isolated from fur bearing animals. *Revista-Romana-de-Medicna-Veterinara,* **3**:63-69.

Shandera W.X., Tacket C.O. and Blake P.A. (1983). Food poisoning due to *Clostridium perfringens* in United States. *J. Infect. Dis.* **147**:167-170.

Silva R.O.S., Salvarani F.M., Assis R.A., Martins N.R.S., Pires P.S. and Lobato F.C.F. (2009). Antimicrobial susceptibility of *Clostridium perfringens* strains isolated from broiler chickens. *Brazilian. J. Microbiol.* **40**:262-264.

Singh R.V., Bhilegaonkar K.N. and Agarwal R.K. (2005). Studies on occurrence and characterization of *Clostridium perfringens* from select meats. *J. Food. Safety.* **25**:146-156.

Songer J.G. (1996). Clostridial enteric diseases of domestic animals. *Clin. Microbiol. Reviews.* **9**:216-234.

Stevens D.L., Maier K.A. and Mitten J.E. (1987). Effect of antibiotics on toxin production and viability of *Clostridium perfringens. Antimicrob. chemother.,* **31**:213-218.

Tansuphasiri U., Matra W. and Sangsuk L. (2005). Antimicrobial resistance among *Clostridium perfringens* isolated from various sources in Thailand. *South. East. Asian. J. Trop. Med. Pub. Heal.,* **36**:954-961.

Vaz E.K. (2009). Resistência antimicrobiana: como surge e o que representa para a suinocultura. *Acta. Scien. Vet.,* **37**:147-150.

van den Bogaard A.E.J.M., London N. and Stobberingh E.E. (2000). Antimicrobial resistance in pig faecal samples from The Netherlands (five abattoirs) and Sweden. *J. Antimicrob. Chemother.,* **45**:663-71.

The Blue Cross Book July 2015 Vol. 32 : 54-57

Effect of Methanolic Extract of *Caesalpinia sappan* Lin on Lead induced oxidative stress in Rats

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Abstract :

A study was conducted to evaluate the ameliorating effect of Caesalpinia sappan L on lead toxicity using albino rats. Four experimental groups with six rats in each, Group 1 (vehicle control), Group 2 (lead acetate @90mg/kg body weight), Group 3 (methanolic extract of Caesalpinia sappan @ 100mg/Kg b.wt) and Group 4 (Lead acetate @ 90mg/Kg b.wt. and methanolic extract of Caesalpinia sappan L @ 100mg/Kg b.wt) were treated for 28 days. The concentrations of TBARS and glutathione were increased significantly ($P<0.05$) in Group 2. The activities of GST, SOD and membrane ATPases in liver, kidney and heart and Vitamins E concentrations in liver, kidney revealed a significant decrease ($P<0.05$) in Group 2. Treatment with Caesalpinia sappan extract in group 4 resulted in marked improvement in all the above parameters as compared to Group 2 and evidenced the ameliorating effect.

Key words: Caesalpinia sappan; antioxidant activity; Lead toxicity

Introduction :

Lead is non essential, toxic heavy metal widely distributed in the environment (Alghaza *et al.,* 2008). For animals, main source of lead contamination is through soil, industrial pollution, agricultural technology and feed processing. Lead may cause acute and chronic toxicity, inducing broad range of physiological, biochemical and behavioural dysfunctions resulting in reduced performance and death in livestock. Lead also affects metabolism of other minerals and has affinity for bone, where it acts by replacing calcium. Thus highest concentration of lead is usually found in bone, followed by kidney and liver (Gurer and Ercal, 2000). Lead has potential to induce oxidative stress and acts as catalyst in oxidation reactions of biological molecules by producing free radicals. Lead interferes with different anti oxidant defences (Gayathri *et al.,* 2007) Many plants are known to exhibit antioxidant properties, *Caesalpinia sappan* L is one such plant belonging to family of Caesalpiniaceae reported to have strong antioxidant activity (Shrishailappa *et al.,* 2003). A study was conducted to evaluate the effect of methanolic extract of *Caesalpinia sappan* against experimental lead toxicosis in Wister rats.

Meterials and Methods:

A total of twenty four male Wister rats weighing 120-150g were procured and assigned randomly into four groups of six animals each. All the animals were acclimatized to lab conditions for 15 days prior to the start of the experiment and maintained as per the guidelines of CPCSEA. Rats were provided with feed and water *ad libitum* throughout the experiment.Group 1 received Gum acacia (0.5ml

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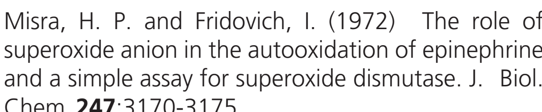
– 1%) orally, Group 2 was given Lead acetate @ 90mg /Kg body weight and group 3 was given methanolic extract of *Caesalpinia sappan* @ 100mg/Kg body weight orally and Group IV was administered with the combination of both, lead acetate and methanolic extract of *Caesalpinia sappan* at the doses mentioned in groups 2 and 3. The protocol was got approval of Institutional animal ethics committee. Animals were sacrificed on day 29 and organs like liver, kidney and heart were collected to study TBARS (Niehius and Samuelsson, 1968), glutathione (Ellman, 1959), SOD (Misra and Fridovich, 1972), catalase (Beer and Sizer, 1952), glutathione-S-transferase (Habig et al., 1974), vitamin C (Omaye *et al.,* 1979), vitamin E (Baker et al., 1951) and mitochondrial ATPases (Veldsema curie and Slater, 1969). The data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's post hoc test using statistical package for social sciences (SPSS) version 15. $P<0.05$ was considered as significant.

Results and Discussion:

Heavy metals like lead are known to increase levels of ROS generated in the body by virtue of excessive number of electrons in the outer orbits. Lead generated ROS include superoxide and hydroxyl radicals which will deteriorate biological macromolecules and deplete major cellular antioxidants (Gayathri *et al.,* 2007). The concentration of TBARS in liver, kidney and heart of Group II was significantly ($P<0.05$) increased at the end of 28 days. The increase in ROS, generated by lead could be attributed to the rise in serum MDA level. Increase in serum MDA levels could be used as a marker for free radical mediated destruction of liver parenchyma (Patil *et al.,* 2006). The levels of glutathione in liver, kidney and heart which were decreased in lead toxic control group, however, the significant difference was observed only with liver tissue (Table 1). Glutathione is one of the important thiol compounds present in the cell which helps in detoxification, binding and excretion of heavy metals. Exposure of animals to lead resulted in a parallel decrease in GSH in different organs (Siva Prasad *et al.,* 2003). The level of vitamins E (µM/mg) in liver, kidney and heart in group II showed significant ($P<0.05$) decrease than in group I. The reduction in the levels of vitamin E in different tissues which might be due to the alteration in the lipid content of the cells as evidenced by oxidative damage, thus reduced the storage of vitamin E (Upasani and Balaraman, 2001). However, vitamin C levels were not affected in any treatment group. *Caesalpinia sappan* by virtue of its antioxidant properties could have protected the cells and restored the levels of TBARS, glutathione, vitamin E in different tissues.

The activity of antioxidant enzymes, GST, SOD and membrane ATPases in liver, kidney and heart were significantly ($P<0.05$) decreased in group 2 (Table 2). Glutathione-s-transferases (GSTs) are cytosolic enzymes involved in detoxification of xenobiotics by conjugating with glutathione. SOD and catalase are the major enzymes present in RBC's and tissues to counteract the toxic effects of ROS such as superoxide radicals and hydrogen peroxides (Patra and Swarup, 2000). Lead induced generation of superoxide radicals could diminish the activity of SOD due to over utilization of SOD in neutralizing excess superoxide radicals. Further, lead binds to – SH groups in these enzymes (Wang *et al.,* 2006). The interaction of lead with copper also results in decreased availability of copper, which is necessary for proper functioning of SOD could be attributed to the reduced activity of SOD. Catalase is responsible for the breakdown of hydrogen peroxide produced during metabolism. Membrane ATPases are lipid dependent membrane bound enzymes. Alteration in membrane lipids leads to change in membrane fluidity, which in turn alters the ATPase activities and cellular functions. The decrease in the levels of these enzymes could be due to enhanced lipid peroxidation by free radicals generated by lead and binding of lead



with - SH group of lipid dependent ATPases (Ycebiligic *et al.,* 2003). Administration of *Caesalpinia sappan* to lead treated rats restored the activities of GST, SOD and catalase in all tissues and brought back the activity of membrane ATPases of lead treated group to normalcy thereby maintained the normal cellular functions.

In conclusion, the present study revealed that lead induced changes on antioxidative defence system in tissues were reversed by methanolic extract of *C. sappan* L by virtue of its strong oxidative property. Further studies are warranted on its combination with lead chelating agents to counter the lead toxicity in various biological systems.

References:

Alghaza, M.A., Lenartova, V., Holovska, K., Sobekova, A., Falis, M and Legath, J. (2008) Activities of antioxidant and detoxifying enzymes in rats after lead exposure. ACTA VETERINARIA. BRNO **77**: 347-354.

Backer, H., Frank, O., Angelis, B. and Feingold, S. (1951) Plasma tocopherol in man at various times after ingesting free or acetylated tocoferol. Nutri. Rep. Int. **21**:531-6

Beer, R. F. and Sizer, I. W. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195**:133-140

Ellman, G. L. (1959) Tissue Sulph hydryl groups. Arch. Biochem. Biophysic. **82**:70-77

Gayathri, M. R., Beena, V. S. and Sudha, K. (2007) Evaluation of lead toxicity and antioxidants in battery workers. Biomedical Research 19:1-4.

Gurer, H. and Ercal, N. (2000) Can antioxidants be beneficial in the treatment of lead poisoning? Free Radical Biol. Med. **29**: 927-945.

Habig, W. H., Pabst, M.J. and Jakoby, W. B. (1974) Glutathione-S-transferase. First enzymatic step in mercapturic acid formation.J. Biol. Chem. **249**:7130-7139

Misra, H. P. and Fridovich, I. (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. **247**:3170-3175

Niehius, W. G. and Samuelsson, D. (1968) Formation of Melondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem. **6**:126-130

Omaye, S. T., Turbull, T. D. and Sauberlich, H. C. (1979) Selected method for the determination of ascorbic acid in animal cells, tissues and fluids. McCormic D B, Wright D L eds. Enzymol Academic press, New York 62:3-11

Patil, A. J., Bhagwat, V. R., Dongre, N.N., Ambedkar, J. G., Rama, J. and Das, K. K. (2006) Effect of lead exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of western Maharashtra (India) with reference to heme biosynthesis. Int. J. Environ. Res. Pub. Health **3**: 329-337.

Patra, R.C. and Swarup, D. (2000) Effect of lead on erythrocytic antioxidant defence, lipid peroxide level and thiol groups in calves. Research in Veterinary Science **68**: 71-74.

Shrishailappa, B., Sudheer, M., Sujay, R., Kannan, E. and Suresh, B. (2003) Antioxidant activity of Caesalpinia sappan Heart wood. Biol. Pharmaceutical Bull. **26**: 1534-1537.

Sivaprasad, R., Nagaraj, M. and Varalakshmi, P. (2003) Combined efficacies of lipoic acid and meso 2,3 dimercaptosuccinic acid on lead induced erythrocyte membrane lipid peroxidation and antioxidant status in rats. Human Exp. Toxicol. **22**:183-192.

Upasani, C. D. and Balaraman, R. (2001) Effect of Vitamin E , Vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead. Ind. J. Pharmacol. **33**:185-191.

Veldsema curie, R. D. and Slater, (1968) Inhibition by anions of dinitrophenol induced ATPase of mitochondria. Biochem. Biophys. Acta **162**:310-319

Wang, J., Wu, J. and Zhang, Z. (2006) Oxidative stress in mouse brain exposed to lead. Ann. Occu. Hyg. **50**:405-409.

Ycebiligic. G., Bilgin, R., Tamer, I. and Tkel, S. (2003) Effect of lead on Na+ - K+ ATPase and Ca+2 ATPase and lipid peroxidation in blood of workers. Int. J. Toxicol. **22**: 95-97.

Table 1: Antioxidant and MDA levels in different tissues of experimental groups

Parametes	Group 1			Group 2			Group 3			Group 4		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
TBARS (n moles of MDA/100 g of tissue)	18.4± 2.2^{a}	18.2± 1.5^{a}	28.2± 3.3^{a}	35.6± 5.8^{b}	39.5± 1.6^{b}	40.0± 1.9^{b}	18.9± 2.4^{a}	18.7± 1.5^{a}	30.5± 2.6^{a}	23.8± 4.2^{a}	27.0± 1.6^{a}	32.6± 2.9^{a}
Glutathione (mg/100 g tissue)	10.9± 1.12^{a}	30.3± 1.6^{a}	36.7± 3.2^{a}	3.40± 0.48^{b}	25.1± 0.9^{a}	29.4± 1.1^{a}	10.4± 0.52^{a}	29.0± 1.1^{a}	36.2± 3.2^{a}	6.05± 0.35^{a}	27.1± 1.9^{a}	33.± 1.0^{a}
Vit E (µM/mg)	0.65± 0.05^{a}	2.9± 0.04^{a}	1.30± 0.03^{a}	0.40± 0.07^{b}	1.2± 0.02^{b}	0.66± 0.05^{b}	0.60± 0.08^{a}	2.8± 0.01^{a}	1.22± 0.03^{a}	0.50± 0.05^{a}	1.9± 0.01^{ab}	1.20± 0.06^{a}
Vit C (µM/mg)	0.07± 0.01^{a}	0.88± 0.04^{a}	0.19± 0.03^{a}	0.04± 0.01^{a}	0.43± 0.02^{a}	0.13± 0.01^{a}	0.07± 0.01^{a}	0.69± 0.01^{a}	0.16± 0.01^{a}	0.05± 0.01^{a}	0.65± 0.03^{a}	0.15+ 0.01^{a}

Table 2: Activity of antioxidant enzymes and membrane ATPases in different tissues of experimental groups

Parametes	Group 1			Group 2			Group 3			Group 4		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
GST (U/mg protein)	0.014± 0.003^{a}	0.010± 0.001^{a}	0.040± 0.000^{a}	0.006± 0.003^{b}	0.005± 0.001^{b}	0.020± 0.001^{a}	0.015± 0.003^{a}	0.010± 0.001^{a}	0.030± 0.001^{a}	0.008± 0.001^{a}	0.009± 0.001^{a}	0.030± 0.006^{a}
SOD (U/mg protein)	1.92± 0.29^{a}	6.60± 0.50^{a}	2.30± 0.6^{a}	0.90± 0.11^{b}	3.30± 0.40^{b}	1.40± 0.3^{b}	1.50± 0.14^{a}	5.70± 0.45^{a}	2.10± 0.3^{a}	1.30± 0.29^{a}	5.50± 0.80^{a}	2.01± 0.3^{a}
Catalase (KU/mg protein)	0.010± 0.005^{a}	0.08± 0.007^{a}	0.05± 0.001^{a}	0.003± 0.009^{a}	0.04+ 0.002^{a}	0.01± 0.005^{a}	0.008± 0.001^{a}	0.08± 0.001^{a}	0.03± 0.000^{a}	0.004± 0.001^{a}	0.05± 0.005^{a}	0.02± 0.002^{a}
Total membrane ATPases (µM of pi released/mg protein)	0.49± 0.01^{a}	0.26± 0.01^{a}	0.28± 0.04^{a}	0.26± 0.01^{b}	0.16± 0.01^{b}	0.16± 0.02^{a}	0.40± 0.02^{a}	0.24± 0.01^{a}	0.26± 0.01^{a}	0.38± 0.02^{a}	0.23± 0.01^{a}	0.21± 0.03^{a}

Values are Mean + S.E. of six observations
Means with alphabets as superscripts differ significantly ($p < 0.05$)

The Blue Cross Book July 2015 Vol. 32 : 58-60

Standardization of Polymerase Chain Reaction (PCR) for diagnosis of Dermatophilosis from Cattle & Buffalo in Andhra Pradesh.

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Abstract

Dermatophilosis is an economically important contagious zoonotic skin disease of livestock affecting a wide range of domestic and wild animals including man, is caused by Dermatophilus congolensis. The disease was previously diagnosed by stained impression smears, isolation and characterization of the organism from skin scabs of infected animals, though laborious and time consuming. In the present study, PCR was standardized for the detection of *Dermatophilus congolensis* directly from skin scabs of bovines without any preparatory DNA purification protocol, targeting 500bp 16s ribosomal RNA fragment of the organism.

Keywords: Diagnosis, Dermatophilosis, *Dermatophilus congolensis*, PCR, Bovines.

Introduction

Dermatophilosis is an economically important contagious zoonotic skin disease of livestock affecting a wide range of domestic, wild animals and humans is caused by *Dermatophilus congolensis* (Zaria, 1993). The disease was first reported in Belgian Congo by *Van Saceghem* in 1915, later it was reported worldwide (Zaria, 1993). The disease has a wide host range from domestic to wild and aquatic animals (Zaria, 1993). The domestic animals include cattle, buffaloes, sheep, goats and horses, which are most frequently affected and pigs, dogs and cats, which are rarely affected. The disease is characterized by acute (or) chronic, local (or) progressive exudative epidermatitis with serous exudation and subsequent drying to form characteristic matting of hair and scab formation (Zaria, 1993 and Loria et al., 2005). The economic losses result in the form of loss of condition, decrease in milk and meat production, reduced working ability, reproductive failures and depried hide values (Zaria, 1993). Though the conventional methods exist for diagnosis of the disease culturally and serologically (Abu-samra 1978), they are slow and laborious. The present study was aimed to standardize a simple, specific diagnostic method of PCR for rapid diagnosis of *Dermatophilosis* from skin scabs of infected animals.

A total of 33 samples were collected from clinically infected animals (cattle-18 and buffaloes-15) of Kadapa, Chittor and Kurnool districts of Andhra Pradesh. Initially the samples were screened by examination of Giemsa stained skin scab impression smears and results were compared with PCR.

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Polymerase chain reaction

The DNA extraction from infected skin scabs was followed according to the method of Johnson et al., (1995). The PCR was standardized for the diagnosis of *Dermatophilus congolensis* from skin scab samples, according to the method of Shaibu et al., (2010) with some modifications. The sequences of oligonucleotide primers specific for *Dermatophilus congolensis* employed in the PCR were as follows.

Forward primer: 5' ACATGCAAGTCGAACGATGA-3'

Reverse primer : 5' ACGCTCGCACCCTACGTATT-3'

PCR was set up in 25 µl reaction. The reaction mixture consisted of 2.5 µl of 10x Taq buffer A, 0.4µl of 10mM dNTP mix, 1.0µl of 25mM MgCl2, 0.4µl of Taq DNA polymerase (5U/ µl), 0.3µl of each primer (15p mol) and 1.0µl of template DNA. The volume was made up to 25µl with nuclease free water. The cycling conditions for amplification of 16s ribosomal RNA were as follows: Initial denaturation at 95°C for 1 min, 32 cycles of denaturation at 94°C for 30sec, annealing at 60°C for 30sec and extension at 72°C for 30sec. The final extension was performed at 72°C for 7min. Purified DNA from isolates of *D. congolensis* served as positive control, while DNA from *Staphylococcus aureaus* isolate was used as negative control. The amplified products were checked by electrophoresis on a 1.7% agarose gel at 80 volts for 60 minutes and visualized using gel documentation system (Alpha Innotech, Alphaimager HP). The results of PCR assay were compared with results of conventional impression smear examination as shown in table-1.

Table-1: Comparison of PCR with direct smear examination for diagnosis of Dermatophilosis.

S. No	Name of the district	Number of samples examined		Number positive for PCR		Number positive for direct smear examination		Number positive for PCR and negative for direct smear examination		Number positive for both PCR and direct smear examination	
		Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
1	Kadapa	-	15	-	12	-	9	-	3	-	9
2	Chittor	13	-	11	-	7	-	4	-	7	-
3	Kurnool	5	-	5	-	2	-	3	-	2	-
		18	15	16	12	9	9	7	3	9	9
Total		**33**		**28**		**18**		**10**		**18**	

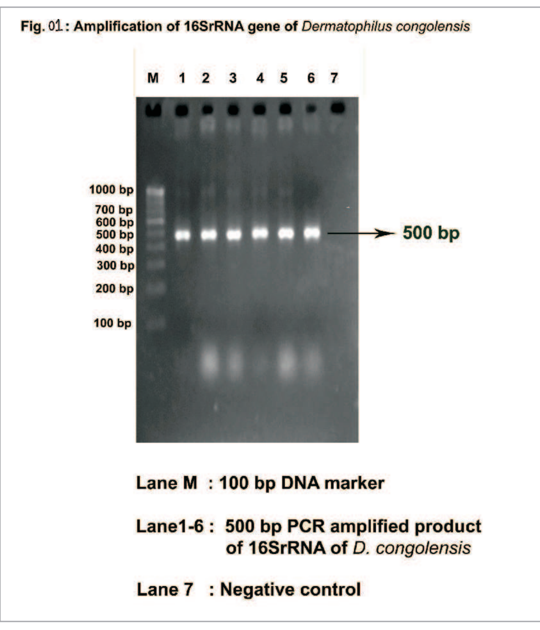
Results

In the present study, PCR was standardized as a 32 cycles PCR with annealing temperature of 60°C for 30sec and 1.0µl of 25mM MgCl2 was found optimum for the amplification of 500 bp product of 16s ribosomal RNA gene of *Dermatophilus congolensis*. The size of the amplified product observed was 500 bp (Fig.1).

Out of 33 samples, 18 samples (cattle-9 and buffaloes-9) found positive on direct smear examination. Similarly out of 33 samples, 28

samples (cattle-16 and buffaloes-12) were found positive on PCR. The results indicated that the PCR could detect all the positive samples which are positive on direct smear examination as positive and it also detected a total of 10 (cattle-7 and buffaloes-3) samples which were negative on direct smear examination as positive showing the sensitivity of the test (table-1) and no amplification in the negative control showed the specificity of the primers.

Fig. 01 : Amplification of 16SrRNA gene of *Dermatophilus congolensis*



Lane M : 100 bp DNA marker

Lane1-6 : 500 bp PCR amplified product of 16SrRNA of *D. congolensis*

Lane 7 : Negative control

Discussion

Diagnosis of *Dermatophilosis* in animals is often based on clinical findings, examination of Giemsa`s stained impression smears prepared from affected skin lesions, isolation and identification of causative agent *Dermatophilus congolensis* (Mannan et al., 2009). In the present study PCR was standardized and used for the detection of *Dermatophilus congolensis* directly from skin scabs of bovines. The PCR results of this study was in agreement with the research findings of Han Wen-Xing et al., (2007) and Shaibu et al., (2010) in detection as well as in sensitivity and specificity

Conclusion

A total of 33 skin scab samples (cattle18; buffaloes15) were collected from clinically suspected cases of Kadapa, Chittor and Kurnool districts of Andra pradesh. The clinical samples were subjected for direct smear and cultural examination initially later for PCR. Out of 33 samples 28 samples (cattle16; buffaloes12) were detected as positive by PCR showing the sensitivity and specificity; was found to be simple and rapid test for detection of *Dermatophilus congolensis.*

References

Abu-Samra M.T. (1978) Morphological and biochemical Characteristics of *D. congolensis.* Zentralbalt fia veterinarimeddizin Zbl, *Vet. Med,* **25**: 668-688.

Han WenXing., Chen Yu., Wang JingMei., Wang YuanZhi. and Yan GenQiang. (2007) Establishment of PCR for detection of dermatophilosis in sheep, *Chinese Journal of Veterinary Science* **29**: 49-51.

Johnson M.L., Quednau M., Molin G. and Ahme, S. (1995) Random amplified polymorphic DNA (RAPD) for rapid typing of Lactobacillus *plantarum* strains, *Lett. Appl. Microbiol.* **21**: 155-159.

Loria G.R., Babera Ela., Monteverde V., Piraino C. and Caracappa, S. (2005) Dermatophilosis in goats in Sicily, *Veterinary Record,* **156**: 120-121.

Mannan M.A., Khan M.S.R., Rahman M.M., Begum F. and Uddin, M.Z. (2009) Isolation and identification of Dermatophilus Bacteria from skin lesions of cattle, Bangl. *J. Vet. Med.* **7**: 342 – 347.

Shaibu S.J., Kazeem H.M., Abdullahi U.S. and Fatihu, M.Y. (2010) The use of Polymerase Chain Reaction in the diagnosis of dermatophilosis from cattle, sheep, and goats in Nigeria, *Journal of animal and veterinary advances* **9**: 1034-1036.

Zaria L.T. (1993) Dermatophilus congolensis infection (Dermatophilosis) in animals and man, an update, *Comp. Immunol. Infect. Dis.,* **16**: 197-222.

The Blue Cross Book July 2015 Vol. 32 : 61-63

Occurrence of Marek's disease associated immunosuppression in unvaccinated commercial broilers

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Abstract

The occurrence of MD in unvaccinated commercial broilers was investigated. MD lesions were observed in 15.6% (15/96) of slaughtered birds at 42 days of age and 40.0% (6/15) of dead birds examined during 28-42 days of age. Affected birds showed enlargement of spleens 3-5 times bigger than normal. The immune response to ND vaccines as determined by HI antibody titers was poor in these birds. This suppression of immunity can be attributed to early cytolytic infection of B-lymphocytes. DNA isolated from enlarged spleens and feather follicles was found positive for serotype-1 MD by PCR. These findings suggest that MD is prevalent in young broilers and vaccination of commercial broilers can prevent immunosuppression and associated losses in broilers.

Key words : Broilers, Marek's disease, immunosuppression, polymerase chain reaction

Introduction

Marek's disease (MD) is a highly contagious lymphoproliferative disease of chickens caused by serotype-1 MD virus belonging to Herpesviridae family (Davison, 2002). Indian poultry industry suffered from serious outbreaks of MDV during 2003-06 and part of the response to this has been the introduction of a cell associated bivalent (serotype 2 and 3) vaccine. Layers and breeders are routinely vaccinated against MD all over the world but broilers are vaccinated only in some countries including Europe, USA and Australia (Witter and Schat, 2003). In India broilers are not vaccinated against MD and information of occurrence of MD is scarce. The present paper describes the occurrence of MD associated immunosuppression in unvaccinated commercial broilers.

Materials and Methods

Commercial broilers died (15) and slaughtered (96) at 6 weeks of age were examined during the year 2007. Samples from grossly enlarged spleen, liver, kidney were collected in 10% formalin for histopathology. Feather follicles and tissues from enlarged spleen and liver were frozen for DNA isolation and PCR detection of MD virus. Commercial broilers were vaccinated against Newcastle disease (ND) at 7th and 28th day by live ND Lasota vaccine. Serum samples were obtained from broilers at 42 days of age for estimation of antibody levels against Newcastle disease.

For histopathology, formalin fixed tissues were processed by standard paraffin embedding technique, sections were cut at 5 microns thickness, processed and stained with haematoxylin and eosin (H&E) for examination under light microscope for characteristic lesions.

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Tissue homogenates were lysed in tissue lysis buffer (4M guanidine hydrochloride, 25 nM sodium citrate, 1% Triton x100 and 200 µg proteinase K/ml) and feather follicles were lysed in feather lysis buffer (50 mM Tris (pH 8), 20 mM EDTA, 2% SDS and 200 µg proteinase K/ml) at 37°C overnight. DNA was isolated using standard phenol-chloroform method (Sambrook et al., 1989). PCR was used to test DNA for the presence of 132 bp repeat sequence specific for MDV as has been described (Becker et al., 1993). The oligonucleotide primers used in PCR detection of MDV-1 included the forward primer M1 (5'-TACTTCCTATATAGATTGAGACCGT-3') and the reverse primer M2 (5'-GAGATCCTCGTAAGGTGTAATATA-3'). This pair of primers is known to be specific for MDV-1 and result in a 300 bp PCR product. MDV-1 DNA was amplified in a 25 µl reaction volume, using a mixture composed of 2.5 µl 10x Taq DNA polymerase buffer (Promega), 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.0 mM MgCl2, 200 mM dNTPs, 20 pmol each primer, 0.5 U of *Taq* DNA polymerase and 3 µl of template DNA. Reaction volumes were made up to 25 µl using nuclease free water. Amplification was performed in thermocycler (DNA Engine) with PCR conditions consisted of an initial denaturing step of 4 min at 94°C followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 55°C for MDV- and 60°C for HVT for 30 sec and an elongation step at 72°C for 30 sec and a final elongation step at 72°C for 10 min. Amplification products were resolved by electrophoresis through 1% agarose gels containing 0.5 µg/ml ethidium bromide and examined under U V transillumination. Samples were considered MDV-1 positive if the expected 300 bp product was able to be visualized after electrophoresis.

Humoral immunity in affected flock was assessed by measuring antibody levels to ND vaccination. Serum samples obtained two weeks after booster vaccination against ND were tested for antibody levels by haemagglutination inhibition test using 4 HA units of ND virus as antigen. Titers were expressed as the log2 of the reciprocal of the highest dilution of serum in which agglutination was inhibited.

Results and Discussion

The present study investigated the occurrence of Marek's disease in unvaccinated commercial broilers. MD lesions were observed in 15.6% (15/96) of slaughtered birds at 42 days of age and 40.0% (6/15) of dead birds examined during 28-42 days of age. The most frequent lesion observed in affected birds was diffuse enlargement of spleen (2-5 times the normal) with grayish-white discoloration (Figure 1). Diffuse enlargement of liver, proventriculus and kidney was also observed in some cases. Microscopically, diffuse infiltration of lymphoid cells was observed in spleen, liver, kidney and proventriculus. DNA samples extracted directly from spleen and feather follicles yielded a MD specific 300 bp product in the PCR assay using M1 and M2 primers (Figure 2). PCR results in this study confirmed the presence of MD sequences in DNA, which was extracted directly from enlarged spleen and feather follicles. The genomic DNA extracted from MD negative birds were used as negative controls (Figure 2). Direct extraction of genomic DNA from these tissues can rapidly establish the presence of MDV-1 (Handberg, 2001). Antibody titers against ND were low (log2, 3.8) in MD affected broilers as compared to MD negative broilers (log2, 5.7). Atrophy of bursa and thymus was observed both in dead and slaughtered birds. Marek's disease virus is a lymphotropic virus and targets lymphocytes, the principal cells of the immune system. B-lymphocytes are first targeted by the virus in a lytic infection which is followed by cytolytic infection of activated T-lymphocytes (Witter and Schat, 2003). These early cytolytic events result in atrophic changes in the bursa of

Figure 1. Diffuse enlargement of spleen in 6 week old commercial broilers

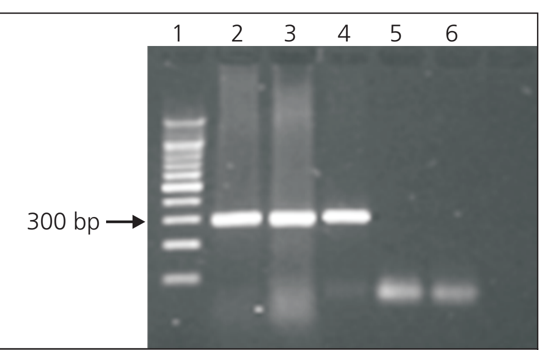

Figure 2. PCR Detection of MD in commercial broilers. Lane1. DNA marker, lane 2.DNA from feather follicles, Lane 3. DNA from spleen, lane 4. positive control, and lane 5 and 6 negative control.

Fabricius and thymus, leading to severe debilitation of the immune system and marked immunosuppression (Calnek, 2001). Humoral and cell mediated immunity can be suppressed by MDV infection leading to reduced antibody responses to vaccines and alteration in T-cell function (Islam, 2002, Witter and Schat, 2003).

Because of the ubiquitous nature of the infection and the ability of MDV to survive for long periods outside the host, flock infections usually occur early in the life of the bird. Currently, vaccination represents the principle strategy for the prevention and control of MD in layers and broiler breeders. Vaccination of commercial broilers in-ovo on 18th day of incubation or immediately after hatch in the hatchery would prevent MD, immunosuppression, shedding of virus and reduce condemnations (Witter and Schat, 2003).

References

Becker, Y., Tabor, E., Asher, Y. E., Davidson, I., Malkinson, M. and Witter, R.L. (1993). PCR detection of amplified 132 bp repeats in Marek's disease virus type 1 (MDV-1) DNA can serve as an indicator for critical genomic rearrangement leading to the attenuation of virus virulence. *Virus Genes,* **7**: 277-287.

Calnek, B. W. (2001). Pathogenesis of Marek's disease virus infection *Current Topics in Microbiology and Immunology*. **255**:25-55.

Davison, A.J. (2002). Evolution of the herpesviruses. *Veterinary Microbiology*. **86**: 69-88

Handberg, K. J., Nielsen, O. L., Jorgensen, P. H., (2001). The use of serotype 1- and serotype 3-specific polymerase chain reaction for the detection of Marek's disease virus in chickens. *Avian Pathology*. **30**: 243-249.

5Islam, A. F. M. F., Wong, C. W., Walken-Brown, S. W., Colditz, I. G., Arzey, K. E., and Groves, P. J., (2002). Immunosuppressive effects of Marek's disease virus (MDV) and herpesvirus of turkeys (HVT) in broiler chickens and the protective effect of HVT vaccination against MDV challenge. *Avian Pathology,* **31**: 449-461.

Sambrook, J., Fritsch, E. F, and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Vol 1, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Schat, K. A and Nair, V (2008). Marek's disease. In *Diseases of Poultry,* 12th edn. Blackwell Publishing, Ames: Iowa, USA. pp.452-514.

The Blue Cross Book July 2015 Vol. 32 : 64-67

Efficacy of Tube Cystostomy for the treatment of obstructive urolithiasis in Buffalo calves

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Abstract

The study was conducted on 32 buffalo calves suffering from obstructive urolithiasis. The animals were divided into three groups based on the status of urinary bladder and urethra. Percutaneous tube cystostomy was done in all the cases under epidural anesthesia. The success rate of tube cystostomy was observed more in animals presented with intact bladder because subserous or incomplete rupture may cause atony of the bladder and such cases are more difficult to treat. The success of treatment also depends on how early the condition is surgically managed.

Introduction:

Urolithiasis is the formation of urolith anywhere in the urinary system and is reported worldwide. In India, urolithiasis has mostly been reported in bullocks, goat, sheep and buffaloes from different parts of the country (Tyagi and Singh, 1993). In recent years, occurrence of urolithiasis has increased in caprines and bovines (Amarpal et al.,2004). Predisposing factors like age, type of feed and water, season, castration etc have been identified to play important role in pathogenesis of the disease. The obstruction in the flow of urine due to calculi may occur in any part of the urinary tract, but mostly it is in urethra. In bovines,clinical signs of retained urine become apparent within 24 hours after complete urethral obstruction. In most of the cases, urethral or urinary bladder rupture occurs within 72 hours of obstruction and untreated animal may die in four to five days. Subserous or incomplete rupture may cause atony of the bladder and such cases are more difficult to treat than following complete rupture. The present study was conducted to study the efficacy of tube cystostomy for the treatment of obstructive urolithiasis in buffalo calves.

Materials and Methods

Thirty two buffalo calves presented with the history of anuria and dysuria to the Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar were selected for the present study. All the animals were subjected to thorough anamnesis and clinical examination and the status of urinary bladder and urethra were recorded. The animals were divided into three groups based on the status of urinary bladder and urethra. Animals with intact bladder and urethra (n = 15) were included in group 1. Eleven animals with ruptured bladder (n = 11)and six animals (n = 6) with ruptured urethra were included in group 2 and group 3, respectively.

Heart rate, respiratory rate and rectal temperature were recorded preoperatively. In all the three groups, tube cystostomy was performed using Foley's catheter and the results were recorded. Lumbosacral epidural analgesia was given with 2% lignocaine and the animals were secured in right lateral recumbency for performing laparotomy

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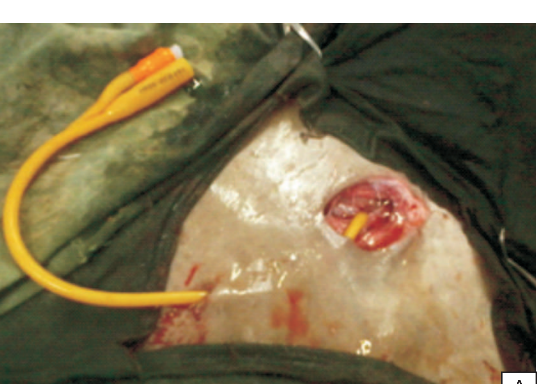


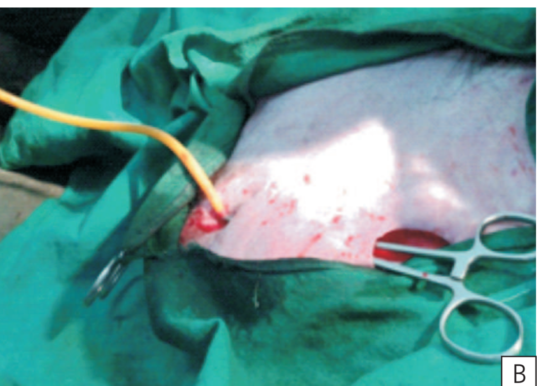
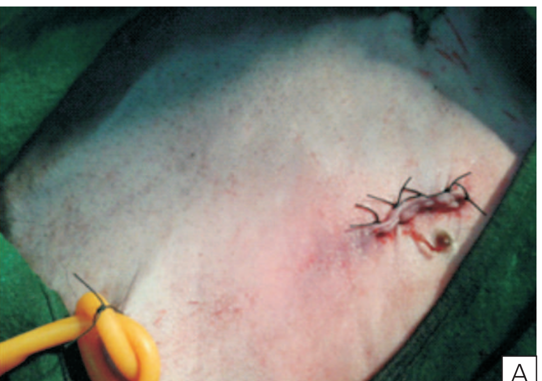


Fig.1.Intra operative photographs of one of the animal during tunneling (A) and after abdominal wall repair (B)



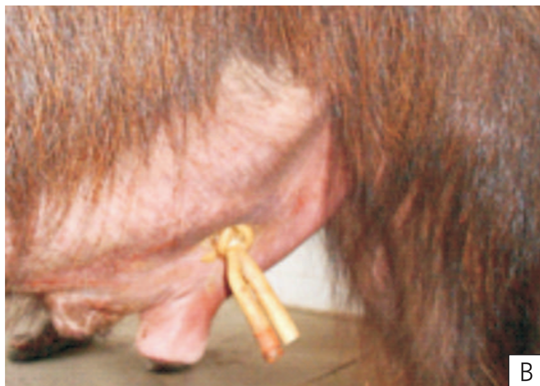


Fig.2.Post operative photographs of the same animal just after surgery (A) and one day after surgery (B)

In group 1, an incision was made at caudoventral abdomen lateral and parallel to the rudimentary teat. Abdominal muscles were separated by blunt dissection. After performing laparotomy, a tunnel was made subcutaneously extending from cranial end of incision to the umbilical region. A Foley's catheter was introduced through the tunnel and with the help of a k-wire anchored in the eye of catheter; it was inserted into the bladder through its ventral aspect with a sudden thrust without incising the urinary bladder if the bladder was intact (Fig.1). The balloon of the catheter was inflated with 10 ml of normal saline to prevent it from dislodging from the bladder and the k-wire was pulled out slowly. The laparotomy incision was sutured in continuous pattern with catgut and the skin was sutured with silk in horizontal mattress pattern with silk. The catheter was fixed to the abdominal wall with simple interrupted silk sutures (Fig.2).

In group 2 where animals were presented with ruptured urinary bladder,laparotomy was performed and the tear in the bladder was sutured with Lembert's pattern using 2/0 catgut rest of the procedure was similar to group 1.

The animals with ruptured urethra were included in group 3. In the cases with intact bladder along with ruptured urethra, the surgical correction was similar to that of group 1. In another cases where both urethra and urinary bladder were found ruptured, the surgical method of group 2 was used. Ruptured urethra was left as such in all the cases.

The postoperative care included daily dressing of the wound with 0.5% povidone iodine solution till healing. Antibiotic (Tab Norflox@ 22mg/kg BW) for five days, meloxicam @ 0.2 mg/kg BW and Tab Ammonium chloride @ 500mg/kg BW for two weeks. Heart rate, respiratory rate and rectal temperature were also recorded post-operatively.

The owners were advised to flush the foley's catheter at an interval of 2 hours for first three days and then to keep it closed for 2 hours after flushing until the animal urinated through the external urethral orifice.The animals were observed for any untoward reactions against the catheter.

Results and discussion

All the animals in the present study were 3 to 5 months old intact males. The urethral diameter and the strength of urethralis muscle are controlled by testosterone hormone, which is low in younger male. The proper development of the urethral muscle is not expected in younger animals as it would occur in adult animals. Thus the younger animals are unable in expelling even the small calculi from the urethra resulting in urethral obstruction

Elevation in heart rate and respiratory rate were noticed pre-operatively and post-operatively in all the three groups of animals. This increase could be due to pain caused by urethral obstruction before surgery, surgical pain at the operation site, traumatic inflammatory reaction and post- surgergical stress.

In animals with intact bladder, the tube cystostomy procedure was easier to perform and complications encountered were much less. Out of 15 animals, two animals were presented again due to blockage of Foley's catheter. Immediate complications that were encountered during cystostomy were death (n=1) due to hypovolemic shock which might be due to the sudden siphoning of urine from the peritoneal cavity in animals with ruptured bladder (In group 2) and death due to hypothermia (n=1) during laparotomy in extreme winter. Late complications were blockage of Foley's catheter, abscess, deflation and dislodgement of catheter.Subserosal tear on the dorsal surface of bladder healed without complication when the obstruction to the urine flow was relieved in group 2. Tear on the ventral surface resulted in leakage of urine through the site of tear in group 2. Among the eleven animals presented with ruptured bladder, three were with severe bladder necrosis and they died during post-operative treatment. In Group 3, there was necrosis and sloughing of skin due to subcutaneous accumulation of urine in all the animals. Out of the six animals of this group, three animals died at different days post-surgery. Remaining three animals survived and urine was dribbling out of a pre scrotal opening formed after urethral slough. The wound at the sloughed off skin healed as an open wound.

Occurrence of urolithiasis in buffalo calves in the present study varied considerably with season and maximal incidence was recorded from December to March. The incidence of urolithiasis has been reported to be influenced by season and higher incidence has been recorded during peak summer (Bhatt et al., 1973) and winter (Singh and Singh, 1990; Amarpal et al., 2004). High occurrence of urolithiasis during winter is

probably due to reduced water intake by buffalo calves during this period.

Reduced availability of milk, reduced intake of water during winter along with feeding rice bran and wheat bran which are rich in phosphorus leads to precipitation and crystallization of minerals in urine. This results in formation of uroliths, which may obstruct the narrowest path of urethra i.e. in the region of sigmoid flexure or glans penis and cause obstructive urolithiasis. In the present study the reduced intake of water during winter and feeding of wheat bran were possibly the two factors responsible for the obstructive urolithiasis.

Rupture of urinary bladder is more likely to occur with complete obstruction of urethra. Administration of diuretics in case of complete obstruction may increase the chances of rupture of urethra. Larson (1996) opined that castration caused the reduction in the urethral diameter and thus predisposed the animal for calculus obstruction. In this study subserous or incomplete rupture was recorded in 6 out of 11 animals of group 2. Complete rupture was in five animals of this group. The subserous or incomplete rupture led to low success rate in this group because it may cause atony of the bladder and such cases are more difficult to treat than following complete rupture. Lowest success rate was obtained in group 3 because of sloughing of skin which led to infection at the site and further complications. More over, these types of cases were presented late to the clinics and were in compromised condition.

Urolithiasis occurs equally in male and female animals but obstruction is not generally caused in the female urethra due to the short length and flexible lumen of the urethra as tunica albuginea is not present over the female urethra. This could be the reason that obstructive urolithiasis was recorded only in male buffalo calves in the present study.

From the results of the present study, it may be concluded that the success rate of tube cystostomy is more in animals presented with intact bladder, because subserous or incomplete rupture may cause atony of the bladder and such cases are more difficult to treat. The success of treatment also depends on how early the condition is surgically managed.

References

Amarpal, Kinjavdekar, P., Aithal, H.P., Pawde, A.M., Singh, T. and Pratap, K. (2004). Indian J. *Anim. Sci*. **74**: 175-177.

Bhatt, G.A., Ahmed, S.A., and Prasad, B. (1973). *Indian Vet. J.* **70**: 459- 464.

Ewoldt, J.M., Anderson, D.E., Miesner, M.D. and Saville, W.J. (2006). *Vet. Surg*. **35**: 417-422.

Larson, B.L. (1996). *Vet. Med*. 366-377.

Radostitis, O.M., Blood, D.C., Gay, C.C and Hinchcliff, K.W. (2000) Veterinary Medicine: A text Book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9th ed. Bailliere Tindall, London. 1877p.

Singh, J. and Singh, K. (1990). *Indian J. Vet. Surg*. **11**: 1-20

Tyagi, R.P.S. and Singh, Jit. (1993) Ruminant Surgery. CBS Publishers and Distributors, New Delhi. 484p.

The Blue Cross Book July 2015 Vol. 32 : 68-70

Photosensitization due to ingestion of *Lantana camara* in Buffaloes

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Abstract:

Lantana camara , a shrub with yellow, pink, or red clustered flowers is known for its hepatotoxic effects, exhibited by jaundic and photosensitization. The successful clinical management in three buffaloes with active charcol (2.5 kg/animal orally) and other supportive treatment is described.

Introduction

Lantana camara is a toxic weed also known as Spanish flag or West Indian *Lantana*. The most important toxic principle of *Lantana camara* is lantedene A, a penta cyclic triterpene acid (Ross, 1999). *Lantana camara* is a noxious weed that grows in many tropical and subtropical parts of the world. Ingestion of lantana foliage by grazing animals causes injury to bile canalicular membrane leading to intrahepatic cholestatis and hepatocellular damage. The intrahepatic cholestasis prevents the excretion of phylloerythrin in bile leading to accumulation of phylloerythrin in blood and skin leading to photosensitisation (Noble *et al.,* 1998). Present paper describes about the successful management of photosensitization in buffaloes.

Case History and Observation

Three adult female Murrah buffaloes were presented to Out Patient Department, Veterinary College, Bidar with the history of anorexia,

Lantana camara at flowering

lethargy, depression, jaundice and dermatitis since three days. On detailed anamnensis, it was revealed that animals were exposed to *Lantana camara* plants while grazing. On Clinical examination of the animals showed exudative dermatitis lesions on dorsum and lateral sides of the body. Severe swelling of muzzle and eyelids were noticed. Samples like skin scrapings, ruminal fluid and blood were collected from all three buffaloes. Skin scraping examination did not reveal any ectoparasites and fungal spores.

Ruminal fluid was viscous, dark green colored and of alkaline pH (8.4). Microscopic examination of ruminal fluid revealed that all protozoa were dead and reduced in number. Haematobiochemical changes are depicted in

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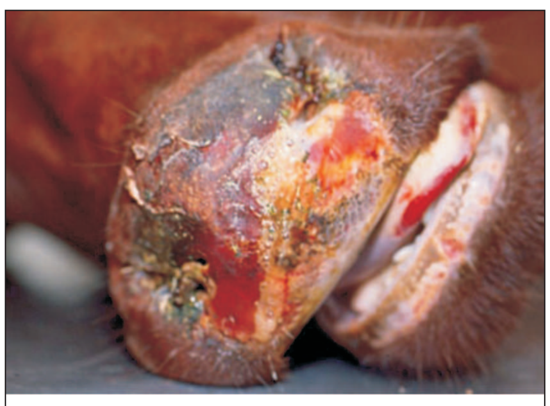
Table 1 and the major abnormalities were normocytic normochromic anaemia, hyperbilirubinemia and elevated serum aspartate amino transferase and serum alkaline phosphatise enzyme levels. Based on these findings, a diagnosis of photosensitization was made.



Swelling of muzzle and eyelids

Treatment and Discussion

Animals were moved indoors and treatment was begun with the administration of Activated Charcoal. About 2.5 kg of activated charcoal was administered to each animal. Other supportive treatment like antihistaminics (Chlorpheneramine maleate @ 0.5mg/kg BW, IM, BID), antibiotics (Ceftriaxone @ 5mg/kg BW, IV, BID for 5 days), multivitamins (Tribivet @10ml, IM, for 3 days) and fluid therapy were given to all the three animals. Topical steroidal application was done on skin lesions. Improvement was seen in affected animals 2 days post treatment. Animals started to take feed and water normally. Complete improvement was seen after 5 days post treatment. Cases were discharged by advising the owner not to graze the animals on *Lantana camara* rich areas.

Lantana camara has spread as an intractable weed in many parts of the world. It has been found in nearly fifty countries and is the principal

Table 1. Haematobiochemical Findings in Cases with Photosensitization

Parameters	Buffalo 1	Buffalo 2	Buffalo 3
TEC ($10^6/mm^3$)	5.9	6.27	6.13
Hb (g%)	9.3	10.85	9.85
PCV (%)	26.25	28.25	29.01
TLC ($10^3/mm^3$)	11.89	10.85	11.06
Total Protein (g/dl)	6.69	7.17	6.84
Albumin (g/dl)	2.75	3.02	2.95
Globulin (g/dl)	3.94	4.15	3.89
Total Bilirubin (mg/dl)	2.49	1.75	2.28
Direct (mg/dl)	1.19	1.15	1.24
Indirect (mg/dl)	1.30	0.60	1.04
AST(IU/L)	255.78	145.85	195.28
ALP(IU/L)	299.85	123.25	130.25

weed in twelve countries (Misra et al, 1997). Cases of Lantana poisoning in livestock has been reported from India, the USA, Australia, Brazil, Indonesia, and Africa (Noble *et al.,* 1998). Lantana was introduced into India in the nineteenth century and has spread all over the country. Present cases were presented in summer season. This is justified by Pass (1991), they opined that scarcity of other fodder during draught period makes livestock susceptible for ingestion and development of Lantana poisoning. Jaundice noticed in present cases might be due to hepatocellular injury by lantadines. This is supported by Day *et al* (2003), they stated that lantadines are hepatotoxic. Treatment protocol followed in present cases is justified by Sharma et al (1988). They suggested the use of activated charcoal and other supportive therapies in the management of photosensitization in livestock.

References:

Day, M.D., Wiley, C.J., Playford, J. and Zalucki, M.P. (2003). Lantana: current management status and future prospects. Australian Centre for International Agricultural Research Canberra. Pp29.

Misra, L.N., Dixit, A.K. and Sharma, R.P. (1997). High concentration of hepatoprotective oleanolic acid and its derivatives in Lantana camara roots. *Planta Medica.,* **63**:582-584.

Noble, D., Lane, S. J., Sidebettom, P. J., Lynn, S..M., Hayes, M. V. and Dix, C. J. (1998). Isolation of translactone- containing triterpenes with thrombin inhibitory activities from the leaves of *Lantana camara*. *J Nat. Prod.,* **61**:1328-1331.

Pass, M. A. (1991). Poisoning of livestock by lantana plants. In: Handbook of Natural Toxins, Vol. 6, Toxicology of plant and fungal compounds (Keeler, R. and Anthony, T., eds.), 297-311.

Ross, L. A. (1999). Lantana camara. In: Medicinal plants of the world. Chemical constituents, traditional and modern medicinal uses. Humana Press, Totowa, New Jersey, pp. 179-182.

Sharma, O. P., Makkar, H. P. S. and Dawra, R. K. (1988). A review of the noxious plant *Lantana camara*. Toxicon, **26**:975-987.

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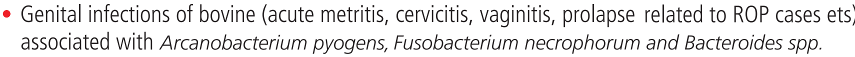
COMPOSITION

Ceftiofur Sodium sterile powder equivalent to Ceftiofur.....1g
One ampoule of sterile water for Inj. IP 20ml

INDICATIONS

- Genital infections of bovine (acute metritis, cervicitis, vaginitis, prolapse related to ROP cases ets) associated with *Arcanobacterium pyogens, Fusobacterium necrophorum and Bacteroides spp.*



- Respiratory diseases of cattle, buffalo, sheep and goat (shipping fever, pneumonia) associated with *Pasteurella haemolytica, Pasteurella multocida* and *Haemophilussomnus.*
- Acute interdigital necrobacillosis (Foot rot, Pododermatitis) caused by *Fusobacterium* & *Bacteroides.*

DOSAGE AND ADMINSTRATION

Cattle, Buffalo, Sheep & Goat : 1.1 to 2.2 mg ceftiofur per kg body weight by IM route for 3 to 5 days.

WITHDRAWAL PERIOD
Milk : 0 (Zero) days
Meat : 4 days

The Blue Cross Book July 2015 Vol. 32 : 71-74

Management of atonic bladder in a Bitch following an Ovariohysterectomy

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Abstract

A 3-year-old bitch with a atonic bladder, anorexia, distention of abdomen following the ovariohysterectomy 5 days back, was presented for the treatment. On clinical examination, abdomen was found grossly distended and the fluid was coming out through the midventral celiotomy incision. Biochemical examination showed raised blood urea nitrogen and creatinine levels. Ultrasonographic findings showed distention of urinary bladder along with the thinning of the wall of the bladder resulting in the seepage of urine into the peritoneal cavity leading to uroperitoneum with anechoic fluid. Ultrasonographic findings further revealed enlargement of kidney (hydronephrosis) with dialated pelvis and diverticuli. The case was diagnosed as atonic bladder.The therapeutic management resulting into clinical recovery is discussed.

Key words: Bitch, Bladder atony, Ultrasonographic evidence, Catheterization.

Introduction

Atonic bladder is a condition in which bladder enters a state of "permanent fullness", where urine may passively leak at a point or overflow through the urinary sphincter which cannot withstand the constant pressure. It is also known as "detrusor atony" or "flaccid bladder". Atonic bladder and the resulting incontinence are caused by atrophy, or some form of damage, to the detrusor muscle. This muscle weakness results in an inability to properly empty the bladder leading to frequent and unsuccessful attempts to urinate. Bladder atony may be primary or secondary to an increase in urethral resistance of anatomical or functional origin. When bladder atony is secondary to bladder over-distension, clinical presentation includes stranguria or overflow incontinence. When the bladder is excessively dilated, the detrusor tight junctions are torn apart, resulting in weaker, uncoordinated or absent bladder contractions (Azadzoi *et al.,* 1996). It can also result from any lesion in the spinal cord. It may be either due to lesion in upper or the lower motor neuron. In case of a lower motor neuron (LMN) lesion due to sacral or lumbar plexus lesions, the voluntary nervous control and the micturition reflex are no longer present (Fenner, 1993). Urethral tonicity is decreased leading to overflow incontinence (Gregory, 1996, Labato, 2005; Fischer and Lane, 2007). In case of a upper motor neuron (UMN) lesion, an incomplete contraction of the detrusor muscle is observed as well as an increase in urethral tonicity preventing complete bladder voiding. With severe lesions, all impulses from

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the higher motor neurons on the micturition reflex are lost. Micturition occurs when bladder pressure is sufficient to force the urethral opening, but the bladder cannot be emptied completely and residual urine persists. After the insult, an involuntary micturition reflex reappears gradually, leading to an 'autonomous' or 'automatic' bladder (Fenner, 1993; Labato, 2005). An UMN bladder can thus be described as a bladder in which a reflex of micturition is present but without voluntary control.

Material and Methods

A 3-year-old bitch was presented to the Division of Veterinary Surgery and Radiology, Shuhama, Srinagar with the history of anorexia, distention of abdomen, following ovariohysterectomy 5 days back.On Clinical examination, animal was found to be dull and depressed. The abdomen was grossly distended and the fluid was coming out through the dehiscenced midventral celiotomy incision.The rate of dribbling of fluid through the dehiscenced midventral celiotomy incision increased 10-15 minute after intravenous fluid administration. The sample of peritoneal fluid was taken for physical and biochemical examination. The physical examination of fluid revealed a pH value of 6.4 and specific gravity of 1.052. Ammoniacal smell was noted once the sample was heated. Biochemical examination showed blood urea nitrogen (BUN) concentration of 0.77 g/L, (normal range: 0.13 to 0.51 g/L), while creatinine level was 17.2 mg/L, (Normal range: 4 to 15 mg/L).Haematological examination revealed total erythrocytic count of (4.39 x 10^6 cumm), total leucocytic count (66.7 x 10^3 cumm), haemoglobin (8 gm/dl), neutrophils 95%, eosinophil 1%, and lymphocyte 4%. Hypersegmented neutrophils were observed along with neutrophilia. Ultrasonographic findings showed distention of urinary bladder along with the thinning of the wall of the bladder resulting in the seepage of urine into the peritoneal cavity leading to uroperitoneum with anechoic fluid (Figure 1). Ultrasonographic findings further revealed enlargement of kidney (hydronephrosis) with dialated pelvis and diverticuli (Figure 2).On the basis of clinical, haematological, biochemical examination and ultrasonographic evidence, the case was diagnosed as atonic bladder.

Animal was prepared for bladder catheterization as routine procedure. The vagina of the bitch was flushed with the normal saline so as to prevent any kind of contamination. A well lubricated catheter was passed from the external urethral orifice in an ascending manner up to the

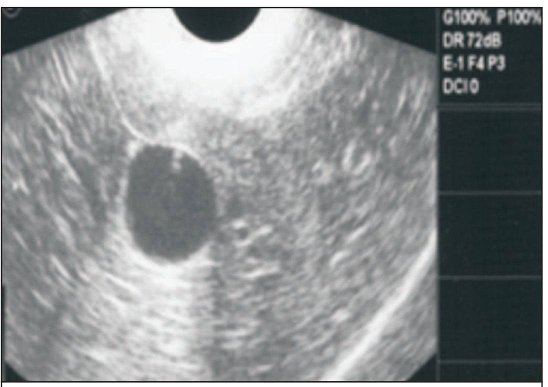

Figure 1: Ultrasonographic image of catheterization in urinary bladder (arrow)

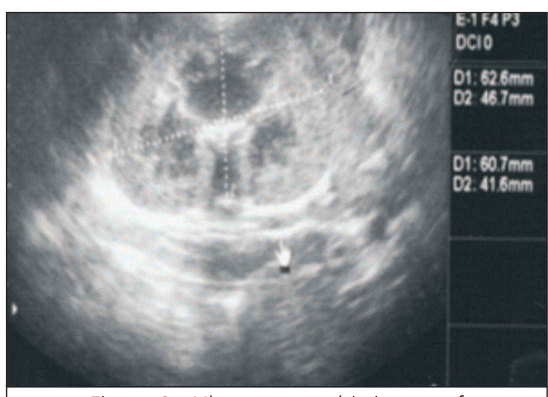

Figure 2 : Ultrasonographic image of enlarged kidney

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bladder so as to facilitate the passage of urine. The patient was given phenylpropanolamine (1.5 mg/kg) twice in a day orally, lespedeza capitata (1ml/kg) twice in a day for 2 days through intramuscular injection, estriol (2 mg) orally daily for 5 days and cefalexin (20mg/kg) every 12 hours orally for 3 days.

Results

Bitch had increased BUN and creatinine along with presence of urine in the peritoneal cavity. Apart from surgical intervention, medicinal therapy was also followed. After passage of catheter, complete uninterrupted flow of urine followed. The catheter was kept in situ to ensure normal flow of urine. Drugs like α1-adrenergic receptor agonists, estrogen and lespedeza capitata increases bladder outlet resistance to improve symptoms of stress urinary incontinence.The animal started signs of recovery from second day. Ultrasonographic findings revealed decreased size of the urinary bladder and kidneys on day 2. On day 5, both regained normal size and healing of the midventral celiotomy incision wound had taken place. Bitch started voiding urine without any difficulty and with normal micturation reflex.

Discussion

Ovariohysterectomy is a surgical procedure widely employed in practice by veterinarians. It is indicated in cases of pyometra, uterine tumours, or other pathologies. This procedure should only be undertaken if the bitch is in a fit state to withstand general anaesthesia. However, the procedure is contraindicated if the bitch presents a generalized condition with hypothermia, dehydration, and mydriasis. Ovariohysterectomy is one of the major contributing factors to the development of the urinary bladder atony, the exact mechanism is poorly understood. It occurs most commonly in middle-aged spayed female dogs and the etiology is believed to be multi-factorial (Djemil *et al.,* 2010; Stephanie *et al.,* 2011).

Decreased urethral length and tone, altered bladder neck position, structural and functional alterations in bladder and urethral musculature, alteration in hormones are the major contributing factors. It has been estimated that there is almost an eight-fold increase in the risk of developing urinary incontinence due to atonic bladder in neutered compared to entire bitches (Thrusfield *et al.,* 1998).In the present case, ovariohysterectomy results in the decreased levels of oestrogen which is essential for maintenance of bladder and urethral tone, as a result of which the urethra and urinary bladder lose their normal tonicity leading to atony and subsequent urinary incontinence. Phenylpropanolamine is a α_1-adrenergic receptor agonists drug effective in treating urge incontinence because they inhibit involuntary bladder contractions and are useful in treating urinary incontinence associated with urinary frequency and urgency. It has been used for the treatment of stress urinary incontinence in women and for the treatment of urethral sphincter mechanism incompetence in bitches (Stephanie *et al.,* 2011).Estrogen therapy (estriol) increases urethral closure pressure, theoretically by increasing the density and responsiveness of α-adrenergic receptors in urethral smooth muscle. Estrogen therapy is effective in approximately 65% of female dogs with urethral incompetence (Mandigers and Nell, 2001). Combination therapy with both estriol and phenylpropanolamine is often suggested. Administration of Lespedeza capitata used as a mild diuretic, hypoazotemic agent that acts via renal vasodilatation and stimulates the activity of the renal parenchyma. Antibiotic prophylaxis with Cefalexin is advisable to prevent bacteraemia (Noel *et al.,* 2010).Hence, it may be concluded that combination of bladder catheterization and medicinal therapy can be

used to manage atonic bladder successfully induced by ovariohysterectomy in bitches.

References

Azadzoi, K.M, Pontari, M., Vlachiotis, V. and Siroky, M.B.(1996). Canine bladder blood flow and oxygenation: changes induced by filling, contraction and outlet obstruction. *J. Urol.* **155**: 1459-1465.

Djemil, B., Lamia, A., Annabelle, G. and Daniel, T.(2010). Ovariohysterectomy in the bitch. Obs Gyne Inter, Article ID 542693, 7 pages, http://dx.doi.org/10.1155/2010/542693.

Fenner, W.(1993). Neurologic disorders of micturition. In: Borjab, Mechanism in small animal surgery, Lippincott Williams and Wilkins, Pennsylvania, pp: 520-527.

Fischer, J. and Lane, I.(2007). Incontinence and urine retention. In: Brainbridge J and J Elliott, Manual of canine and feline nephrology and urology, Gloucestershire, pp: 26-40.

Gregory, S.P.(1996). Urinary incontinence. In: Brainbridge J and J Elliott, Manual of canine and feline nephrology and urology, Gloucestershire, pp: 42-51.

Hamaide, A.J., Grand, J.G., Farnir, F., Le Couls, G., Snaps, F.R., Balligand, M,H. and Verstegen, J.P.(2006). Urodynamic and morphologic changes in the lower portion of the urogenital tract after administration of estriol alone and in combination with phenylpropanolamine in sexually intact and spayed female dogs. *Am J Vet* Res,**67**: 901-908.

Labato, M.A.(2005). Micturition disorders. Textbook of veterinary internal medicine, Ettinger S and E Feldman, Elesvier USA, pp: 105-109.

Mandigers, P.J.J. and Nell, T. (2001). Treatment of bitches with acquired urinary incontinence with oestriol. *Vet. Rec.* **149**: 765-767.

Noel, S., Claeys, S. and Hamaide, A. (2010). Acquired urinary incontinence in the bitch: update and perspectives from human medicine. Part 1: The bladder component, pathophysiology and medical treatment. *Vet. J.* **186**: 10-7.

Stephanie, C., Frederico, R., Stephanie, N. and Annick, H.(2011). Clinical evaluation of a single daily dose of phenylpropanolamine in the treatment of urethral sphincter mechanism incompetence in the bitch. *Canadian Vet. J.* **52**: 501-505.

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The Blue Cross Book July 2015 Vol. 32 : 75-77

Therapeutic management of Downer Syndrom in a cow with supportive Physiotherapy

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(received 21/05/2015 - accepted 01/06/2015)

Abstract :

A cow was presented to Mukhymantri Mobile Veterinary Unit, Nasirabad, Ajmer with the history of dystocia due to big size calf. After forceful traction, the calf was delivered. After delivery, cow remained down and was unable to stand up. The cow was bright and alert with normal appetite, defecation and urination. Milk production was reduced. All the physiological and clinical parameters were within normal range at the time of presentation and cow was in sternal recumbency and frequently tried to get up. The therapeutic management was supported with physiotherapy, which included soft bedding of sandy soil, massage of hind limbs with vegetable oil and supportive slings. Cow was able to stand up without help of the slings after complete recovery.

Keywords: Cow, downer syndrome, dystocia, sternal recumbency, physiotherapy

Introduction:

One of the most challenging problem for Veterinarians is management of recumbent/ downer animals. Prolonged recumbency due to inadequately treated and unresponsive hypocalcaemia is one of the common causes of downers syndrome. Cox (1982) proposed that downer cow was any cow which was down in sternal recumbency for more than 24 hours without evidence of a systemic involvement. Blood et al. (1983) defined the downer cow syndrome is a condition occurring following parturient paresis, characterised clinically by prolonged recumbency even after 2 successive infusions with calcium. Ninety four per cent downer cows were average to high producers. About 30 % of cows treated for milk fever will not stand for up to 24 hours after treatment and

turn to downer animals (Radostits et al., 2000). Allen and Davies (1981) suggested that downer syndrome occurred as a result of one of the combination of the factors like milk fever, hypophosphataemia, fat cow syndrome, excessive protein intake, Vitamin E & Selenium deficiency, recumbency due to trauma and malnutrition. Jonsson (1983) suggested that

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downer syndrome occurred as a result of one of combination of the factors like muscular injuries, nerve injuries, persistent hypocalcaemia, persistent hypophosphataemia, myocardosis, hepatosis, septic mastitis and other factors. Jonsson (1983) also observed that 65% cases of injuries of muscles, nerves and hind limbs terminate as downers.

Case History

When a Veterinary Camp was organized in narsingh goshala, Nasirabad by Mobile Veterinary Unit, Nasirabad, Ajmer a cow was presented with the history of dystocia due to big size calf. After forceful traction, the calf was delivered. After delivery cow remained down and was unable to stand up in spite of repeated attempts to make her rise up. Cow tried to stand up repeatedly but could not rise on her feet.

Clinical Signs and Observations

The cow was bright and alert with normal appetite, defecation and urination. Milk production was reduced. All the physiological and clinical parameter were within normal range at the time of presentation such as body temperature 100.5^{0}F, heart rate 56 per minute, pulse rate 66 per minute and respiratory rate 21 breaths per minute. Cow was in sternal recumbency and frequently tried to get up. She was unable to put up weight on hindquarters. Cow was able to stand with some assistance by lifting the tail and head or with the help of wooden sticks below thorax and abdomen with precaution to avoid any injury to udder. All the limbs were checked for sensitivity to pain by pricking with a needle to rule out nerve paralysis. All the fore limbs and hind limbs showed sensitivity to pain.

Treatment

Treatment was undertaken with medicines along with physiotherapy to correct the etiological factor. Injection Oxytetracycline at the dose rate 10 mg/ kg body weight and Meloxicam at the dose rate of 0.5 mg/kg body weight were administered intramuscularly for five and three days respectively. Injection Calcium magnesium borogluconate (Mifex) 450ml (300ml slow i/v and 150ml s/c and Injection 5% DNS 2000ml i/v once were administered. Injection Tribivet (Vit. B1, Vit. B6, Vit. B12) 10 ml i/m. and injection Tonophospan 10ml i/m for 3 days were administered. Injection Triamcinolone (Vetalog) 5 ml i/m for 3 alternate days. Powder chelated Agrimin Forte 50 gm bid for 10 days was orally given. Powder Potassium chloride 30 gm orally for 5 days was given. Physiotherapy comprised of soft bedding (paddy straw and sandy soil) and supportive slings. Sand was used as bedding material because cow would easily be rolled from one side to other and for the regular removal of excreta. The cow was rolled from one side to the other every 3 hours to prevent bed sores. Cow was assisted to stand for 30 minutes four to six times a day using supportive slings. The sling was designed in such way that the weight of the animals could be equally distributed (Fig. 1). Massage of hind limbs with vegetable oil to increase muscular activities was carried out and hot water fomentation was performed. Cow was responding well to the treatment and physiotherapy and was able to stand up without help of slings after complete recovery.

Discussion

The cow appeared bright, alert with normal defecation and urination with normal appetite after recovery. Insufficient calcium in heavy

Fig.1. Cow with specially designed sling in which weight of the animals is equally distributed

animals suffering from parturient paresis may result in incomplete response and lead to failure of animal to rise. If these animals are not treated soon, ischemic necrosis of muscle may occur leading to permanent recumbency, even if the animal is subsequently treated with sufficient calcium (Radostits et al., 2000). The reliable indicator of muscle injuries is the creatine kinase activity in serum which increases many folds (Prasad et al., 1988). Hypophosphataemia is supposed to be one of the commonest causes of downer syndrome and have been observed in recumbent animals. About 32 % of the downer cows had phosphorus concentration below 4 mg/dl (Wadhwa and Prasad, 2007). Fenwick (1969) observed that there was definite increase in number of downers and deaths with decreasing potassium concentration. The animal should be shifted to suitable site for proper nursing preferably on sandy soil floor. Animal should be turned from side to side regularly to minimize the degree of ischaemic necrosis. Lifting of the animal is beneficial and can be done with manual labour or with the use of slings using chain and pulley. Lifting should be done carefully to avoid further damage to muscle and nerves. When the animal is hoisted or able to stand unaided, the blood circulation should be stimulated by massaging the limbs. A downer cow can be successfully treated if the correct diagnosis of the cause of the recunbency is noted early. A lot of special handling, care and patience are required to help the recumbent cow. Massaging of the limbs, turning of the cow and lifting the cow onto it feet will help the cow to recover faster (Muthoni MS and Nganga K, 2009)

Referance

Allen, W. M. and Davies, D.C. (1981). Milk fever, hypomagnesaemia and the downer cow syndrome. *Br. Vet. J.* **137**: 435-441.

Blood, D.C., Henderson, J.A. and Radostitis, O.M. (1983). Veterinary Medicine. 6th edn. The ELBS and Bailliere Tindall, London

Cox, V.S. (1982). Pathogenesis of downer cow syndrome. *Veterinary Record.* **111**: 76-79.

Fenwick, D. C. (1969). Downer cow syndrome. *Aust. Vet. J.* **45**: 345-351.

Jonsson, G. (1983). The downer cow syndrome. *Indian J. Vet. Med*. **3**: 1-8.

Muthoni MS and Nganga K (2009). Successful management of downer cow in Limuru, Kenya. *Journal of Animal & Plant Sciences.* **4**: 379 - 383.

Prasad, B. Rathor, S.S. and Wadhwa, D.R. (1988). A note on serum creatine phosphokinase and transaminases in downer cows. *Indian J. Vet. Med*. **8**: 159-160.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. (2000). Veterinary Medicine. 9th edn. W.B. Saunders Co., USA.

Wadhwa, D.R. and Prasad, B. (2007). Mineral profile in downer cow syndrome. *Indian J. Vet. Med*. **27**: 22-24.

The Blue Cross Book July 2015 Vol. 32 : 78-80

Ehrlichiosis in a Cow - A case report

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(received 29/04/2015 - accepted 01/06/2015)

Abstract:

A five year old Holstein Friesian cross bred cow was brought to Teaching Veterinary Clinical Complex with the history of anorexia, shivering and respiratory distress since a week. Closed physical examination revealed enlarged prescapular lymph nodes and injected conjunctival mucous membrane with petechiae. Physiological parameters like rectal temperature, heart rate and respiratory rate were found to be 105.3°F, 86 beats per minute and 48 per min respectively. On hematological examination, hemoglobin and total erythrocyte count were found to be low. Peripheral blood smear examination revealed the presence of *Ehrlichia bovis* organisms in the monocytes. Based on these findings a diagnosis of ehrlichiosis was made and the case was treated with oxytetracycline @20mg/kg, slow IV, b.i.d for seven days and Meloxicam @0.5mg/kg, IM for five days. Improvement was noticed after 3 days of treatment.

Keywords: Cow, Ehrlichiosis

Introduction:

Ehrlichiosis is a systemic tick borne infectious disease caused by the Rickettsial organisms belonging to the family Ehrlichiae (Ramesh et al., 2008). Ehrlichia are obligate intracellular organisms which infect leukocytes of specific mammalian hosts eg: *E.canis* causing canine monocytic ehrlichiosis, *E.risticii* causing equine monocytic ehrlichiosis and *E.bovis* causing bovine ehrlichiosis (Abuhammour, 2002). Canine ehrlichiosis has been reported widely both in India and abroad, but the reports of bovine ehrlichiosis are few. Karunamurthy et al ., (1992) and Ramesh et al., (2008), Kolte et al., (2003) and Gupta et al., (2005) reported the occurrence of Bovine Ehrlichiosis in Tamilnadu, Maharashtra and Northen India respectively. Present paper reports the occurrence of bovine ehrlichiosis in Karnataka and to authors knowledge, this is the first report from Karnataka.

Case History and Clinical observations:

A five year old Holstein Friesian cross bred cow was brought to Teaching Veterinary Clinical Complex of Veterinary College, Hassan with the history of anorexia, marked reduction in milk yield, shivering and respiratory distress since a week. On closed physical examination, dullness, enlarged prescapular lymph nodes (Fig.1), serous nasal discharge, severe tick infestation and petechial hemorrhages on conjunctival mucous membrane were noticed. Physiological parameters like rectal temperature, heart rate and respiratory rate were found to be 105.3°F, 86 beats per minute and 48 per min respectively.

On hematological examination, hemoglobin and total erythrocyte count were found to be low i.e.,

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5.2g/dl and 3.2 million cells /cmm respectively. Marked monocytosis (11 monocytes per 100 leukocytes) was also noticed. Peripheral blood smear was taken from ear vein and was stained with giemsa stain. Microscopic examination revealed the presence of *Ehrlichia bovis* organisms in the monocytes (Fig.2). Ticks were collected and processed as per standard procedure and were identified as *Haemophysalis* Spp. Based on all these findings a diagnosis of Bovine ehrlichiosis was made.

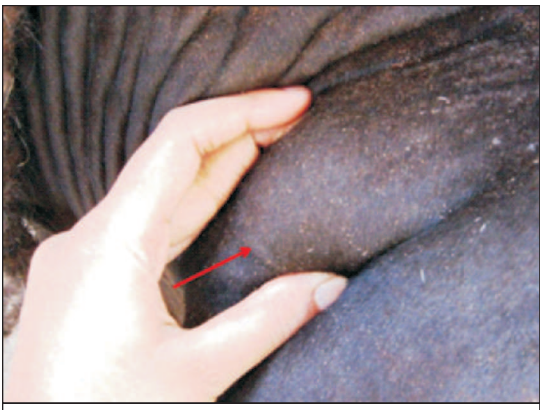

Fig.1: Enlarged prescapular lymph node

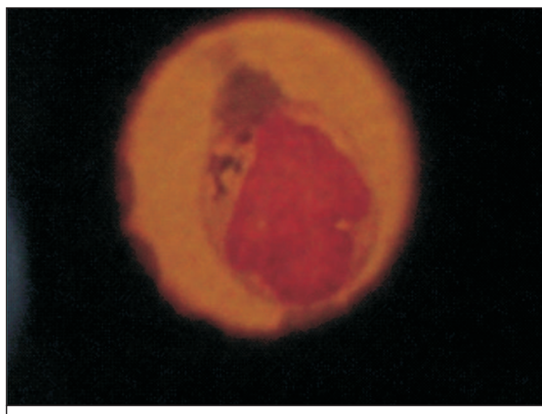

Fig. 2: Ehrlichia organisms in a monocyte

Treatment and Discussion:

The case was treated with oxytetracycline @20mg/kg, slow IV with 1 litre of Normal Saline, b.i.d for seven days. Other supportive therapies like Meloxicam @0.5mg/kg, IM for five days and B-Complex Injection @10ml, IM, once in three days was given. Significant clinical improvement was noticed after 3 days of treatment and the case was discharged after complete clinical recovery.

Karunamurthy et al., (1992) and Kolte et al., (2003) reported the presence of pyrexia, enlargement of lymphnodes and in-appetence which is similar to the present case. Symptoms like grinding of teeth (Karunamoorthy et al., 1992); staggering gait and inco-ordination (Ramesh et al., 2008) were not observed in the present case history. This might be attributed to the fact that the temperature recorded in the present case was slightly lower (105.3°F) when compared to the findings (108°F) of Ramesh et al., (2008).

Faster recovery might be attributed to the early diagnosis and higher dose of oxytetracycline used in the present case. This is in agreement with the findings of Anand et al., (2009) wherein they opined that Oxytetracycline is more effective at higher doses. Although other haemoprotozoan diseases like Theileria, Anaplasma and Babesia are reported from various parts of Karnataka (Anand et al., (2005) and Murulidharan et al., (1995)), but *Ehrlichia bovis* has not been documented. Hence this is the first report of *Ehrlichia bovis* infection from Karnataka.

References:

Abuhammour, W. (2002). Ehrlichiosis [monograph online].eMedicine.com. Available at: http://www.emedicine.com/ped/topic655.html.

Ananda. K. J., D'Souza, P. E. and Puttalakshmamma. G.C. (2009). Prevalence of Haemoprotozoan diseases in crossbred cattle in Bangalore north. *Vet. World.*, **2**: 15-16.

Gupta, M.P. (2005).Clinical and therapeutic observations of babesiosis during outbreak in crossbred cattle. *Indian. Vet. J.*, **82**:1103.

Karunamoorthy, G., Varadharajan, V. and Balachandran, C. (1992). Prevalence of Haemoprotozoan diseases in crossbred cattle in Tamilnadu.ibid., **69**:262.

Kolte, S.W., Maske, D.K., Gaholod and Kurkure, N.V. (2003). Ehrlichiosis in cattle and buffaloes from Vidarbha, India. *Indian Vet. J.*, **80**: 399-400.

Ramesh.S., Rajendran, A., Veeraselvam, M., Rajesh, N.V. and Jayathangaraj, M.G. (2008). Concurrent infection of *Babesia bigemina* and *Ehrlichia bovis* in a cow. *Indian. Vet. J.*, **85**:87.

Muraleedharan, K., K. Syed Ziauddin, P. Margoob Hussain, R. Puttabyatappa and S. J. Seshadri. (2005). Incidence of Anaplasma sp., Babesia sp. and Trypanosoma sp., in cattle of Karnataka. *J. Vet. Parasitol.*, **19**: 71-72.

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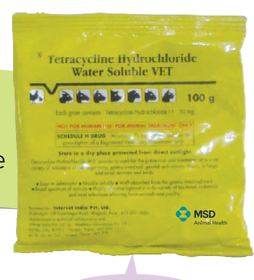

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The Blue Cross Book July 2015 Vol. 32 : 81-83

Mummification of foetus in a crossbred cow and its management.

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(received 10/03/2015 - accepted 01/06/2015)

Abstract :

A five year crossbred cow with the history of 290 days of gestation was brought to Veterinary hospital. The cow was not showing any signs of completion of gestation period. The per rectal examination revealed closed cervix and bony palpable material in the right uterine horn. The ultrasonography confirmed the mummification. The therapeutic management with PGF2α @25mg/kg is described.

Introduction :

Mummification is rare in cows, occurring in 0.13 to 1.8 % of conceived animals (Arthur et al., 1996). Foetal mummification occurs after the first trimester of gestation (Roberts, 1986). It usually goes undiagnosed, because the placenta and corpora lutea (CL) are capable of producing sufficient progesterone. If left undiagnosed, the foetus can remain in the uterus for between 150 and 200 days or for a normal gestation period (Johnson et al., 1981). Most mummified foetus remain in the uterus until treatment is given to expel them or until they are removed by caesarean section (Wenkoff and Manns, 1977). The choice of treatment of this problem is injection prostaglandin $F2_{\alpha}$ and in failure case, The caesarean section (Arthur et al., 1996). In present communication, a rare case of foetal mummification in a crossbred cow and its successful treatment with injection prostaglandin $F2_{\alpha}$ has been placed on record.

Case history and observation

A five year old crossbred cow, weighing 400 kg was referred to the Zonal Veterinary Dispensary of Goa Milk Union at Sankhali with the history of 290 days of gestation. The owner reported that though the pregnancy was confirmed after 3rd month of insemination, there was no development of udder and the animal was not showing any sign to deliver a foetus even after completion of gestation period. Per rectal examination revealed closed cervix, in addition to palpation of hard bony mass in right uterine horn while there was absence of cotyledons, fremitius and foetal fluid. Transrectal ultrasonography (Fig.1) revealed bone fragments directly related to the foetus in the right uterine horn. The animal was apparently healthy and taking food and water normally. Accordingly, the case was diagnosed as foetal

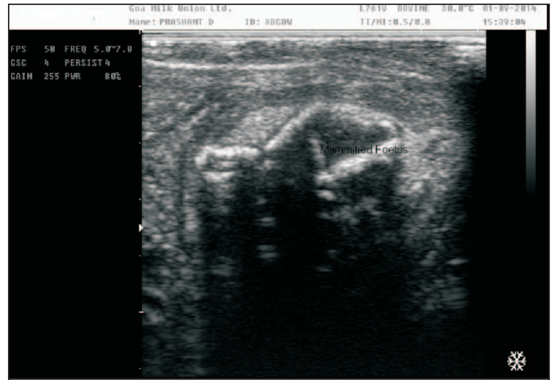

Fig 1.Ultrasonographic image of a mummified foetus in crossbred cow

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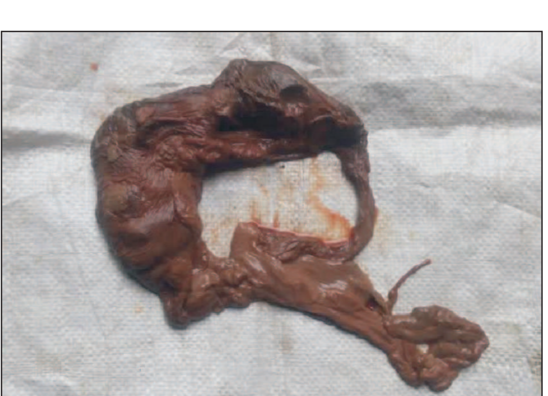

Fig. 2. Mummified foetus wrapped in foetal after births

mummification and was treated accordingly.

Treatment and Discussion

The animal was given Prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) @ 25 mg, intramuscularly and kept under observation. After 48 hour of the therapy, a thick brownish mucoid discharge from vulva was reported. On vaginal examination, the cervix was found not fully relaxed and only two fingers could be inserted. After 72 hour of the therapy, four fingers were passing through the cervix. Inj. Valathamate bromide (Epidosin @ 15ml) administered intramuscularly, subsequent to which perfect cervical dilatation could be achieved one hour later. Further, deep exploration revealed a bony mass draped within the foetal membranes. Eventually a dead foetus, partially dry up in appearance and covered with dark brown foetal membranes was delivered following a mild traction. The placental fluids were found absorbed and the afterbirths were firmly adhering to dehydrated foetus (Fig. 2). The mummified foetus had fetal crown-rump length of 28 cm, skull and bony skeleton were present and the age of the mummified foetus was assessed as approximately 122 days.

After removal of the foetus, four boli of Oriprim-U were put intrauterine, followed by Utriguard I.U. @ 60 ml for 3 consecutive days. Uterovet tablets @ 10 bid were fed for 10 days. The animal recovered uneventfully and exhibited oestrous on 28th day post partum.

Bovine fetal mummification may occur from 3rd to 8th months of gestation, and is usually accompanied by slight to severe inter placental hemorrhage, resulting into separation of the maternal and fetal placentas (Roberts, 1986). The clinical observation related with a present case is noteworthy as the mummification of foetus had taken place at 122 days of pregnancy based on the fetal crown rump length of foetus. The mummification described here is a haematogenous type due to partially dry up appearance of the foetus and presence of bloody and viscous tissue (Roberts, 1986). The main goal when treating an animal with an abnormal pregnancy related to the foetus is to expel the abnormal foetus, so the cow can become pregnant again within the shortest possible time (Erb and Morrison, 1957). The treatment of choice in cases of foetal mummification is medical and surgical treatment. Medical treatment may consist of administration of a PGF2α analogue to induce luteolysis, leading to expulsion of the foetus within two to four days (Wenkoff and Manns, 1977) In the present case, functional regression of CL and expulsion of mummified foetus was achieved with $PGF_{2\alpha}$ injection. The surgical intervention could have lead to long hospitalization period. In the present case, mummified foetus was easily delivered by mild traction after 72 hours of the therapy. The intrauterine therapy used in the present case prevented probable uterine infection after expulsion of the foetus and animal showed oestrus on 28th day post partum due to luteolysis of corpus luteum.

References

Arthur GH, Noakes DE, Parkmson TJ (eds.) (1996): Veterinary Reproduction and Obstetrics. WB Saunders, London. 127-128.

Erb RE, Morrison RA (1957): Effect of mummified fetuses on the prolificacy of Holsteins. *Journal of Dairy Science* **40**, 1030-1035.

Johnson WH, Manns JG, Adams WM, Maplefot RJ (1981): Termination of pregnancy with cloprostenol and dexamethasone in intact or ovariectomized cows. *Canadian Veterinary Journal* **22**, 288-290.

Lefebvre RC, Saint-Hilaire E, Morin I, Couto GB, Francoz D (2009): Retrospective case study of fetal mummification in cows that did not respond to prostaglandin F2α treatment. *Canadian Veterinary Journal* **50**, 71-76.

Roberts SJ (ed.) (1986): Veterinary Obstetrics and Genital Diseases. Theriogenology. 3rd ed. Woodstock Vermont. 213-217, 230-233, 337-343.

Wenkoff MS, Manns JG (1977): Prostaglandin-induced expulsion of bovine fetal mummies. *Canadian Veterinary Journal* **18**, 44-45.

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Therapeutic management of ulcerative mamillitis in buffaloes

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Abstract:

Bovine ulcerative mamillitis (Bovine herpes virus II), an acute ulcerative condition of teats and udder skin, mainly observed in primiparous cows and buffaloes, was diagnosed in five primiparous graded Murrah buffaloes. The clinical management included an antiviral ointment (acyclovir) locally and systemic antibiotics (Cefquinone), antihistaminic (avilin) and immuno-modulator (levamisole).

Key words : Buffalo, Mamillitis

Introduction:

Ulcerative mamillitis is a disease of primiparous buffaloes immediately after calving, characterized by painful acute enlargement (fig.1&2)of one or more teats and subsequent ulceration and necrosis followed by complete sloughing of teats. This condition poses major economic implication to the farmer economy in terms of milk production due to teat sloughing. Malleswara Rao et al., (2003). Sharma et al (1998) and Malleswara Rao et al., (2003) identified the virus particles in the affected purified material tissue by electron microscopy and attributed the probable etiology as bovine herpes virus. Ulcerative mamillitis was treated by several therapeutic regimens like antibiotics with anti-inflammatory and antihistamines drugs like acyclovir (Lokanadhamu et al., 2005), immuno modulators like levamisole, Vitamin E and Selenium (Rao, 2009) and Anthiomaline (Sridhar et al., 2014) with variable efficacy. The present report narrates the successful therapeutic management of bubaline ulcerative mamillitis in primiparous buffaloes.

History and Clinical Observations:

Five primiparous graded Murrah buffaloes with a history of recent calving (within last 2 weeks) showing painful swollen teat (n=3) initially and ulceration (n=2) were presented to the clinic. All other vital parameters were within the normal range. None of the milk samples showed abnormalities either physically or chemically (pH, CMT & BTB). In all the cases, hind quarter teats were only affected and animals were evincing severe pain during milking resulting in incomplete milking. All the milk samples collected were subjected to cultural examination and were found negative for microorganisms.

Treatment

The milking was done by using infant feeder tube without applying much pressure on the swollen teat and advised the farmer to follow the same procedure for milking. All the clinical cases were given cefquinome (Cobactan) @ 10 mg/kg.bwt,meloxicam (Melonex) @ 0.3 mg/kg.bwt, pheneramine maleate (Avilin) 10 ml, intra muscularly for 5days. Apart from the above

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treatment, acyclovir topical ointment and serratiopeptidase (Serakind) orally was also given in case of ulceration. Levamsiole hydro chloride (Lemasole) @ 2.5 mg/kg.bwt was administered subcutaneously every alternative day for five times.

Results and Discussion

The recovery signs like reduction in swelling and edema were appreciable with the initiation of treatment. The ulcerated teat cases showed complete healing after 12 days. As the condition is suspected to be herpes virus in etiology, topical application of acyclovir ointment yielded good results in case of ulcerated lesions which is corroborating with the findings of Lokanadhamu et al., (2005) who recorded 62.5% success rate. Serratiopeptidase is a proteolytic enzyme isolated from non-pathogenic enetero bacteria, SerratiaE15 was given orally to stimulate immunity, reduce edema, and to fight inflammation (Misraulia et al., 2013). In the current study, levamisole administration gave beneficial results due to its immunomodulation property at lower dose. Similar finding was reported by Rao (2009) who used Levamisole as chemoprophylaxis in buffalo heifers.

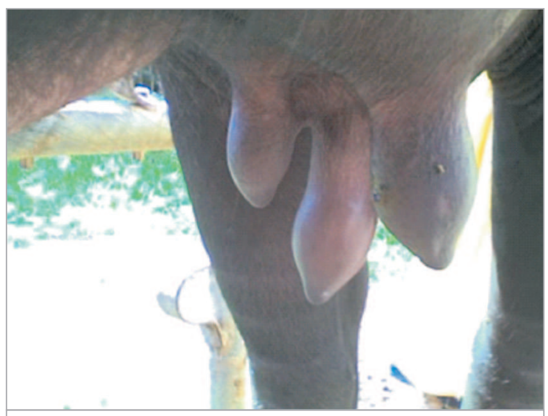

Fig.1: Acute swelling of teats

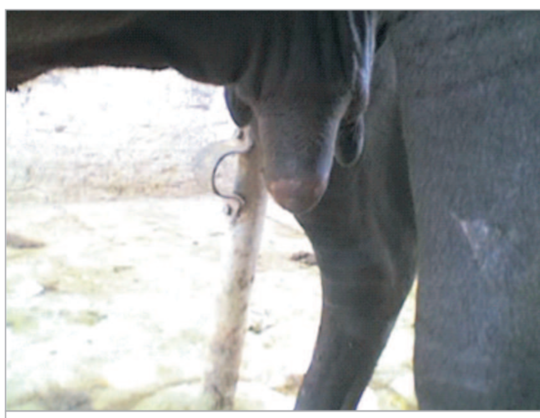

Fig.2: Teat showing ulceration

References:

Lokanadhamu M, Sreedevi B and Venkata Reddy T. (2005). Studies on etiology, symptomatology, diagnosis and therapy in ulcerative thelitis of buffaloes in Andhra Pradesh (India). *Buffalo Bulletin.* **24**: 56-69.

Malleswara Rao U.V.N., Sreedevi B and Venkata Reddy T. (2003). Studies on ulcerative mamillitis of buffaloes in Andhra Pradesh (India). *Buffalo Bulletin.* **22**: 80-90.

Misraulia K.S, Mahswari P and Dongre J.(2013). Studies on the efficacy of non-steroidal anti-inflammatory drugs and serratiopeptidase in dogs.Haryana Vet.**22**:46-47.

Rao V.V.(2009).Chaemoprophylaxis against ulcerative mamillitis in buffaloes. *Indian Veterinary Journal.* **86**:852.

Sharma S, Singh K.B, Oberoi M.S and Sood N. (1998).Studies on the occurrence of bovine herpes mamillitis in buffaloes. *Buffalo Bulletin.* **17:** 79-81.

Shridhar N.B, Santhosh Kumar C.N and Mahesh Kadagi. (2014).Therapeutic management of mamillitis-Areport of 5 buffaloes. *Intas Polivet.* **15**:83-84.

The Blue Cross Book July 2015 Vol. 32 : 86-88

Congenital preputial orifice stenosis and Its surgical management in a Kid

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Abstract

Stenosed prepucial orifice is sporadically reported in kids and lambs. A three-day old local non-descript kid was brought with a complaint of difficulty in urination and a presence of swelling in penile region on the ventral abdomen. A polyvinylchloride tubing was tried to pass through the prepucial opening with no success. Prepucial orifice was found stenosed. A case of congenital stenosis of prepucial orifice was thus diagnosed. Timely surgical management of the stenosed prepucial orifice not only relieved the condition of dysuria, but also prevented the development of balanoposthitis and urine induced necrosis of the overlying skin in the present case. No post-operative complications were reported by the owners of animal subsequently.

Key words: Kid, Preputial orifice stenosis, Congenital, Surgical management

Introduction

Prepuce of the animals may be affected with many congenital abnormalities like hypoplasia and absence or failure to fuse normally (Booth, 1993). Prepucial stensosis is defined as the narrowing of the prepucial orifice. Stenotic or an absent preputial orifice may lead to dysuria, retension of urine and phimosis (Kahn, 2006; Fossum, 2002). Congenital prepucial stenosis has been described in young dogs, cats, and stallions(Roberts, 1986). This case describes the clinical findings and successful surgical management of congenital stenosis of prepucial orifice in a kid.

Case history and observations

A three-day old local non-descript kid was presented with a complaint of difficulty in urination and a presence of swelling in penile region on the ventral abdomen. The clinical examination revealed increased heart rate (104/minute) and respiration rate (36/minute). Physical examination revealed fluid filled balloon shaped dilatation of prepucial cavity just caudal to the prepucial orifice (Figure 1). Urine dribbled drop wise through stenosed prepucial opening. On compression of prepucial dilatation, urine fountained out through prepucial orifice in a fine stream. A polyvinylchloride tubing was tried to pass through the prepucial opening with no

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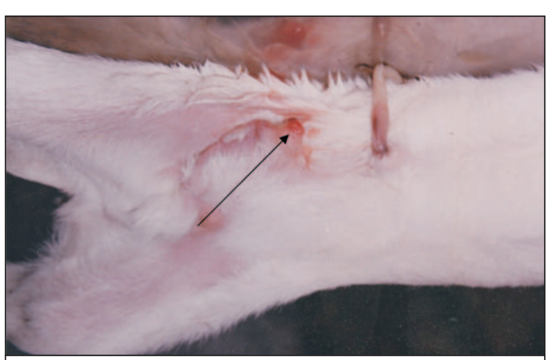

Figure 1: Dilatation of prepucial cavity just caudal to the prepucial orifice with urine (arrow head)

success. Prepucial orifice was found stenosed. All the physiological parameters were within normal limits. A case of congenital stenosis of prepucial orifice was thus diagnosed.

Surgical management and results

A teat dilator was introduced into the prepucial cavity to dilate the prepucial orifice. The accumulated urine in the prepucial cavity was drained out. Prepucial orifice was then widened with the help of teat tumor extractor. The animal was given local antiseptic dressing, antibiotics and painkillers. After a lapse of three days, animal was again presented with the same but severe complaint as urine outflow has completely stopped due to complete closure of the prepucial orifice. Complete surgical enlargement of the prepucial orifice was decided to be undertaken.

After epidural anaesthesia with 2% lignocain hydrochloride (Xylocaine, Astra IDL, Bangaluru) and preparation of the site, polyvinyl chloride tubing was introduced into prepucial cavity and a triangular incision with the base of the triangle on the prepucial orifice was made on the ventral surface of the prepuce.The skin, subcutaneous tissue and prepucial mucosa were excised. The excised edges of the triangle were sutured separately with through and through sutures using no. 2 black braided silk.Bleeding vessels were also ligated.

Post-operatively the animal was given injections of ampicillin and cloxacillin (Megapen, Aristo Private limited, Mumbai, India) dosed at 5 mg/ kg twice in a day, meloxicam (Melonex, Intas Pharma, Ahmedabad, India) 0.5ml and B-complex (Tribivet, Intas pharma, Ahmedabad, India) 1ml once in day for a period of three days. The suture line was cleaned daily with antiseptic povidone iodine (Betadine solution, Win Medicare, Mumbai, India) for a period of 10 days. Sutures were removed on 12th post-operative day. Recovery was uneventful. Urine flow was normal.

Discussion

Prepucial stenosis may be congenital or acquired and can result from a developmental anomaly of the prepuce. The prepuce is embryologically derived from the ectoderm; a layer of epithelium which is trapped between the urogenital folds and the glans of the penis. This epithelial lamina eventually splits, and the folds are transformed into flaps of skin covering the glans, and the preputial orifice is formed by the edges of the flaps (Latshaw, 1987). Prepucial stenosis may cause retension of urine and consequent balanoposthitis. The infected area may ulcerate (Booth, 1993). Timely surgical management of the stenosed prepucial orifice not only relieved the condition of dysuria but also prevented the development of balanoposthitis and urine induced necrosis of the overlying skin in the present case. Further, persistent stenosed prepucial orifice might otherwise would have led to other surgical conditions like kinking and phimosis of the penis in the latter life of the animal. Kinking of penis due to stenosis of prepucial opening has been reported in a buffalo (Chandrasekhar *et al.,* 2002).Congenital preputial stenosis leading to phimosis, or

entrapment of the penis inside the prepuce has been reported in both dogs (Elam and Randle, 1952) and cats (Elkins, 1983). However, stenosed prepucial orifice is sporadically reported in kid and lamb and reported first time from Kashmir Valley.

References

Arthur, G.H., Noakes, D.E. and Pearson, H.(1996). Veterinary reproduction and obstetrics. 7th edition Saunders, London, pp: 714-724.

Booth, H.W., (1993). Penis, prepuce and scrotum. In: Textbook of Small Animal Surgery. (Douglas Slatter edited), 3rd edition WB Saunders Co, Philadelphia, pp:1531-1542

Chabdrasekhar, E.L., Haragopal, V. and Dhanvanthari, E.(2002). Stenosis of prepucial orifice and penile kink in a buffalo. *Indian J. Vet. Surg*.**23**: 57.

Elam, C.W. and Randle, P.O. (1952). Peculiar preputial condition in a five week old puppy. *Vet. Rec.* **64**: 98.

Elkins, A.D.(1983). Surgical correction of congenital Stricture of the preputial orifice in the feline. Fel. Pract. **12**: 20-25.

Fossum, T.W. (2002). Small animal surgery. 2nd edition St Louis, Mosby Inc, pp:666-668.

Kahn, C.M.(2006). The Merck veterinary manual. 9th edition Whitehouse Station, NJ Merck & Co, p: 1159.

Latshaw, W.K.(1987). Veterinary Developmental Anatomy. Toronto, Philadelphia, BC Decker, p: 245.

Roberts, S.J.(1986).Veterinary obstetrics and genital diseases (theriogenology). 3rd edition Woodstock, David and Charles Inc, pp: 793-805.

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The Blue Cross Book July 2015 Vol. 32 : 89-90

Surgical management of Intussusception in a Calf

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Abstract

A 4 month old calf was presented at Polyclinic IVRI, with a history of bloat for almost 2 days. It was treated for the same in the Medicine Unit for 5 days. Animal was suspected of having gastrointestinal obstruction and was referred to the Surgery Division. Rumenotomy was performed via left paralumbar fossa, no obstruction could be felt inside. Abdomen was explored for any obstruction in intestine and an intussusception could be felt at the region of small intestine. Enterectomy and enteroanestomosis was performed under distal para-vertebral anaesthesia.

Introduction:

Intussusception occurs when a segment of the gastrointestinal tract telescopes into an adjacent segment, causing intestinal obstruction. The outer receiving segment and the inner inverting segment are called intussuscipiens and intussusceptum, respectively(Pravettoni *et al,* 2009). The blood supply to the affected portion of the intestine is interrupted when in-tussusception occurs, and the intestinal wall becomes oedematous, ischaemic, and turgid (Smart *et al.* 1977; Horne 1991; Levitt and Bauer 1992). Intussuception appears to be most common in calves up to two-months age (Pravettoni *et al.* 2009). A total of 336 cattle were identified, comprising 281 (84%) cases of small intestinal, 7 (2%) ieocolic, 12 (4%) cecocolic, and 36 (11%) colocolic intussusceptions (Constable *et al.* 1997). Because of the minimal fat-filled mesentery in the caecal area of calves compared with that in adult cattle, mobility of the intestine is increased and intussusception is more common. Intestinal intussusceptions within the caecal area can be classified into four different types: cecocecal, cecocolic, ileocecal, and ileocecocolic (Steiner 2004).The present study is about the successful surgical management of ileocecocolic intusussusception in a calf.

Case History and Observation

A 4 month old calf was presented at Polyclinic IVRI with a history of bloat for almost 2 days. It was treated in the medicine unit for 5 days.Animal was suspected of having gastrointestinal obstruction and was referred to the Surgery Division. Rumenotomy was performed via left paralumbar fossa, no obstruction could be felt inside. Abdomen was explored for any obstruction in intestine and an intussusception could be felt at the region of small intestine. An enterectomy and enteroanestomosis was performed under distal paravertebral anaesthesia.

Treatment

Left paralumbar fossa was prepared for performing rumenotomy. Animal was given distal paralumbar anaesthesia with lignocaine (2%). Animal was restrained in standing position. The surgical site was prepared for aseptic surgery. A 10 cm long incision was made on the skin caudal to the last rib and the abdominal cavity was exposed. The rumen was exteriorized and was sutured with skin using stay sutures so that the rumen contents could not spill into the abdomen. Then the rumen was incised and the contents were removed. Rumen was explored for any obstruction and rumen wall

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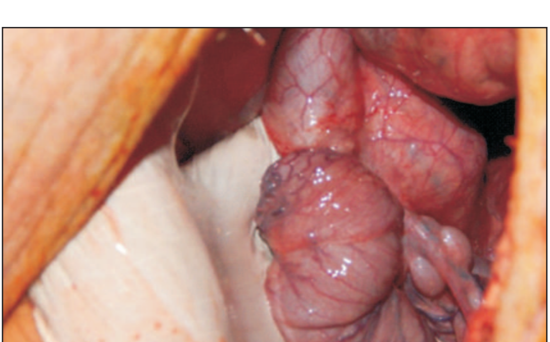
Fig. 1. Ileocecocolic intussusceptions in a calf.

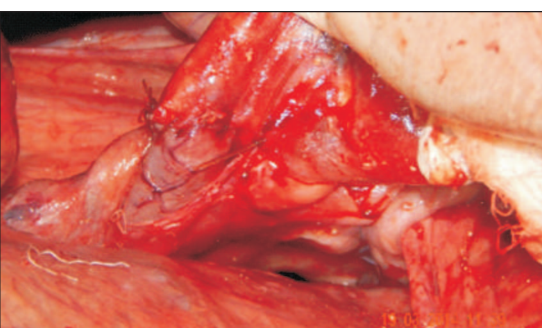
Fig.2. Intestinal resection was performed to correct intussusception.

was washed with normal saline and were sutured using double row of inversion sutures (Lembert's pattern). The abdomen was explored for any obstruction in intestine. Intussusception was observed in small intestine region which was corrected but the lumen was very narrow in the region (Fig.1.). Part of the intestine from that region was excised and using 2-0 absorbable suture material(catgut) anastomosis was performed to avoid leakage. For end to end anastomosis, the mesenteric suteures were placed first and tagged (Fig.2.). Anastomosis completed with a simple continuos pattern. After anastomosis, liberal lavage with sterile isotonic fluids was done. Gloves and instruments were changed and then the muscles were sutured in continuous pattern. Subcuticular sutures were applied and finally the skin was opposed by interrupted suture pattern using silk. Animal was given intravenous fluids and antimicrobial. Owner was advised not to feed the animal for 4 days and to give complete GI rest. Intravenous fluids were given in the morning and evening. Antimicrobials were given for 7 days. Anti-inflammatory drugs were given for 3 days. Animal recovered successfully 15 days post operatively.

Discussion

Classic signs of intussusception are, initially, either chronic low grade pain or acute signs of abdominal pain, followed by progressive loss of appetite, abdominal distention, a reduction in fecal volume and lethargy (Karapinar *et al*, 2007). Calves have a higher prevalence of intussusception than adult cattle because of the thin, fragile nature of the mesentery, which is more susceptible to tearing under tension, and which allows increased movement of adjacent intestinal segments (Pearson 1971). The high prevalence of intussusception in calves results from the common problem of enteritis at this age group, before two months of age. Prolonged diarrhoea causes abnormal peristalsis and thinning of the intestinal wall. These risk factors, along with the lower amounts of fat-filled mesentery, contribute to ileocecocolic intussusceptions (Lee *et al,* 2013). The treatment goal of intussusception is to restore gastrointestinal tract patency, which was achieved in the present case.

References

Constable P, St Jean G, Hull B, Rings D, Morin D, Nelson D (1997): Intussusception in cattle: 336 cases (1964–1993). *Journal of the American Veterinary Medical Association* **210**, 531-536.

Steiner A (2004): 14.2.2 Cecal intussusceptions. In: Fubini SL, Ducharme N (eds.): Farm Animal Surgery. 1st ed. Elsevier Inc., New York. 470-472.

Karapinar T, Kom M (2007): Transrectalultrasonographic diagnosis of jejunoileal intussusception in a cow. *Irish Veterinary Journal* 60, 422-424.

Pravettoni D, Morandi N, Rondena M, Riccaboni P, Zani DD, Scandella M, Belloli AG (2009): Repeated occurrence of jejuno-jejunal intussusception in a calf. *Canadian Veterinary Journal* **50**, 287-290.

The Blue Cross Book July 2015 Vol. 32 : 91-94

Diagnosis and therapeutic management of Horses infected with *Trypanosoma evansi*

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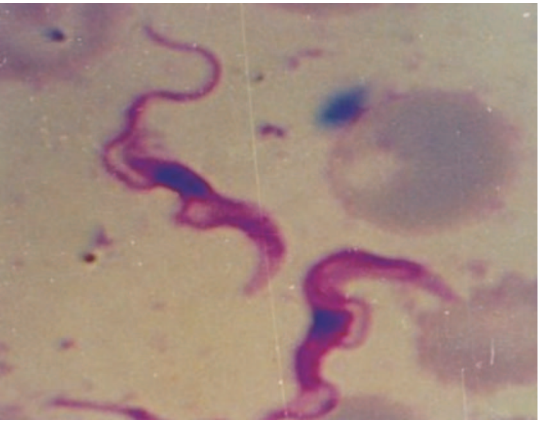
Abstract

Equine trypanosomiasis caused by *Trypanosoma evansi* is an important disease owing to high susceptibility and mortality throughout the world including India. In the present study, a total of seventeen (n=17) horses tested positive for presence of *T. evansi* were evaluated for diagnosis and therapeutic management with two drugs (Quinapyramine sulphate and chloride, Isometamidium HCl) along with supportive therapy, including fluids and NSAIDs. The horses were diagnosed for presence of *T. evansi* by two methods i.e. based on clinical signs, thin blood smear with Geimsa stain. The possible reinfections post treatment is reported.

Key words: Horse, *Trypanosoma evansi*, Diagnosis, Therapeutic management

Introduction

Trypanosomiasis, an arthropod borne blood protozoan disease commonly known as Surra is caused by *Trypanosoma evansi.* Several species of haematophagous flies, including Tabanids and Stomoxys are implicated in transferring infection from host to host, acting as mechanical vectors. The disease is characterized by fever, progressive emaciation, anaemia, subcutaneous oedema, stiffness, dullness and depression, nervous signs and death (Radostits *et al.,* 2009). Equine trypanosomiasis is an important disease owing to high susceptibility and mortality throughout the world. Equines affected by surra mostly die within 3 months (Gill, 1991). Surra in India is generally considered as a disease prevalent mostly in animals of Northern India and prevalence of the disease in equines of



Northern India have been reported earlier (Singh *et al.,* 1995 and Soodan *et al.,* 1995). A detail investigation about diagnosis and therapeutic management was undertaken and is being reported.

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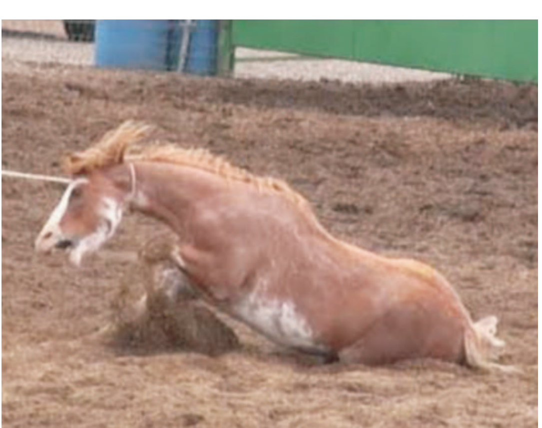

Materials and Methods

The present study was conducted in different districts of Gujarat state. The horses were diagnosed for presence of *T. evansi* by two methods i.e. based on clinical signs, thin blood smear with Geimsa stain. It was observed that 17 affected horses out of 72 horses showed clinical signs like anorexia, general weakness, dullness and depression, intermittent fever, inappetance, oedema of legs or abdomen, pale mucous membrane, serous nasal discharge, congested mucous membrane of eyes and lacrimation, incoordination of legs and paraplegia. Smears were prepared by taking the blood of these affected horses and stained by Giemsa stain. The dry stained smears were then examined first under high power of microscope and then under oil immersion for detection of parasites. Parasitaemia in the Giemsa stained blood smears were denoted as '+'=1 to 4 number of parasites/field (X1000), '++'=5 to 9 number of parasites / field (X1000) and '+++'=more than 9 parasites/field (X1000) as described by Laha and Sasmal (2004). The rest 55 horses (total 72) were screened for detection of 'surra' by examination of Giemsa stained blood smears. Affected animals (n=17) were selected for therapeutic management with two drugs (Antitrypanosomal). Surra positive animals were divided into two groups. Gr-I (n=8) received quinapyramine sulphate and chloride @ 4.4 mg/kg, s/c whereas gr-II (n=9) were treated with isometamidium chloride HCl @ 0.5 mg/kg, i/m. The supportive therapy was given to horses of both the groups in the form of Dextrose 25% @ 3lit i/v, DNS @ 4lit i/v and Tribivet @ 10ml, i/m. Moreover, Meloxicam @ 0.3 mg/kg was used as common NSAID for both the groups. The horses which manifested stiffness as one of the clinical sign were administered with Phenylbutazone (NSAID) @ 4.4 mg/kg, i/m. Supportive therapy and NSAID were continued for at least 3 days or depending upon severity of infection.

Results and Discussion

Among 72 horses, 17 horses (23.61%) showed clinical signs of surra whereas 14 horses (19.44%) were found positive for *T. evansi* infection as observed by examination of Giemsa stained blood smears. The temperature of affected animals varied from 98ºF-104.3ºF. The main clinical signs observed in clinically infected horses (n=17) included anorexia, general weakness, dullness and depression, intermittent fever, inappetance, oedema of legs or abdomen, pale mucous membrane, serous nasal discharge, congested mucous membrane of eyes and lacrimation. Three horses showed nervous signs like incoordination of legs, paraplegia and two horses fell down. The rectal temperature, heart rates and respiration rates were also increased in affected horses.

Presence of fever in trypanosomiasis is due to the toxin liberated by the parasites which were present in the blood, irrespective of concentration of the parasite. This leads to the change in the body temperature set point in the hypothalamus under the influence of pyrogenic stimuli released during infection (Singh *et al.* 2011). Nervous symptoms like incoordination, paraplegia and falling down were observed in three horses which might be due to long standing chronic infection resulting in invasion

of brain by *Trypanosoma evansi.* Similar findings were reported by Yadav and Kumar (2010) in a horse at NRCE farm, which was heavily infested with *T. evansi* and died in 2009. Several factors are responsible for causing anaemia due to *T. evansi* infection. The first one is the production of haemolysin by trypanosomes resulting into haemolysis of RBCs and extravascular destruction of RBCs either immune mediated or by erythrophagocytosis (Laha and Sasmal, 2008). Depression of erythropoiesis and non-specific factors which increase red cell fragility may be responsible for anaemia in *T. evansi* infection. Oedema during *T. evansi* infection may be due to release of kinin from antigen-antibody complexes, which may cause increased endothelial permeability of vessels leading to oedema.

A high percentage (19.44%) of horses was found suffering from *T. evansi* infection. This high prevalence of *T. evansi* in horses, in this area could be considered as an alarming situation which has never been explored previously in horses of different district of Gujarat state.

In gr-I, three horses died on 2nd, 3rd and 38th day post treatment (PT) respectively while remaining five horses successfully recovered from *T. evansi*, in gr II, three horses died on 1st, 2nd and 4th day PT respectively, remaining six horses successfully recovered from *T. evansi.* The blood smears of horses which were infected and treated were re-examined after a period of around one month to observe the status of infection within them. All the animals were found negative for *T. evansi* infection. After a period of two months and 17 days PT, two horses of gr-II were suspected for *T. evansi* by clinical signs viz. slight oedema of hind legs, congested mucous membrane of eyes, anorexia, elevated rectal temperature, heart rate and respiration rate. Examination of Geimsa stained blood smears of these horses revealed presence of *T. evansi* which indicated the reinfection. Recurrence of infection were observed in the horses treated with isometamidium chloride HCl. Similar findings were also recorded by Hardeep *et al*. (2012) and Eisler *et al*. (1996). It could be mentioned that between the period of one month 8 days and two month 17 days of PT, reinfection occurred. Sandhu and Rampal (2011) reported that isometamidium chloride HCl gave protection against *T. evansi* infection for 2 month or more. If the situation is critically analyzed, the treatment of infected horse with isometamidium chloride HCl @ 0.5mg/ kg was unsatisfactory as the disease relapsed. The possible cause of the relapse might be the fact that trypanosomes escaped the action of the isometamidium chloride HCl. *T. evansi* are tissue invasive and penetrate into the brain of the infected host. Isometamidium chloride HCl, however, does not cross the blood brain barrier (BBB) and cannot therefore kill trypanosomes in the brain.

Reasons other than trypanosomiasis, such as environmental stress, insufficient diet, the harsh climate and some other unidentified causes could have contributed to the relapse and death of animals. Treatment was most effective in the early stages of infection while animals with nervous signs and treated at late-stage often succumbed (Shrivastava and Malhotra, 1967). Though *T. evansi* is an extra-cellular parasite, it is possible that organisms may cross the blood-brain barrier via a paracellular route. Parasites that cross the blood brain barrier and remain viable can re-infect the host after treatment with drugs that are not able to cross the barrier. This could result in a relapse that is not due to the presence of resistant parasites once treatment is withdrawn (Brun and Lun, 1994). The infected horses showed good response following administration of quinapyramine sulphate and chloride as compared to isometamidium chloride HCl.

References

Bharathi, S. and Padmaja, K. (2007). Trypanosomiasis in equine - a case report. *Indian J. Vet. Med.* **27**: 72.

Brun, R. and Lun, Z. R. (1994). "Drug sensitivity of Chinese *Trypanosoma evansi* and *Trypanosoma equiperdum* isolates." *J. Vet. Parasitol.* **52**: 37-46.

Derakhshanfar, A., Mozaffari, A. A. and Mohaghegh, Z. A. (2010). An Outbreak of Trypanosomiasis (Surra) in Camels in the Southern Fars Province of Iran: Clinical, Hematological and Pathological Findings. *Res. J. Parasitol.* **5**: 23-26.

Eisler, M. C., Maruta, J., Nqindi, J., Connor, R. J., Ushewokunze, O. U, Holmes, P. H. and Peregrine, A. S. (1996). Isometamidium concentrations in the sera of cattle maintained under a chemoprophylactic regime in a tsetse-infested area of Zimbabwe. *Trop. Med. and Int. Health.* **1**: 535-541.

Gill, B. S. (1991). *Trypanosomes and trypanosomiasis* of *Indian Livestock.* ICAR. New Delhi. Pp. 139.

Hardeep, K., Mohinder, P. G., Pritam, K. S., Vishal, M., Mandeep, S. B., Kamalpreet, K., Sunil, V. and Lachhman, D. S. (2012). An outbreak of acute *Trypanosoma evansi* infection in crossbred cattle in Punjab, India. *J. Appl. Anim. Res.* **1**: 1-2.

Imad, R., Firyal, S. and Muhammad, G. (2010). Use of Quinapyramine in the treatment of an outbreak *Trypanosoma evansi* infection in camel. *J. Camel Pract. and Res.* **17**: 43-44.

Laha, R. and Sasmal, N. K. (2008). Endemic status of *Trypanosoma evansi* infection in a horse stable of eastern region of India - a field investigation. *Trop. Anim. Health. Prod.* **40**: 357-361.

Menon, D, G. and Mathew, L. (2008). Incidence of *Trypanosoma evansi* in Thrissur town. *Veterinary World.* **1**: 275-277.

Radostits, O. M., Gay, C. C., Blood, D.C., Hinchcliff, K. W. (2009). Veterinary Medicine: A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th Ed., W.B. Sounders Company, London, UK, Protozoan parasites in horses. Pp: 1537-1538.

Sandhu, H. S. and Rampal, S. (2011). Essential of Veterinary Pharmacology and Therapeutics. Pp. 1307-1308.

Shrivastava, R. V. N. and Malhotra, M. N. (1967). Efficacy of Mel-W-Trimelarsan-Melarsony1 Potassium and Samorin-Isometamidium Chloride against *Trypanosoma evansi* infection in experimental dogs. *Indian J. Anim. Health.* **6**: 291-297.

Singh, R., Gupta, S. K., Upadhyay, S. and Koul, A. L. (2011). Clinicohaematobiochemical studies in equines infected with *T. evansi. Veterinary Practitioner.* **12** : 142-147.

Singh, V., Chaudhari, S. S., Kumar, S. and Chhabra, M. B. (1995). Polyclonal antibody-based antigen-detection immunoassay for diagnosis of *Trypanosoma evansi* in buffaloes and horses. *J. Vet. Parasitol.* **56**: 261-267.

Soodan, J. S., Singh, K. B., Juyal, P. D. and Khakhaira, S. S. (1995). Incidence of *Trypanosoma evansi* in equines in Punjab state. *J. Vet. Parasitol.* **9**: 133-134.

Yadav, S.C. and Kumar, R. (2010). *Immunological Diagnosis of Trypanosomiasis in Equines.* Compedium Equine Health and Production Management, National Research Centre on Equines, Hisar, Haryana. Pp. 51-53.

The Blue Cross Book July 2015 Vol. 32 : 95-97

Subacute Hemorrhagic Septicemia in a Cow - A Case Report

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(received 04/04/2015 - accepted 01/06/2015)

Abstract:

A five year old cross bred cow was brought with the history of anorexia, respiratory distress and swelling at inter mandibular region since a week. When clinically examined, injected mucous membranes, elevated rectal temperature, inspiratory dyspnea and inter mandibular edema were noticed. Aspirated fluid from the intermandibular space showed the presence of bipolar organisms. Based on these findings, a diagnosis of Subacute hemorrhagic septicemia was made. Successful clinical management with sulphdimidine (110 mg/kg) iv, bid, for 5 days with other supportive drugs is described.

Keywords: Subacute Hemorrhagic Septicemia - Cow

Introduction:

Haemorrhagic septicaemia (HS) is an acute, highly fatal septicemic disease of cattle and buffaloes caused by specific serotypes of *Pasteurella multocida* organisms. The disease is characterized by high temperature, nasal discharge, laboured breathing and submandibular edema. Death usually occurs quickly and mortality is virtually 100% in infected but untreated animals (Dey *et al.,* 2007). True recovery from clinical disease occurs only if the animal is treated in the very early stages, which is often impossible under prevailing field conditions (Dziva *et al.,* 2008).

Haemorrhagic septicaemia has a wide distribution, particularly in tropical countries in Asia and Africa (Tasneem *et al.,* 2009). The organisms are endemic in many Asian countries including India and cause a variety of disease syndromes in many species of animals and accounts for high mortality (Kedrak and Borkowska-Opacka, 2001). In India, HS is ranked as the most important bacterial disease in cattle and buffaloes. The present paper describes about the successful management of subacute Hemorrhagic Septicemia in a Cow.

Case history and Clinical findings:

A five year old cross bred cow was brought to the Department of Teaching Veterinary Clinical Complex with the history of anorexia, respiratory distress, excessive salivation and swelling at inter mandibular region since a week. Animal was in last trimester of pregnancy and was treated by local veterinarian. On clinical examination, injected mucous membranes, elevated rectal temperature (106°F), inspiratory dyspnea and inter mandibular edema (Fig.1) were noticed. Under aseptic conditions fluid was aspirated from the intermandibular space and was subjected for microbiological examination. Bipolar organisms (Fig.2) were observed on bipolar staining.

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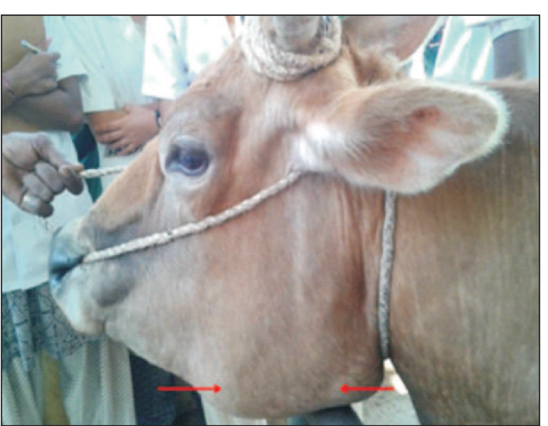

Fig.1: Photograph Showing Sub-mandibular swelling

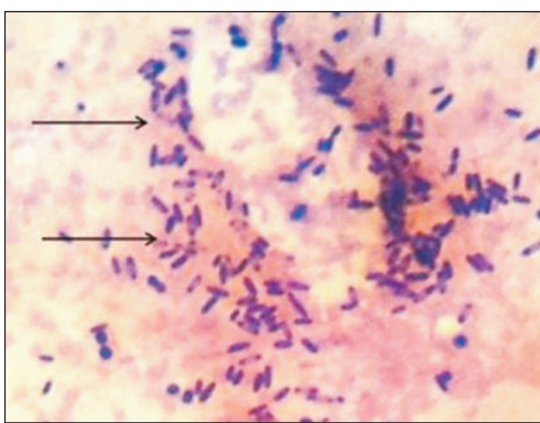

Fig.1: Photograph Showing bipolar organisms in Sub-mandibular edema fluid

Major hematological alterations included leukocytosis (22,000 cells /cumm) and neutrophilia (Neutrophils = 74, Lymphocytes = 24, Monocytes = 1, Eosinophil = 1 and Basophils = 0). Other hematological (Haemoglobin, Packed Cell Volume and Total erythrocyte count) and biochemical parameters (Blood Urea Nitrogen, Serum Creatinine and Serum Alkaline Phosphatase) were within normal physiological range. Based on these findings, a diagnosis of subacute hemorrhagic septicemia was made.

Treatment and Discussion:

The case was treated with Sulphadimidine @110mg/kg, intravenously, b.i.d, for five days, Meloxicam @0.4mg/kg, intramuscular, s.i.d, for three days and Chlorpheniramine maleate @0.5mg/kg, intramuscular, s.i.d, for three days. Significant clinical recovery was noticed after three days of treatment and the case was discharged after five days of treatment.

Hemorrhagic septicemia is one of the most important diseases of bovines in many Asian countries. Epidemiological studies (1974-1986) in India indicated that mortality-wise, HS was placed first and morbidity-wise, second as compared to four other epizootic diseases namely, foot and mouth disease, rinderpest, anthrax and black quarter (Dutta *et al.,* 1990). Many states in India are marked as high risk zones and outbreaks are commonly noticed during monsoon (Saini *et al.,* 1991). Present case was also observed during monsoon. Case fatality rates vary from 94-96%, Hence HS is responsible for severe economic loss to the poor livestock farmers. Medical treatment alone results in poor survival rate (Khan *et al.,* 2006). Successful outcome in present case might be attributed to the early diagnosis.

References:

Benkirane, A. and M.C.L. De Alwis. (2002). Haemorrhagic septicaemia, its significance, prevention and control in Asia. *Vet. Med. - Czech.,* **47** : 234-240.

Carter, G.R. (1982). Whatever happened to hemorrhagic septicemia?. *J. Am. Vet. Med. Assoc.,* **180**: 1176-1177.

Dey, S., Singh, V.P., Kumar, A.A., Sharma, B., Srivastava, S.K. and Singh, N. (2007). Comparative sequence analysis of 16S rRNA gene of Pasteurella multocida serogroup B isolates from different animal species. *Res. Vet. Sci.,* **83**:1-4.

Dutta, J., Rathore, B.S. Mulick, S.G. Singh, R. and Sharma. G.C. (1990). Epidemiological studies on occurrence of Hemorrhagic septicemia in Inida. *Ind. Vet. J.*, **67**: 893-899.

Dziva, F., Muhairwa, A.P., Bisgaard, M. and Christensen, H. (2008). Diagnostic and typing options for investigating diseases associated with Pasteurella multocida. *Vet. Microbiol.*, **128**:1-22.

Kedrak, A. and Borkowska-Opacka, B. (2001). Phenotypic characteristics of Pasteurella multocida strains isolated from cattle affected with haemorrhagic septicaemia. *Bull. Vet. Inst. Pulawy.*, **45**: 171-176.

Saini, S.S., Sharma, D.R. Gill, B.S. Kwarts, M.S. Singh, J. Sharma, J.K. Dhillon, S.S. and Ramneek, R. (1991). Re-emergence of hemorrhagic septicemia in Punjab. *Indian. J. Anim. Sci.*, **61**: 1178-1180.

Tasneem, K., Zamir, B., Ali. S., Gill, Z.J. and Raza.A. (2009). Haemorrhagic Septicemia: A review. *Pak. J. Sci.*, **61**: 10-17.

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The Blue Cross Book July 2015 Vol. 32 : 98-99

Successful expulsion of Mummified Fetus in a Cow

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(received 12/01/2015 - accepted 01/06/2015)

Abstract

A case of fetal mummification in a pleuriparous cow managed successfully by single i/m injection of cloprostenol 500 μg is reported.

Key Words: Cow, Fetal mummification, Cloprostenol, Clinical management.

Introduction

Mummification occurs when the fetus dies inside the aseptic uterus, the pregnancy maintains and the dead fetus is not expelled but shrivels up. Mummification of bovine fetuses is an uncommon condition with an incidence of 0.13 to 1.8 % of conceived animals (Arthur *et al.,* 1996). In cattle, fetal mummification occurs after formation of the placenta and fetal ossification, most often between the 3rd and 8th months of gestation, without concomitant luteolysis of the corpus luteum (CL) and opening of the cervix. Fetal mummification associated with a persistent CL is observed mainly in cattle and goats, both species being dependent on progesterone (P4) produced by the CL for the maintenance of pregnancy (Mathew *et al.,* 1980).

Although the exact causes of fetal death followed by mummification have not been completely determined but some infections like bovine viral diarrhea (BVD), leptospirosis, and molds, mechanical factors, such as compression or torsion of the umbilical cord, or both (Mahajan and Sharma, 2002); uterine torsion, defective placentation; genetic anomalies; abnormal hormonal profiles; and chromosomal abnormalities etc are considered responsible. The present report places on record a case of mummification in a cow.

Case History and Clinical Observation:

A seven-year old pleuriparous cow in its third lactation and nine months of gestation was presented with history of chocolate color abnormal vaginal discharge. The cow was confirmed earlier pregnant during the third month of gestation. On rectal examination, the hard mass was felt in right uterine horn. The signs of nine month pregnancy like palpation of fetus, cotyledons and freemitus were not observed. Transrectal ultrasonongrahy showed hyperechoic uneven immobile structure in the right horn indicating fetal mass. Based on the history, clinical symptoms, per-rectal and ultrasonographic examination, the case was diagnosed as of fetal mummification.

Treatment and Discussion

The cow was treated by i/m administration of cloprostenol sodium 500 μg (Cyclix) and was

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Fig. 1: Expelled Mummified fetus separated from placenta.

kept under observation. After approximately 70 hours, a dry hard mass with chocolate brown color along with placenta was expelled out (Fig.1). The mass was rigid and all of the four legs and neck were covered by placenta. The chocolate red colored discharge was indicative of fetal mummification as haematic type. After expulsion of mummified fetus, the cow was treated intramuscularly with methyl ergometrine maleate 5 mg (Nexbolic) on first day and antibiotics Amoxycillin and Dicloxacillin 3.5 gm (Intamox-D) and Clorpheniramine maleate 10 ml (Anistamin) for three days. The cow recovered uneventfully and exhibited normal estrus 25 days after treatment.

The object of treating an abnormal pregnancy is either to save the fetus, or expel the abnormal fetus, in order to have the cow pregnant again within the shortest possible time. The treatment of choice in cases of fetal mummification is induction of luteolysis by injection of PGF2α, which is followed by the expulsion of the fetus within 2 to 4 days (Jackson and Cooper, 1977). Expulsion of mummified fetus by administration of luteolytic agents and betamethasone has been reported as early as by 12-14 hr (Saxena *et al.,* 2001) to 8 days (Srinivas *et al.,* 2007). The expulsion of mummified fetus after 30 hr without any assistance after valethamate bromide 150 mg and 25 mg prostaglandin F2 alpha treatment was reported by (Markandeya et al, 2003). However, in non-responding cases, fetus may be delivered by caesarean section as reported by Phogat and Gupta (1996).

References

Arthur, G.H., Noakes, D.E. and Parlmson, T.J. (1996). Veterinary Reproduction and Obstetrics. WB Saunders, London. p.127-128.

Jackson, P.S. and Cooper, M.J. (1977).The use of cloprostenol for the termination of pregnancy and the expulsion of mummified fetus in cattle. *Vet. Rec.,* **100**: 361-363.

Mahajan, M. and Sharma, A. (2002). Haematic mummification due to umbilical cord torsion in a cow: A case report. *Indian Vet. J.,* **79**: 1186-1187.

Markandeya, N.M., Desphande, A.V., Bharkad, G.P. and Bhikane, A.U. (2003). Succesful termination of mummified fetus in a primiparous Deoni heifer. *Indian J. Anim. Reprod.,* **24**: 173-174.

Mathew, J., Madhavan, E. and Iyar, CPN. (1980). *Livestock Adviser*, **80**: 61-65.

Phogat, J.B. and Gupta, S.L. (1996). Foetal mummification and uterine rupture in crossbred cow. *Indian J. Anim. Reprod.,* **13**: 92.

Saxena, A., Mishra, S.K., Chandra, R. and Tiwari, H.A. (2001). Vaginal delivery of mummified fetus from buffalo heifer-a case report. *Indian Vet. J.*, 78: 750-751.

Srinivas, M., Sreenu, M. and Laxmi Rani, N. (2007). Per vaginal expulsion of mummified fetus in a crossbred cow. *Indian Vet. J.* **84**: 288.

The Blue Cross Book July 2015 Vol. 32 : 100-102

A foreign body in a Cow at the floor of Oral Cavity and its management

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(received 22/04/2015 - accepted 01/06/2015)

Abstract :

A 3 year old female Gir crossbred cow was presented with the history of dysphagia, excessive salivation, and ventral submandibular hard swelling from last two weeks. Physical examination of oral cavity with the help of mouth gag revealed a visible (approximately 3% tip portion) of foreign body (needle) below the base of tongue. Foreign body pierced vertically downward to floor of mandible. Inflammatory swelling was noticed at surrounding area of foreign body. With the help of haemostatic artery forceps, tip of foreign body was grasped and by gentle traction, the foreign body was removed. Cow was kept on antibiotic, analgesic and anti-inflammatory therapy. In the present case, recovery was uneventful and uncomplicated

Keywords: Cow, foreign body, submandibular region.

Introduction

Any foreign body in the oral cavity produces pain which prevent the animal from eating or drinking. The tongue of cattle is firm and plump, highly mobile and protrusive and has an important function in the prehension of feed (Radostitis, 2005). The tongue grasps forage and drags it into the mouth, where the ventral incisors pressure against the dental pads and cuts it (Ducharma, 2004). Insensible dental pad and indiscriminate eating habits make the animal prone to foreign body in the oral cavity. It is commonly lodged in the oropharynx, including the tongue base and tonsils (Verma et al., 2007).

Case History

A 3 year old female gir crossbred cow was presented to First Grade Veterinary Hospital, Nasirabad, Ajmer, Rajasthan with the history of dysphagia, excessive salivation, pain and ventral submandibular hard swelling from last two weeks. Owner reported that animal was previously treated by three different veterinarians. According to the history, out of three, two veterinarians diagnosed as bottle jaw condition and treated accordingly. One veterinarian diagnosed as tumor and without treating, referred it to hospital for surgery.

Clinical Observation and Diagnosis

Clinical examination revealed excessive salivation, pain and ventral submandibular hard swelling (Fig. 1). Physical examination of oral cavity with help of mouth gag was performed. The rostral aspect of the mouth was inspected. Examination of the oral cavity revealed a visible, approximately 3% portion (tip portion) of a foreign body (looked like stainless steel wire) below the base of tongue. The foreign body had pierced vertically downward to mandible. Inflammatory swelling, ulceration and mild

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Fig. 1 Clinical examination reveals ventral submandibular hard swelling

bleeding was noticed at the area surrounding the foreign body.

Treatment

The animal was restrained properly in trevis. The tongue was pulled out on one side. With the help of haemostatic artery forceps (Fig. 2), tip of foreign body was grasped and by gentle traction, the foreign body was removed. Recovered foreign body was long suturing needle (Fig. 3) which is mostly used for suturing the gunny bags

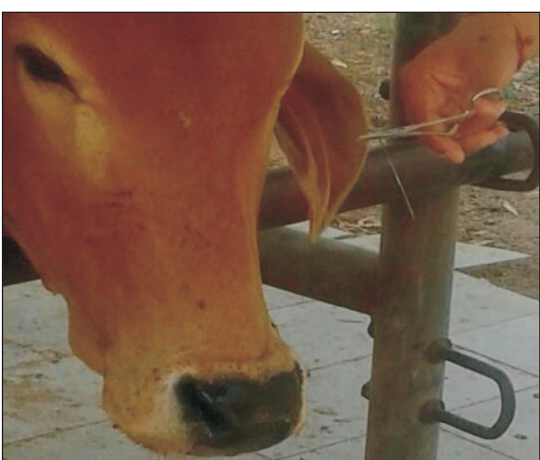

Fig. 2 Recovered foreign body with haemostatic artery forceps

Fig. 3 Recovered foreign body, a long suturing needle.

and bedding materials. 5 gm of Streptopenicillin was administered intramuscularly for two days. Pheniramine maleate 10 ml and Melonex at the dose rate 0.5mg/kg body weight were administered intramuscularly for three days respectively. Oral cavity was irrigated with light potassium permanganate solution for 7 days and animal was offered rough ages with soft dry leaves with minimum straws. A submandibular abscess developed on 4th day which was drained by giving a criss-cross incision on the ventral aspect of the mandible. The wound was dressed with 5% povidone iodine and fly repellent ointment. The wound healed after 15 days.

Discussion

If cases are reported with the history of ventral submandibular swelling, salivation, pain and dysphagia, they should be differentiated between parasitic load, actinomycosis, neoplasm, abscess, cyst and foreign body. Any foreign body in the oral cavity produces pain which prevents the animal from eating or drinking. The mechanical cause of dysphagia might be foreign body, anatomical defects, peripharyngeal masses such as neoplasia and abscess. The most important foreign bodies encountered in the tongue and oral cavity are fish hooks, needle, wire, rubber band and tooth pieces etc which may become fixed and lead to protrusion of the organ, difficulty in swallowing, salivation and dysphagia (Boden and West,

1998). Development of sub-mandibular abscesses was a complication in present study due to location of foreign body and could have led to osteomyelitis if not drained and treated in time. Similar technique with the same complication has also been described by Dudi and Gahlot (2003) in case of mandible fractures.

Literature cited :

Boden, E. and Geoffrey Philip West (1998). Tongue foreign body in cattle. In : Black Veterinary Dictionary, Saunders, p. 530.

Ducharma (2004). Surgery of the bovine digestive system. In: Farm Animal Surgery. S. L. Fubini and N. G. Ducharma, Saunders, p. 161.

Dudi, P.R. and Gahlot, T.K. (2003). A survey of ethnoveterinary treatments of common surgical conditions of large ruminants and camels. *Journal of Camel Practice and Research* **10**:211-212.

Radostitis, O. M., Mayhew, I.G. and Houston, D.O. (2005). Tongue foreign body in cattles. In: Veterinary Clinical Examination and Diagnosis, Saunders, p. 448.

Verma, R., Kapil Sikka and Thakar, A. (2007). Autologous embedded foreign body of mobile tongue. Indian J. Otolaryagology and Head Neck Surg. **59**: 291-92.

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Horn Cancer in a cow and its surgico-medical management

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(received 21/04/2015 - accepted 01/06/2015)

Absract

A 8 years old cow was presented with the history of trauma to left horn, which was later showing cauliflower like growth and swelling at the base of the horn and hanging of the horn. A blood tinged serosanguinous nasal discharge was also observed from left sided nasal passage. The animal was treated by electric dehorner under local anaesthetic infiltration. The cavity was curetted to remove the neoplastic cells. Postoperatively, cow was kept on antibiotic (Oxytetracycline), analgesic and anti-inflammatory (Meloxicam) treatment. Later, Vincristine therapy and alternate dressing with sprit was followed. The horn cancer recovery was uneventful and uncomplicated

Keywords : Cow, horn cancer, cornual nerve, vincristine

Introduction

Horn cancer is predominantly reported in bullocks and less in cows (Yadav et al., 2002). Highest incidence in cattle at the age group of 6-8 years has been observed (Nair & Sastry, 1954). Irritation is a major predisposing factor for horn cancer. Yoke, trauma, chronic irritation due to tying a rope at the base of the horn, rubbing against hard object, fighting, pairing of horns, painting, solar radiation, flies, worms and viruses, either alone or in combination, have been reported as predisposing factor. Bamne et al. (2007) stated that diagnosis of cases can be made according to manifestations like frequent shaking of horn, tilting at the affected side, bending of affected horn and increase nasal discharge on the affected side in advance cases (Joshi et al., 2009). rubbing on hard objects. Kulkarni (1953) stated that horn cancer was more common in Kankrej breed as compared to Gir and other breeds. The most consistent clinical signs are frequent head shaking,

Case History and Observation

A eight year old cow was brought to the Mobile Veterinary Unit, Nasirabad, Rajasthan, with the history of trauma and swelling at the base of the horn from last 3 months. History further revealed that the horn of the animal was hanging for last 20 days. Clinical observations showed

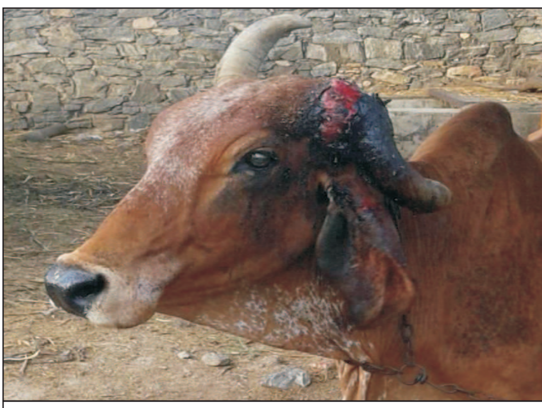

Fig. 1. Cauliflower like growth and swelling at base of horn and hanging of horn.

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Fig. 2. Cow was restrained by rope squeeze method.

cauliflower like growth and swelling at the base of horn and hanging of horn (Fig. 1) was observed. A blood tinged serosanguinous nasal discharge was also observed from left sided nasal passage. Animal was restless, frequently shaking horn, rubbing and striking affected horn against hard objects.

Fig. 3. Postoperative dressing with gauze of Povidone iodine.

Surgical Management

The cow was kept off feed for 24 hours prior to surgery. It was restrained by rope squeeze method (Fig. 2). The operation was performed in lateral recumbency. After aseptic preparation of site, Xylazine was administered for sedation at 0.03mg/kg body weight. 2% Lignocaine hydrochloride was infiltrated to desensitize the cornual nerve. Pre-operative systemic haemostatic (Adrenochrome 10 ml intramuscularly) was administrated. After adequate analgesia, dehorning was done by using electric dehorner. The cavity was thoroughly curetted to remove the neoplastic cells. Cauterisation was done at affected area with Copper sulphate. For control of haemorrhage, gauze soaked in Tincture benzoin was applied in the cavity for 2 minute and later dressed with gauze of Povidone iodine (Fig. 3). Post-operatively, Oxytetracycline at the dose rate 10 mg/ kg body weight and Meloxicam at the dose rate of 0.5 mg/kg body weight were given intramuscularly for seven and five days

respectively. Vincristine sulphate (0.025 mg/kg intravenously) was administered 3 times at the interval of 7 days. Alternate day dressing was performed with spirit and povidone iodine ointment.

Discussion

Cancerous growth of horn of cattle is reported from India with serious economic losses (Lall, 1953). Carcinoma of horn core in cattle is primarily squamous cell neoplasm (Patra, 1963). The origin of cells has not been determined, although it is claimed that the neoplasm arises from the mucous membrane of horn core sinus and then invades the horn core (Moulton, 1961). Horn cancer is generally unilateral and is encountered in cattle in the age group of 5-10 years (Tyagi and Singh, 2006).The bullocks appear to be highly susceptible as compared to bulls and cows. The disease is associated with chronic irritation of horns at their base (Sastry, 2001). In the present case of horn cancer, recovery was uneventful and uncomplicated. Complete cure and non recurrence of horn cancer could be due to action of Vincristine on mitotic figures of rapidly multiplying neoplastic cells (Udharwar et al. 2008). Histological examination of cancerous tissue was not performed in the present case.

References

Bamne, S.S., Yerolkar, A.J., Khangal, P.S., Londhe, S.P., Yadav, G.U. and Moregaonkar S.D. (2007). Horn cancer in a non descript cow – A case report. *Intas polivet.* **8**:178-179

Joshi, D.O., Karle, A.S. and Vedpathak, H. S. (2009). Epidemiological status of surgical affections of the buffalo horn at hospital population and ambulatory villages. *Indian J. Field. Vets.* **5**:910.

Kulkarni, H. V. (1953) Carcinoma of the horn in bovines of the old Baroda state. *Indian Vet. J.* **29**:415-421.

Joshi, B.P., Soni, P.B., Ferar, D.T., Ghodasara, D.J. and Prajapati, K.S. (2009). Epidemiological and pathological aspects of horn cancer in cattle of Gujarat. *Indian J. Field Vet.* **5**: 15-18.

Lall, H.K. (1953). Incidence of horn cancer in Meerut Circle, Uttar Pradesh. *Indian Vet. J.* **30**: 205-09.

Moulton, J.E. (1961).Tumors in Domestic Animals, pp. 44-48 (University of California Press, Berkeley). Patra, B.N. (1963). Horn cancer in cattle in India. *Vet. Rec.* **71**: 216.

Patra, B.N. (1963). Horn cancer in cattle in India. *Vet. Rec.* **71**: 216.

Sastry, G.A. (2001). Veterinary Pathology. 7th Edn., CBS Publisher and Distributors. New Delhi. pp. 205-49.

Tyagi, R.P.S. and Singh J. (2006). Ruminant Surgery, 1st Edn., CBS Publisher and Distributors. New Delhi. pp. 415-16.

Udharwar, S.V., Aher, V.D., Yadav, G.U., Bhikane, A.U. and Dandge, B.P. (2008). Study on incidence, predisposing factors, symptomatology and treatment of horn cancer in bovine with special reference to surgery and chemotherapy. *Vet. World* **1**: 7-9

Yadav, G.U.; P. T. Jadhav; S.D.Moregaonkar; A.U.Bhikane and R. R. Mugale (2002). An unusual horn cancer in a cow - A case report. *Indian Vet. J.* **79**:515-516

The Blue Cross Book July 2015 Vol. 32 : 106-107

Sarcoptic Mange in a Persian Cat

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Abstract:

Sarcoptic mange is a highly contagious skin infection, caused by a *sarcoptic scabie* mite, affecting primarily the domestic pets. The successful therapeutic management of sarcoptic mange in a 2 year old Persian cat with Ivermectin (200 mg/kg s/c), thrice, at weekly interval along with an antihistaminic, given daily for 7 days is described. There were no side effects of the drugs, neither the recurrence of the mange.

Key words : *Sarcoptes scabiei,* Mange, Persian Cat

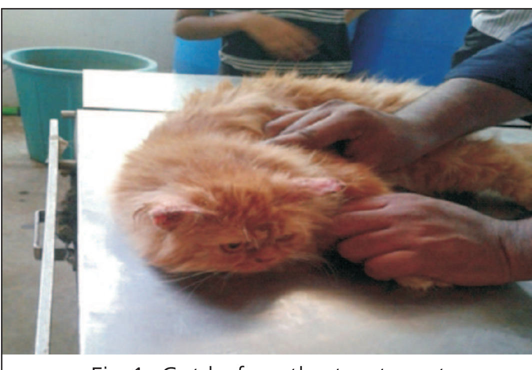

Fig.1. Cat before the treatment

Introduction:

Sarcoptic mange (also known as scabies) is a highly contagious parasitic disease caused by a microscopic mite, *Sarcoptes scabiei* that affects animals and humans. These mites invade the skin of healthy cats and kittens and create a variety of skin problems. Cats of all ages may be affected, but Sarcoptic mange is more common in young animals. Cats living in close contact with affected dogs may develop the disease (Rosanna, 2014). The present paper reports a case of sarcoptic mange in a persian cat.

Case history and Observations:

A two year old Persian cat was presented to the Teaching Veterinary Clinical Complex, College of Veterinary Science, Proddatur with the history of pruritus and alopecia over pinna of both the ears. Characteristic lesions were present at the edges of the pinnae with intense itching and patchy hair loss. On clinical examination, the cat was dull, temperature was 102.4F and conjunctival mucous membranes were pale.

Diagnosis:

Skin scrapings were collected for confirmative diagnosis. The scraped samples were taken in a screw capped test tube and dissolved in 10% NaOH and examined microscopically (Charles and Robinson, 2006). Examination of skin scraping revealed mites that were identified as *Sarcoptes scabiei* mites (Souls by, 2005).

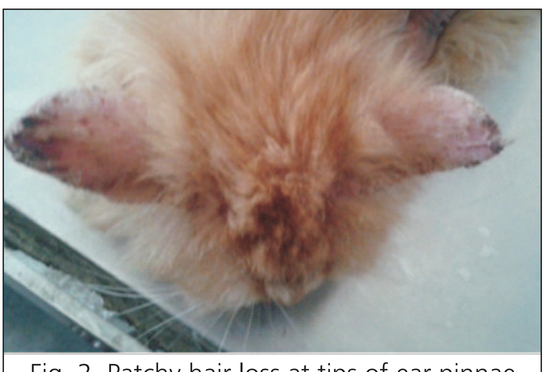

Fig. 2. Patchy hair loss at tips of ear pinnae

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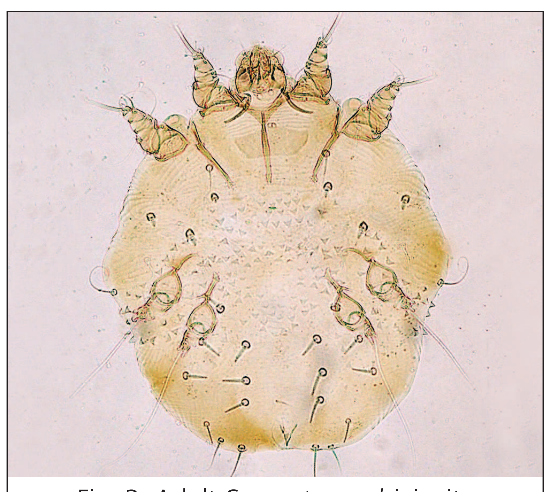

Fig: 3. Adult *Sarcoptes scabiei* mite

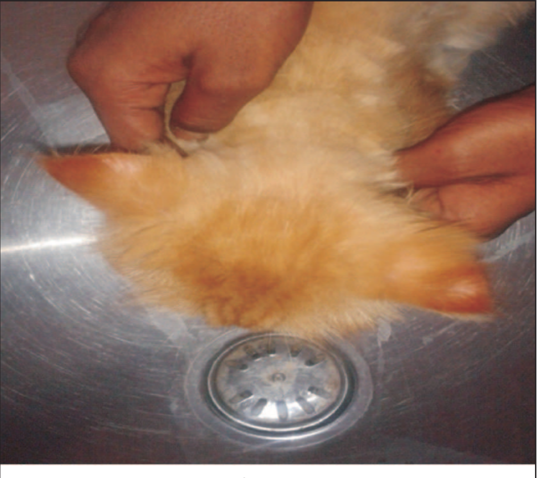

Fig. 4. 3 weeks post treatment

Treatment and Discussion:

Based on the history, clinical signs and microscopic examination of skin scrapings, the condition was diagnosed as Sarcoptic mange infestation. The cat was treated with Inj. Ivermectin @ 200µg/kg body weight subcutaneously. Inj. Avil (0.5 ml intramuscularly) was given daily for 7 days. By day 7, the pruritus had resolved. There was no side effects noticed hence Inj. Ivermectin was continued at weekly interval for 3 weeks. There was a significant clinical improvement after 14 days of treatment. Two weeks post treatment, skin scrapings were examined and found negative for the mites. Treatment was in accordance with Campbell (1985) who reported ivermectin as a highly potent, broad- spectrum and systemic antiparasitic drug. Scott et al., 2001 treated the cat with Inj. Ivermectin @200µg/kg, subcutaneously at weekly intervals for a month. Anil Kumar et al., (2013) found no side effects when ivermectin was used for a month at weekly intervals in the treatment of mange in cat. Supportive therapy was done with antihistamines by parentral route which was also recommended by Mahesh et al., (2013). Vitamin-Mineral supplementation (VM all syrup) orally was also given to hasten the recovery. In the present case, complete recovery of lesions was noticed after three weeks post treatment and no side effects were found.

References:

Anil Kumar, A., Usha N.P., Manju K. M. and Ajithkumar S. (2013) Therapeutic management of Notoedric mange in a cat. *Intas Polivet.* Vol. **14**: 327-328.

Campbell, W.C. (1985) Ivermectin, an update. *Parasitology. Today* 1: 10-16.

Mahesh, G., Shukla, P.C. and Rao, M.L.V. (2013) Therapeutic management of Demodicosis in a Dog. *Intas Polivet*. Vol. **14**: 282-283.

Rosanna, M. (2014) Sarcoptic mange in cats. http://www.petplace.com/article/cats/diseases-conditions-of-cats/skin/sarcoptic-mange-in-cats. Last Modified on December 10, 2014 accessed on 5th march 2015.

Scott, D., Miller, W., Griffin, C., Muller and Kirk. (2001) Small animal dermatology. 6th Edition W.B. Saunders Co. Philadelphia, 1552.

Soulsby, E.J.L. (2005) Helminths, Arthropods and protozoa of domesticated animals. 7th Edition. Lea & Febiger, Philadelphia

The Blue Cross Book July 2015 Vol. 32 : 108-110

Occurrence of Fowl Pox virus in Grey Jungle Fowls - A report

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Abstract :

Fowl pox is a slow spreading viral infection of chickens and other avian species including wild birds, characterized by proliferative lesions in the skin (cuteneous form). Ten Gray Jungle Fowls in a Zoological park showing cutaneous lesions on face, beak, nares etc. were investigated for fowl pox virus through isolation in embryonated chicken eggs (chorioallantoic membrane route). The isolated virus was further adopted in chicken embryo fibroblast cell culture to detect characteristic CPE with degeneration, rounding and aggregation with synctia formation.

Introduction

Fowl pox is a contagious disease of domestic and wild birds affecting all ages, sexes and breeds (Adbajo et al 2012; Weli and Tryland 2011). It is caused by Fowl pox virus of the genus Avipox of the family poxviridae and sub family chordopoxvirinae . Disease is characterized by three clinical forms, namely, cutaneous or skin form showing nodular leision on unfeathered parts of body, diphtheritic form showing psedodiptheretic membrane having fibro necrotic leisions in the mucosa of mouth and upper respiratory tract and the systemic form affecting internal organs (Fenner et al 1999). The disease causes economic losses and mortality rate upto 50% (skinner 2008) and the disease is complicated when the diphtheritic form is accompanied by secondary bacterial infections (Macanchlan et al 2009). Fowl pox is an emerging disease and diagnosed either by isolation in embryonated chick eggs through chorioallantoic membrane (CAM) route or by cell cultures or by both (Carulei et al 2009 and Farres et al 2010). In the present study, occurrence of Fowl pox virus was reported in Grey Jungle Fowls in SV zoological park, Tirupati. Virus isolation was attempted for the first time in Andhra Pradesh and methods have been described.

Material and Methods:

Ten out of fifty Grey jungle fowls in SV zoological park, Tirupati, Andhra Pradesh were affected with cutaneous lesions suggestive of Fowl pox during the month of July, 2009. The Grey Jungle fowls were having characteristic pock lesion on face, beak, nares, combs and wattles. The pock lesions were collected aseptically and preserved at 4^{0}C before processing.

Processing of samples:

The skin scabs collected from affected fowl were grounded with pestle and mortar aseptically with sterile PBS (pH 7.4). The material was clarified by centrifugation at 3000rpm for 20min. at 4^{0}C. The supernant was filtered through 0.22µ membrane filters and made 10%

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suspension. The suspension was treated with antibiotics penicillin at the rate of 1000 IU/ml and Streptomycin 100µg/ml of suspension for 30 min. at 37°C, later used as inoculum for inoculation into embryonated chicken eggs.

Virus isolation in embryonated chicken eggs

Fertile chicken eggs were selected from specific pathogen free flock and incubated in egg incubator for 11 days. 0.2ml of 10% suspension of inoculum prepared was inoculated through CAM route in to the chicken eggs according to the method of Cunnigham (1966). The inoculated chicken eggs were incubated at 37°C in egg incubator and checked daily for mortality. Five days of post inoculation, CAM were harvested and examined for pock lesions. Later subsequently, further three more blind passages were given for adoptation of the virus in CAM of chicken eggs.

Adoptation of isolated virus in chicken embryo fibroblast cell culture

The primary chicken embryofibroblast cell culture was prepared from nine day old chicken embryos as described by Cunnigham (1966) using Hank's balanced salt solution(HBSS) growth medium with 10% fetal calf serum. The uniform cell suspension was made using 0.25% trypsin as per the method of Youngner (1954). The uniform monolayer cells were obtained after 36-48 hrs of incubation.

The field virus which was recovered on CAM of chicken embryos was adopted to grow in chicken embryo fibroblast cell culture. The 0.2ml of 10% suspension of CAM was inoculated into monolayer of chicken embryo fibroblast cell cultures and incubated for 2hrs at 37°C for virus adsorption. After adsorption, the excess inoculum from the cell culture bottle was removed and HBSS maintainance medium was added and again incubated at 37°C. Further, four blind passages were given in chicken embryo fibroblast cell cultures for characteristic CPE, and was preserved at -20°C for future use.

Results and Discussion

On gross examination, thick edematous diffuse pock lesions were observed on infected CAM in the initial two passages and on fourth passage the characteristic white necrotic foci were observed during the study. These findings are in agreement with Skinner (2008) and Haligu et al (2009). In the present report, only cutaneous form was observed showing characteristic pock lesions on unfeathered body portions, face, beak, nares, combs and wattles in affected grey Jungle fowls. Observations made during the chicken embryo fibroblast cell culture study revealed that only slight CPE was observed upto third passage level but characteristic CPE with degeneration, rounding and aggregation with syncytia formation were noticed in subsequent fourth and fifth passages. Similar observations were reported by Yadav et al (2007) in their studies.

References:

Adebajo, M.C., Akinyede, O. and Shittu, I.A. (2012) Seroprevalence of fowl pox antibody in indigenous chickens in Jos North and South council areas of Plateau State, Nigeria: Implication for vector vaccine. *ISRN Vet. Sci.,* : 154971.

Carulei, O., Douglass, N. and Williamson, A.L. (2009) Phylogenetic analysis of three genes of penguinpox virus corresponding to vaccinia virus G8R (VLTF-1), A3L (P4b) and H3L reveals that it is most closely related to turkeypox virus, ostrichpox virus and pigeonpox virus. *Virol. J.,* **6**: 52.

Cunningham, C.H. (1966) A Laboratory Guide in Virology. 6th ed. *Burgess Publishing Company, Minneapolis.*

Farias, M.E.M., La Pointe, D.A., Atkinson, C.T., Czerwonka, C., Shrestha, R. and Jarvi, S.I. (2010) Taqman real-time PCR detects Avipoxvirus DNA in blood of Hawaii Amakihi *(Hemignathus virens). PlosOne,* **5**:10745.

Fenner, F.J., Gibbs,E.P.J., Murphy,F.A., Rott, R., Studderi,M.J. and White, D.O.(1999). Veterinary Virology, Fourth Edn. Academic Press Inc., USA.

Haligur, M., Ozmen, O., Vural, S.A. and Berlin, S. (2009) Pathological, mmunohistochemical and electron microscopical examinations on chorioallantoic membrane lesions in experimental fowl poxvirus infection. *Kafkas Univ. Vet. Fak.,* **15**: 345-350.

Maclachlan, N.D. and Dubovi, E. (2009) Fenner's Veterinary Virology. 4th ed. Elsevier Academic Press, Amsterdam. p163.

Skinner, M.A. (2008) Poxviridae. In: Pattison, M., McMullin, P.F., Bradbury, J.M. and 8.Alexander, D.J., editors. Poultry Diseases. 6th ed. WB Saunders Company, London. p333-338.

Weli, S.C. and Tryland, M. (2011) Avipoxviruses infection biology and their use as vaccine vectors. *Virol. J.,* **8**: 49.

Yadav, S., Dash, B.B., Kataria, J.M., Dhama, K., Gupta, S.K. and Rahul, S. (2007) Pathogenicity study of different avipoxviruses in embryonated chicken eggs and cell cultures.Indian *J. Vet. Pathol.,* **31**: 17-20.

Youngner, J.S. (1954). Monolayer tissue cultures. Poliomylitis virus assay in roller tube cultures of trypsin dispersed monkey kidney. *Pro. Soc. Exp. Biol.,* **85**: 527-530

COMPOSITION

Scalibor P B 65 cm contains 1 gm of deltamethrin

Scalibor P B 48 cm contains 0.76 gm of deltamethrin

INDICATIONS

- Anti tick, anti flea, anti sandfly and anti mosquitoes

DOSAGE AND ADMINISTRATION

One collar for six months. 65 cm (medium to large dogs) and 48 cm (smaller dogs)

PRESENTATION

6 x 65 cm and 6 x 48 cm.

The Blue Cross Book July 2015 Vol. 32 : 111-115

Beta-casein protein of bovine milk and its possible effects on human health

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Abstract

The European breeds of cattle, particularly Holstein and Jersey are extensively used for cross breeding of Indian non-descript breeds. The presence of AI Beta-casein variant in Holstein and Jersey milk has been a matter of discussion since last couple of years over the use of these breeds in Indian crossbreeding programme. The scientific information about the presence of A1 and A2 alleles of beta casein in the milk of different breeds is discussed.

Introduction

Milk constitutes about 85% water, 15% milk sugar lactose, protein, fat, and minerals. Bovine milk is the most important food for young calves and a common source of proteins and microelements. In milk, there are two major protein groups known as caseins and whey proteins. Caseins account for 80% of bovine milk protein (Niki et al.1994; Martien et al. 1994), whereas, major whey proteins constitute about 14% (McLachlan 2001; Roginski 2003). Beta-casein is 30% of the total protein content in cow's milk. Bovine milk contains 4 caseins: alpha s1 (CSN1S1, 39–46% of total caseins), alpha s2 (CSN1S2, 8–11%), beta (CSN2, 25–35%), and kappa (CSN3, 8–15%) (Eigel et al. 1984; Roginski 2003). There is also gamma-casein that is a product of degradation of beta-casein (Ostersen et al. 1997; Miller et al. 1990). Each cow carries two copies of the gene encoding beta-casein, with a genotype of A1/A1, A1/A2, or A2/A2. Neither the A1 nor A2 trait appears to be dominant (co-dominant), which means that the milk produced by an A1/A2 cow will likely to contain equal proportions of A1 and A2 beta-casein. A1/A1 cows will obviously produce A1 beta-casein, just as A2/A2 cows will only produce A2 beta-casein. Caseins are encoded by members of a multigene family. The genes encoding 4 caseins are found on bovine chromosome 6 (Rijnkels 2002). The present article describes the beta-casein gene and the potential influence of beta-casein variants on human health.

Beta-casein polymorphism

There are 13 genetic variants of beta-casein: A1, A2, A3, A4, B, C, D, E, F, H1, H2, I and G. The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common, and A3 and C are rare (Farrell et al. 2004). Presence of proline (CCT) and histidine (CAT) amino acid in peptide chain at position 67 of the beta-casein may give rise to two variants A2 and A1 beta-casein respectively (Roginski 2003). The cause for concern with milk containing A1 beta-casein is that histidine at the 67th amino acid position allows a digestive enzyme to cut out a 7 amino acid segment of the protein immediately adjacent to that histidine.

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Table 1: Occurrence of beta-casein gene in various cattle breeds and countries (Kaminski et al., 2007)

Breed	Country	No. of animals	Frequency of beta-alleles casein			References
			B	Al	A2	
Guerney	USA	400		0.010		Swaissgood 1992
Jersey	USA	3861	0.010-0.020	0.010-0.060	0.880-0.970	Hnennam *et at.* 1991
	Germany	43	0.186	0.093	0.721	Ehrmann *el al.* 1997
	Denmark	157	0.350	0.070	0.580-0.650	Bech *et al.* 1990
	New Zealand	1328	-	0.123	0.591	Winkelman and Wickham 1997
Brown	USA	387	0.290-0.370	0.090-0.220	0.490-0.540	Eenennam *et al.* 1991
Swedish	Germany	232	0.170	0.108	0.705	Ehrmann *et al.* 197
	USA	282	0.100-0.180	0.140-0.150	0.660-0.720	Swaissgood 1992
Simmental	USA	259	0.100-0.180	0.140-0.180	0.660-0.720	Eenennam *et al.* 1991
	Croatia	621	0.150	0.190	0.630	Curik *et al.* 1997
HF	Germany	229	-	0.343	0.566	Ehramann *et al.* 1997
	USA	526	0.010-0.060	0.310-0.660	0.240-0.620	Swaissgood 1992
	USA	6000	0.010-0.040	0.310-0.490	0.490-0.620	Eenennam *et al.* 1991
	Hungary	768	0.107	0.418	0.470	Baranyi et al. 1997
	Germany	229	0.026	0.472	0.496	Ehrmann *el al.* 1997
	Poland	143	-	0.402	0.598	Kaminski *el at.* 2006a
	New Zealand	3761	-	0.465	0.510	Winkelman *el at.* 1997
	Norway	306	-	0.100	0.490	Lien *et at.* 1993
Black-and-White	Denmark	223	0,030-0.080	0.550	0.390	Bech *et al.* 1990
Red-and-White	Sweden	394	0.008	0.160	0.531	Lunden *el al.* 1997
	Germany	179	0.020	0.573	0.366	Ehrmann *et al.* 1997
Ayrshire	New Zealand	37	-	0.432	0.527	Winkelman and Wickham 1997
	Finland	686	0.001	0.509	0.490	Ikonen 1997
	United Kingdom	29	0-0.003	0.600	0.400	Swaissgood 1992
	USA	45	0	0.720	0.280	Swaissgood 1992
	Red Denmark	169	0.044-0.060	0.710	0.230	Bech *et al.* 1990

However, proline is present in that location in A2 beta-casein, that same segment is either not separated at all or the separation occurs at a very low rate. The 7 amino acid segment that is separated from A1 beta casein is known as beta-casomorphin-7, often abbreviated as BCM-7 (Kostyra et al., 2004).

Effect of BCM-7 on human health

The A1 beta-casein protein derived BCM-7 can affect many opioid receptors in the endocrine, nervous and immune system. Infants are having more chance of absorption of BCM-7 through their comparatively immature gastro-intestinal tract than the adults who are having a chance of showing local reaction in the intestine. BCM -7 is also considered to be an oxidant of Low Density Lipoprotein (LDL) which may have some role in formation of arterial plaque. It is reported that BCM-7 may function as an immunosuppressant and impair tolerance to dietary antigens in the gut immune system which may contribute to the onset of Type 1 diabetes. BCM-7 has been implicated as a potential etiological factor in Type 1 diabetes mellitus, coronary heart disease, arteriosclerosis, sudden infant death Syndrome and also related with some neurological conditions such as autism or schizophrenia. A2 beta-casein has not been implicated for these conditions (Pattanayak, 2013). As a result the A2 Corporation was setup in New Zealand during 1990s to genotype cattle and market the milk with A2 beta-casein at premium price.

Prevalence of A1 and A2 allele in bovine

A2 beta-casein is found in most of the Western,

Table 2: Allelic frequency of Beta casein gene in zebu cattle (Mishra et al., 2009)

Cattle breed	Type of breed	Sample no. (N)	Allelic frequency	
			Al	A2
Kangayam	Draught	48	0	1
Nimari	Draught	45	0	1
Red Kandhari	Draught	39	0	1
Malnad Gidda	Draught	47	0.096	0.904
Kherigarh	Draught	23	0.109	0.891
Malvi	Draught	44	0	1
Amritmahal	Draught	37	0	1
Kankrej	Milch	32	0	1
Gir	Milch	45	0	1
Sahiwal	Milch	47	0	1
Hariana	Dual	48	0	1
Tharparkar	Dual	44	0	1
Rathi	Milch	46	0	1
Mewati	Dual	40	0	1
Red Sindhi	Milch	33	0	1
Mean		**618**	**0.013**	**10.987**

Table No. 3: Allelic frequency of Beta casein gene in buffaloes (Mishra et al., 2009)

Cattle breed	Sample no. (N)	Allelic frequency	
		Al	A2
Murrah	22	0	1
Mehsana	49	0	1
Marathwada	40	0	1
South Kanara	10	0	1
Mainpur	40	0	1
Assamese swamp	40	0	1
Nilli Ravi	22	0	1
Pandharpuri	8	0	1
Total	231		

African and Indian cattle and water buffaloes. A1 beta-casein is carried only by the cows of European breeds especially Holstein (Bos taurus) as mentioned in table -1 (Kaminski et al., 2007). Jersey has an A2 allele frequency somewhat higher than Holstein. But some Jersey cows carry one 'B' beta-casein allele which can release BCM-7 far more (Pattanayak, 2013). Beta-casein allele frequency in indigenous Indian cattle (Bos indicus) and buffalo breeds reported to have 99 to 100% presence of the A2 /A2 genotype (Mishra et al., 2009) and A1 /A1 genotype is absent among them as mentioned in table- 2 and 3. So, most of the indigenous cattle and buffalo breeds are homozygous for the A2 beta-casein allele and good for health.

Conclusion

The role of different variants of beta-casein on human health is a matter of concern worldwide. The status of A1/A2 beta-casein variants in *Bos taurus* cattle breeds from different countries has shown presence of A1 allele in European cattle. The data of beta-casein variant in zebu/Indian/*Bos Indicus* is not much more available, however, a fewer investigations indicate presence of mostly A2 allele in *Bos indicus* that is safer for the health. Hence, we can conclude that the milk of our native cows and buffaloes is far safer than the milk of European breeds.

References:

Eigel WN, Butler JE, Ernstrom CA, Farrell HM, Halwarkar VR, Jenness R and Whitney RM, (1984). Nomenclature of proteins of cow's milk: fifth revision. *J Dairy Sci*, **67**: 1599-1631.

Farrell HM Jr, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, et al. (2004). Nomenclature of the proteins of cows' milk-sixth revision. *J Dairy Sci*, **87**: 1641-1674.

Kaminski S, Cieoelinska A and Kostyra E. (2007). Polymorphism of bovine beta-casein and its potential effect on human health. *J Appl Genet*, **48**: 189-198.

Kostyra E,Sienkiewicz-Szapka E, Jarmoowska B, Krawczuk S and Kostra H. (2004). Opioid peptides derived from milk proteins. Polish *J Nutrit. Sci*, **13/54**:25-35.

Martien AM, Groenen J and van der Poel J. (1994). Regulation of expression of milk protein genes: a review. *Livest Prod Sci*, **38**: 61-78.

McLachlan CN. (2001). Beta-casein A1, ischaemic heart disease mortality, and other illnesses. *Med Hypotheses*, **56**: 262-272.

Miller MJS, Witherly SA and Clark DA. (1990). Casein: a milk protein with diverse biologic consequences. *Proc Soc Exp Biol Med*, **195**: 143-159.

Mishra BP, Mukesh M, Prakash B, Sodhi M, Kapila R, Kishore A, Kataria RR, Joshi BK, Bhasin V, Rasool TJ and Bhujarbaruah KM. (2009). Status of milk protein, β-casein variants among Indian milch animals. *Ind J Anim. Sci*, **79**: 722-725.

Niki R, Kim GY, Kimura T, Takahashi K, Koyama K, Nishinari K. (1994). Physical properties and microstructure of rennet gels from casein micelles of different sizes. *Milchwissenschaft*, **49**: 325-329.

Ostersen S, Foldaber J, Hermansen JE. (1997). Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. *J Dairy Res*, **64**: 207-219.

Pattanayak S. (2013). Human health impact of bovine Beta-casomorphin-7 of milk - a question for milk products or our breeding policy ? Explor. *Anim. Med. Res*, **3**: 93-94.

Rijnkels M. (2002). Multispecies comparison of the casein gene loci and evolution of casein gene family. *J Mammary Gland Biol Neoplasia*, **7**: 327-345.

Roginski H. (2003). Encyclopedia of dairy sciences. Academic Press, London.

A step forward in the treatment of Mastitis

COBACTAN® LC
(Intramammary)

Control Measures for Mastitis

- Wash the hands with soap and water before hand milking.
- Clean the udder with antiseptic solution before & after milking.
- Use full-hand milking instead of knuckling.
- Allow animals to stand for 30 minutes after milking by providing feed or grass.
- Identify the chronic mastitic cow and milk them at last.

Advantages of Using Cobactan LC in Early Stages:

- Stops the Progression of Mastitis.
- Faster Recovery.
- Symptoms disappear quickly.
- Quick return to normal Milk production.

Withdrawal Period:
Milk- 84 hrs. (7 milking)
Meat- 2 days

Administration of Cobactan LC

Infuse COBACTAN® LC (Intramammary)

At 0 hr. 1st tube — At 12 hr. 2nd tube — At 24 hr. 3rd tube

The Blue Cross Book July 2015 Vol. 32 : 116-117

Studies on biochemical parameters in Leptospira infected cattle

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Abstract:

The bovine serum samples were collected from suspected cases of Leptospirosis from the state of Andhra Pradesh. The serum samples were subjected for seroepidemiological study using MAT (Microscopic agglutination test). The serum samples which were positive on MAT were further subjected for estimation of total bilirubin, SGOT and SGPT. The results of the study revealed the elevated levels of total bilirubin SGPT and SGOT indicating hepatocellular damage.

Key words: Leptospira-Microscopic agglutination test-total bilirubin-SGOT-SGPT-hepatocellular damage.

Introduction:

Leptospirosis is a world wide spread zoonotic disease affecting wide variety of animals and humans. In cattle, economic losses are due to reproductive wastage in the form of abortions, still births, reproductive failures and decreased milk yield (Radostitis et al 2000). The present work was aimed to study the involvement of liver damage through sensitive serum biochemical parameters in leptospira infected cattle.

Material and methods:

A total of 120 sera samples from different places of Andhra Pradesh state were collected from clinically suspected cases of bovines, showing pyrexia, abortions and haemoglobineuria. The serum samples with positive titers on MAT were divided into three groups and analyzed for biochemical parameters like estimation of total bilirubin, SGOT and SGPT using kits from M/S Span diagnostics Pvt. Ltd. India.

Results and Discussion:

Bovine sera samples positive for leptospiral antibodies on MAT were randomly selected and subjected to biochemical analysis. Total bilirubin, SGOT and SGPT were estimated on MAT positive serum samples. Elevated levels of total bilirubin (>0.50mg/dl) SGPT (>40 IU/L) and SGOT (>132 IU/L) were observed in the sera samples tested. The results of the study are shown in the table-I. The increased values in biochemical parameters studied could be due to the liver damage. Similar findings of elevated levels of SGOT and SGPT were also reported by Benzamin (1985) and Chauhan (2003) respectively and concluded that the degeneration and necrosis of the liver is one of the pathognomic changes in leptospirosis. Ananda et al (2008) obseved the elevated levels of SGPT (190IU/L) and SGOT (140IU/L) in case of dogs infected with leptospirosis.

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Biochemical analysis of MAT positive sera samples (cattle).

S. No	No. of samples tested	MAT Titers	Total bilirubin mg/dl > 50mg/dl	SGPT (IU/L) >40 IU/L	SGOT (IU /L) >132 IU/L
1	56	80	0.62-0.64	48-52.8	140-146.0
2	48	160	0.58-0.61	45-47.5	137-140.0
3	16	320	0.55-0.57	42.8-44.9	134-136.5

Conclusion :

The results of the study concluded that the increase in the levels of serum total bilirubin, SGPT and SGOT is a second level of confirmation for the MAT in the detection of Leptospirosis in bovines.

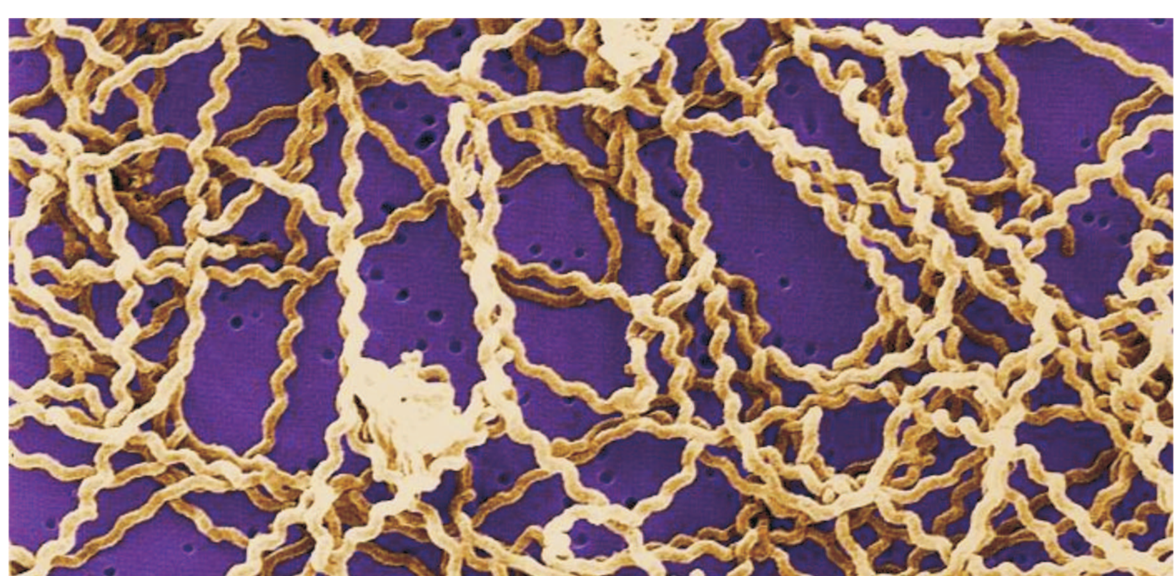
References:

Ananda KJ, Suryanarayana T, Praturst PR, Sharada R and Placid D'Souza (2008) Diagnosis and treatment of leptospirosis in dogs - A case report Veterinary World **9**, 278-279.

Benzamin M (1985) out line of Veterinary clinical pathology third edition. Kalyani publishers, New Delhi.

Chauhan RS (2003) veterinary laboratory diagnosis. International distributing co.,Lucknow, UP, India

Radostitis MO, Gay CC, Blood DG and Hincheliff KW (2000) Veterinary Medicine. A text book of the disease of cattle, sheep, pigs, goats and horses Ninth edition WB Saunders London PP 1228.



Scanning electron micrograph of a number of Leptospira sp. bacteria atop a 0.1 μm polycarbonate filter

The Blue Cross Book July 2015 Vol. 32 : 118-120

Changing pathogenecity of Adenovirus infections in commercial poultry farms

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Abstract:

Adenovirus infection is considered to produce typical histopathological changes in heart, pericardium and liver characterized by mononuclear cell infiltration and/or basophilic intranuclear inclusion bodies. The similar histopathological changes in kidneys, spleen, and intestines are reported, indicating probably the changed tropism and pathogenicity of adenoviruses in avian species.

Key word : Adenovirus, broilers

Introduction:

Adenoviruses are widespread throughout all avian species. Avian adenoviruses in chickens are associated with two important diseases, namely, inclusion body hepatitis (IBH) and hydropericardium syndrome (HP), which may occur singly or as a combined entity. The characteristic lesions include accumulation of straw coloured transudate in pericardium and an enlarged, pale, friable liver. The hystopathology of the affected organs usually reveals myocardial oedema and mild mononuclear cell filtration in the heart and basophilic intranuclear inclusion bodies in the liver. These are considered diagnostic. The present paper demonstrates unusual sites like kidneys, spleen and intestines where histopathological lesions are suggestive of adenovirus infection.

Materials and methods:

Commercial broilers were presented to Omega laboratory for the detailed postmortem examinations from different parts of Maharashtra. Since last three years, 14060 commercial birds were examined for detailed pathology. Amongst these 6842 birds showed mixed type of lesions which were suggestive of adenovirus. Detailed histopathological examination of some representative samples (450) was carried out and recorded.

Observations and results:

After detailed post mortem examination, the following lesions were recorded and analyzed.

Liver : enlargement of liver, pale discoloration, pinpoint hemorrhages, fatty changes, rupture of liver and formation of hematomas within the capsule of liver in some of the cases and reddish bile in gall bladder.

Heart and Pericardium : golden yellow colored fluid in pericardial sac, severe engorgement of epicardial blood vessels, pin point hemorrhages on the epicardium and endocardium,

Kidneys : swollen

Intestine: hemorrhages in distal part of large intestine. Besides this, golden yellow colored fluid was observed in the abdominal cavity which got clotted in many birds after exposure to air.

Spleen : enlargement, pale and red patches, and mottled appearance. was observed.

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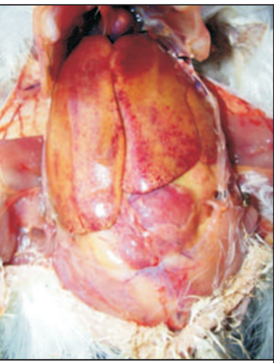
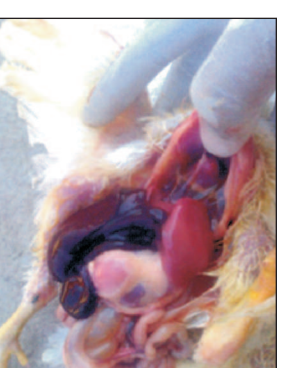
Histopathological examinations of liver reveled swelling of hepatocytes, tiny fatty changes intracellular and intercellar, moderate hemorrhages, infiltration leucocytes. Two types of inclusions bodies were observed in liver viz. intranuclear eosinophilic and intranuclear basophilic. The evidence of intranuclear eosinophilic inclusion bodies was very less i.e only 5 cases out of 450 cases showed eosinophilic inclusion bodies. Rest of the cases i.e. 445 showed basophilic inclusion bodies. Besides these findings, margination of chromatic material was also observed in most of the cases.

Histopathological examination of pericardium and heart revealed presence of basophilic intranuclear inclusion bodies in the epithelial cells of pericardium and epicardium, vascular endothelium of capillaries, moderate necrosis of cardiac muscle fibers, diffuse infiltration of MNC, presence of edematous fluid in the interstitial spaces, some of the capillaries in the epicardium showed presence of partial or complete occlusion of lumen due to thrombus and embolus and these areas were surrounded by leucocytes.

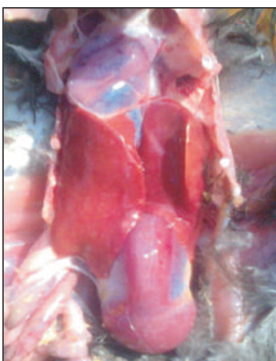
Lecchy heart and liver lesions together



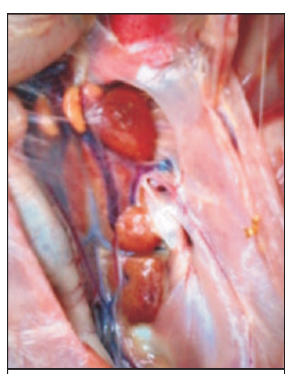
Typical pinpoint hemorrhages on liver and fatty chages



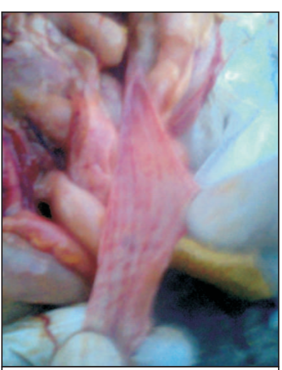
Hematoma, hydropericardium and lesions on liver



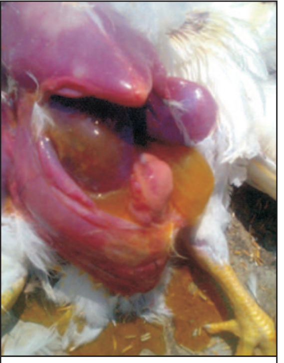
Hemorrhages on epic-ardium and hydroperic-ardium with liver lesions



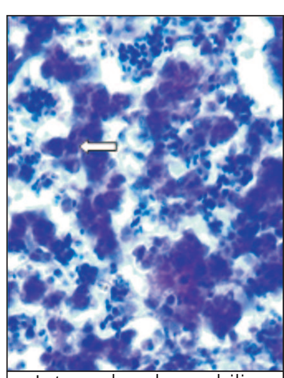
Yellow discoloration and hemorrhages on kidney



Hemorrhages on the terminal part of intestine



Golden yellow fluid in abdominal cavity and pericardium



Intanuclear basophilic inclusion bodies in hepatocytes H& E stain 400X

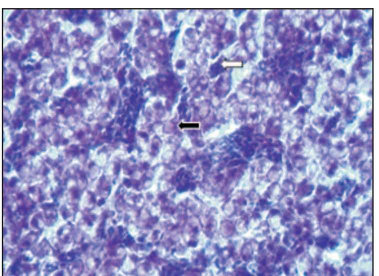
Histopathological examination of kidney revealed presence of intra and inter cellular fatty changes, swelling and degeneration of tubules, infiltration of MNC, few of the epithelial cells showed intranuclear basophilic inclusion bodies.

Histopathology of terminal intestine revealed sloughing and necrosis of enterocytes, hemorrhages, focal MNC in the sub mucosal area.

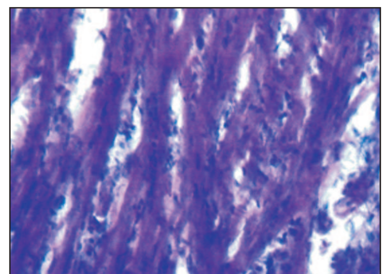
Histopathology of spleen revealed necrotic splenitis, focal hemosiderinosis, aggregations of MNC and hemorrhages.

Conclusions:

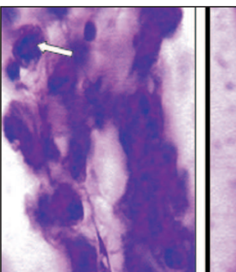
Confirmative histopathology suggests that the adenovirus has changed its pathogenecity as well as tropisms to specific organs. This may be due to changes in the biology of virus, strains subtypes or may be due to entry of wild variety and mutagenesis. Further research is needed to isolate the new field virus, its typing and mutagenesis, if any.



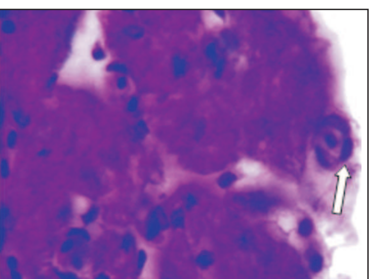
Liver fatty changes, margination of chromatin (black) and intranuclear basophilic inclusion bodies (white arrow) H&E stain 400X



Myocarditis and intercellular edema H&E stain 400X

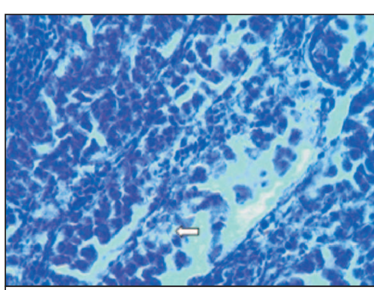


Intranuclear inclusion bodies in pericardial epithelium 1000X H& E stain



Vascular endothelial cells of capillary in epicardium showed intranuclear basophilic inclusion bodies H&E stain 400X

Golden yellow fluid in the abdominal cavity



Kidney tubules epithelial cells with tiny fatty droplets (White arrow) H&E stain 400X

The Blue Cross Book July 2015 Vol. 32 : 121-122

Toxocariosis - Pet's enemy

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Abstract

Toxocariosis is one of the important helminthic disease affecting dogs and cats. Adult worms are usually found in the small intestine and shed large number of unembryonated eggs in the faeces. Affected pups show poor growth, loss of condition and pot belly appearance. Worms may be passed in the faeces or vomitus. Sometimes, the entire litter can succumb to the infection. In cats, infected kittens show less symptoms than puppies. Anthelmintics such as pyrantel pamoate, praziquantel are found to be effective in puppies and kittens. Generally for adult dogs and cats, drugs such as Fenbendazole, Levamisole and Albendazole may be effective. Thorough cleaning of kennels and maintaining kennels dry is very important for effective control of ascarids

Key Words : *Toxocara canis*, *Toxocara cati*, Pyrental pamoate

Pet animals like dogs and cats are owned by animal lovers for various benefits. The primary reason is companionship, more than just being a friend. Keeping a pet at home can help a stress free life, a source of exercise, better social skills and providing safety. These animals are exposed to various diseases due to microbes, parasites and other causes. Parasites play an important role in the well being of the pet animals particularly the puppies and kittens. Among the parasites, various helminths (worms) are found to affect dogs and cats and one such important helminth is *Toxocara*. Species of *Toxocara* in dogs and cats include *Toxocara canis* and *Toxocara cati*.

Distribution

T. canis and *T. cati* eggs are found in the soil worldwide. The eggs of these parasites occur in 2-90% of soil samples as reported in various countries. The high ambient temperature and humidity of the tropics helps in the dissemination of infection by *Toxocara* species.

Transmission

The life cycle stages of *Toxocara sp*. in dogs and cats include; unembryonated eggs excreted in the faeces, infective embryonated eggs containing second and third stage larvae. Usually this stage is present after the eggs develop for at least one to two weeks in the environment. The other stages include, the immature larvae which migrate and wander through the tissues of dogs and cats, dormant immature larvae in various tissues and the adult worms in the intestine.

Tococara canis

Dogs and other canids are the definitive hosts for *T. canis.* Adult worms are usually found in the small intestine and shed large number of unembryonated eggs in the faeces. The eggs become embryonated in the environment. Larvae within the egg do not develop at temperature below 10^{0}C. Eggs containing L2 stage larva is infective. When a dog ingests the egg with L2, the larvae hatch in the intestine. In pups less than 4-5 weeks old, the larvae penetrate the intestinal wall and are carried to the lungs via the blood stream, where they enter the alveoli and migrate up the bronchioles, bronchi and trachea, reaching the pharynx from where the larvae are swallowed. The larvae reach the intestine and develop into eggs releasing adults. In heavy infection, immature larvae can also be found occasionally in the faeces. Adult *T. canis* have a life span of approximately 4 months in the intestine and most of the parasites are expelled within six months of infection. In

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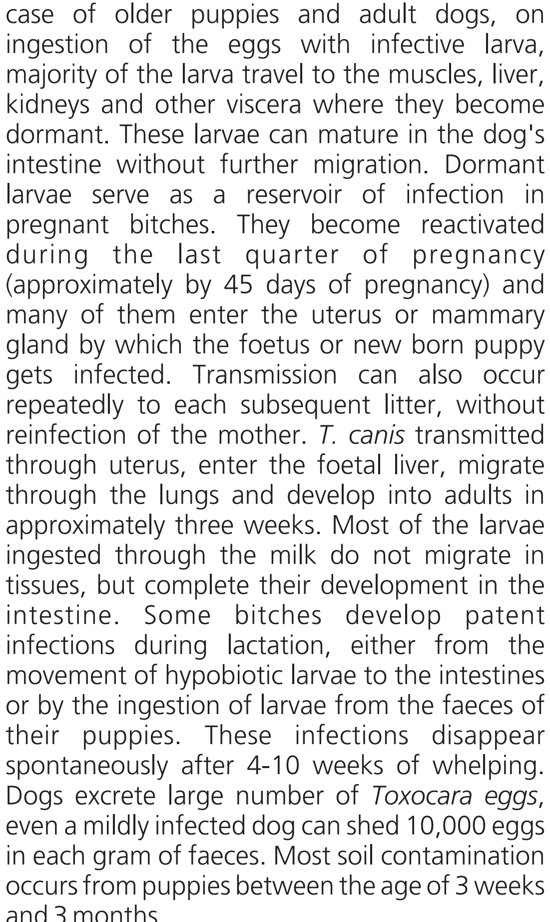


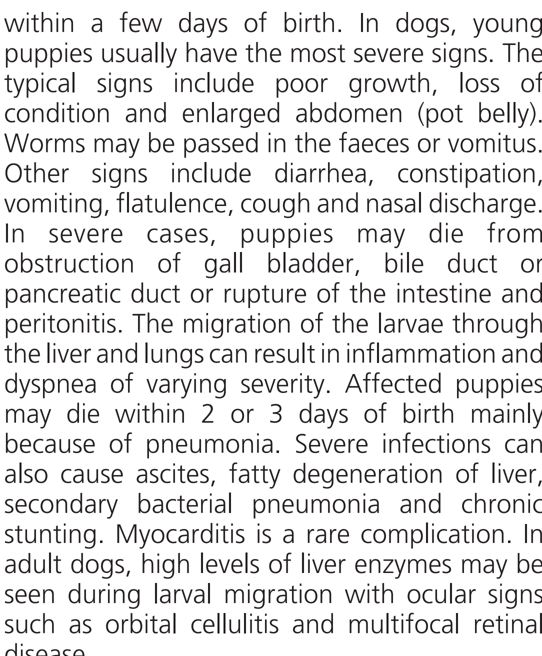
case of older puppies and adult dogs, on ingestion of the eggs with infective larva, majority of the larva travel to the muscles, liver, kidneys and other viscera where they become dormant. These larvae can mature in the dog's intestine without further migration. Dormant larvae serve as a reservoir of infection in pregnant bitches. They become reactivated during the last quarter of pregnancy (approximately by 45 days of pregnancy) and many of them enter the uterus or mammary gland by which the foetus or new born puppy gets infected. Transmission can also occur repeatedly to each subsequent litter, without reinfection of the mother. *T. canis* transmitted through uterus, enter the foetal liver, migrate through the lungs and develop into adults in approximately three weeks. Most of the larvae ingested through the milk do not migrate in tissues, but complete their development in the intestine. Some bitches develop patent infections during lactation, either from the movement of hypobiotic larvae to the intestines or by the ingestion of larvae from the faeces of their puppies. These infections disappear spontaneously after 4-10 weeks of whelping. Dogs excrete large number of *Toxocara eggs*, even a mildly infected dog can shed 10,000 eggs in each gram of faeces. Most soil contamination occurs from puppies between the age of 3 weeks and 3 months.

Tococara cati

Cats are the definitive hosts for *T. cati.* The transmission and life cycle of *T. cati* is thought to be similar to that of *T. canis;* however, *T. cati* is not transmitted through uterus or placenta and kittens are infected only through milk or colostrum. Hypobiotic larvae is not transmitted through lactogenic route. Adult cats can develop patent infections after ingesting either eggs or larvae. In cats, hypobiotic *T. cati* larvae are found mainly in the muscles. Cats shed *Toxocara cati* between the age of 2 and 6 months.

Clinical signs

Puppies infected via uterus can develop enteric signs within first 2-3 weeks. Pneumonia and other symptoms of tissue migration can appear



within a few days of birth. In dogs, young puppies usually have the most severe signs. The typical signs include poor growth, loss of condition and enlarged abdomen (pot belly). Worms may be passed in the faeces or vomitus. Other signs include diarrhea, constipation, vomiting, flatulence, cough and nasal discharge. In severe cases, puppies may die from obstruction of gall bladder, bile duct or pancreatic duct or rupture of the intestine and peritonitis. The migration of the larvae through the liver and lungs can result in inflammation and dyspnea of varying severity. Affected puppies may die within 2 or 3 days of birth mainly because of pneumonia. Severe infections can also cause ascites, fatty degeneration of liver, secondary bacterial pneumonia and chronic stunting. Myocarditis is a rare complication. In adult dogs, high levels of liver enzymes may be seen during larval migration with ocular signs such as orbital cellulitis and multifocal retinal disease.

In cats, infected kittens show less symptoms than puppies. Many infections in kittens are asymptomatic. In heavy infections, the clinical signs may include abdominal distension, rough coat, diarrhea with dehydration. In adult, the primary lesions may be eosinophilic pulmonary endarteritis and medial hyperplasia of the pulmonary arteries due to migration of larvae.

Treatment and Control

Anthelmintics such as pyrantel pamoate, praziquantel are found to be effective in puppies and kittens. Generally for adult dogs and cats, drugs such as Fenbendazole, Levamisole and Albendazole may be effective. With regard to control, puppies and kittens should be dewormed to eliminate the shedding of eggs. Removal of faeces and thorough cleaning of kennels is important. The kennel should be kept as dry as possible. Contamination in public places can be decreased by restricting uncontrolled dogs and cats, particularly in playgrounds and parks. There is no practical method available to remove *Toxocara eggs* from the soil once contamination has occurred.

The Blue Cross Book July 2015 Vol. 32 : 123-127

Zoonotic Diseases of Sheep and Goat

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Abstract :

Most of the zoonoses diagnosed in goats and sheep are transmitted by close contact of human being with sheep and goats and also by the transmitting vectors present in the environment. Occupational diseases mainly affect the breeders, veterinarians and slaughter house workers. Raw goat milk, undercooked meat products of goat and sheep can also transmit the disease. Some of the zoonotic agents such as *B melittensis, Chlamydia abortus, Coxiella burnetti* under natural condition can be transmitted from animals to human being. Zoonotic disease transmission impacts greater on human health as well as productivity and also decreases animal origin food production.

Key words : zoonoses, sheep, Goat

Introduction :

Zoonoses are the infections and diseases those are naturally shared by vertebrate animals and humans. The definition is based on assumptions: that the diseases are shared by man and vertebrate animals through bidirectional movement of disease under natural conditions (WHO/SDE/FOS/2006.1).

Sheep and goat rearing is the backbone of the economy of small and landless farmers in India. They have distinct social, economical, managerial and biological advantages over other livestock species. They significantly contribute to the agrarian economy and play a very vital role in the livelihood security of the small and marginal farmers and landless especially in arid, semi–arid and mountainous regions of the country. Goats have served the mankind earlier and longer than cattle and sheep. Goat milk is prescribed for children, old and sick as it is easily digestible and has medicinal value, Goat meat (chevon) is preferred over other meats because it is leaner and there are no religious taboos against its consumption. A number of rural-based industries use wool and sheep skins as raw material. Sheep in India are mostly maintained on natural vegetation on common grazing lands, wastelands and uncultivated (fallow) lands, stubbles of cultivated crops and top feeds (tree loppings). Rarely are they kept on grain, cultivated fodder or crop residue. According to the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) at least 61% of all human pathogens are zoonotic and about 75% of all emerging pathogens that have affected humans over the past 10 years have been caused by pathogens originating from an animal or from products of animal origin. (WHO, Control of neglected zoonotic disease, 2005)

The important zoonotic diseases from sheep and goat are tabulate in this paper.

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Disease	Causative agent (Disease)	Clinical signs In goats and sheep	Signs in humans	Route of transmission
Brucellosis	*Brucella mellitensis*	Abortions, stillbirths, epididymitis, Orchitis, arthritis	Undulant fever, fatigue, headache, sweats, arthralgia, chills, malaise, weight loss, myalgia, abortion	Oral, respiratory, conjunctival, cutaneous
Listeriosis	*Listeria monocytogenes*	Abortions, meningo-encephalitis, mastitis, septicaemia in lambs	Septicemia, meningitis, abortions	Oral
Anthrax	*Bacillus anthracis*	Cerebral anoxia, pulmonary edema, fever, lameness, edema of the pharynx, dyspea, death	Skin lesions on hands, arms, face or neck, malignant edema, septicemia, airway obstruction, meningitis, toxemic shock, diarrhea, vomiting, abdominal pain, death	Cutaneous, respiratory, oral, close contact with animals and animal products
Vibriosis	*Campylobacter jejuni*	Asymptomatic infection diarrhea, fever, arthritis in kids	Diarrhea, vomiting, abdominal pain, fever, headaches, abortions, endocarditis	oral
Salmonellosis	*Salmonella spp*	Asymptomatic infection, abortions. In kids: diarrhea, fever, arthritis	Diarrhea, vomiting, abdominal pain, fever, headaches	oral
Food poisoining	*Staphylococcus aureus*	Mastitis	Severe vomiting, abdominal pain, diarrhea, headaches resolves spontaneously	Contaminated, oral milk
Pasturellosis	*Pasteurella multocida Mannheimia haemolytica*	Pneumonitis, septicemia and death in kids	Pneumonitis, abscess	Respiratory, cutaneous
Erysepeles	*Erysipelothrix rhusiopathiae*	Arthritis	Erythema, arthritis, endocarditis and sepsis	Cutaneous
Tuberculosis	*Mycobacterium bovis M. caprae*	Lower respiratory tract disease, lymphadenitis	Cough, unexplained weight loss, fatigue, night sweats, chills, loss of appetite, lymphadenitis	Oral, close contact respiratory tract
Caseous lymphadenitis) (CL)	*Corynobacterium pseudotuber-culosis*	Abscesses behind the ears, beneath the jaw, progressive weight loss	Painful skin wounds with pus and dead tissue	Direct skin contact with pus
Johne's disease (Paratuberculosis)	*Mycobacterium avium subsp. paratuberculosis*	Bottle jaw condition, weight loss, progressive pneumonia	Diarrohea, weight loss, Crohn's disease	Eating or drinking raw milk, uncooked meat, unpasteurized dairy products

MSD
Animal Health

Disease	Causative agent (Disease)	Clinical signs In goats and sheep	Signs in humans	Route of transmission
Encephalitis (tick borne)	*Flavivirus*	Inflammation of brain called as spring –summer encephalitis	Inflammation of brain	Tick bite (Ixodes ricinus and I. persulcatus)
Orf Sore mouth/ Contagious ecthyma	*Parapoxvirus* *Rift Valley Fever*	Skin lesion on lips, gingiva, tongue, labia, nostrils and toes, starvation	Skin lesions on fingers, lips	Close contact with animals and fomites
Rift Valley Fever	*virus Family Bunyaviridae, Genus Phlebovirus*	Asymptomatic infection, abortions, fetal malformations, hepatitis, fever, conjunctivitis, nasal discharge, hepatitis and death	fever, headache, muscular pain, nausea, photophobia, hemorrhagic fever with jaundice and death (0.5–2%)	Mosquitoes, close contact, oral
Rabies	*Rhabdoviridae, Genus Lyssavirus*	Change in behavior, apprehension, aggressiveness, hyper-excitability, irritability, hypersalivation, nervousness, solitude, anorexia, change in voice, paralysis, death	Fever, fatigue, headache, vomiting, decrease of appetite, sleepiness, partial paralysis, hypersalivation, agitation,	Cutaneous: bite by an infected animal
Toxoplasmosis	*Toxoplasma gondii*	Abortion, asymptomatic infection	Asymptomatic infection, abortion, congenital infection	Oral, close contact
Cryptosporidiosis	*Cryptosporidium*	fever, diarrhea, and stomach cramps	fever, watery diarrhea, and stomach cramps, vomiting	Oral route by swallowing of contaminated food and faecal matter
Giardiasis	*Giardia spp*	diarrhea, gas or flatulence, greasy stools that tend to float, stomach cramps, or upset stomach or nausea	Intestinal problem like diarrhoea	Oral (drinking water)
Q fever (Queens land fever)	*Coxiella burnetti*	Abortions, stillbirths, asymptomatic infections, pneumonitis	Fever, flu-like syndrome, pneumonitis, hepatitis, endocarditis, fatigue syndrome, abortion	Respiratory, cutaneous, Oral (±), ticks
Chlamydiosis	*Chlamydia abortus*	Abortions, stillbirths, asymptomatic infections, epididymitis,	Flu-like syndrome, pneumonitis, abortion	Close contact, respiratory, cutaneous

Disease	Causative agent (Disease)	Clinical signs In goats and sheep	Signs in humans	Route of transmission
		pneumonitis, conjunctivitis		Direct contact
Ring worm infection	*Dermatophytes (fungi) Microsporum spp Trichophyton spp*	Pimple sore on the skin lesions,ring shaped pink lesions	Pimple sore on the skin lesions, ring shaped pink lesions	Close contact

It is very much clear that the prevention and control of disease status can be effected by breaking of transmission of disease from animal to human. Several attempts have been taken by WHO and CDC for the disease control and prevention. In general following measures help in prevention of disease transmission and control (Smits and Kadri, 2005; Singh *et al.,* 2010; Rodolakis, 2014; Gandham, 2014)

1. Immunization, environmental hygiene, chemoprophylaxis in animals
2. Early diagnosis of disease
3. Quarantine of diseased animal
4. Vector and reservoir control
5. Epidemiological diagnosis
6. Establishment of surveillance
7. Enrollment of individuals in occupational health and safety programme.
8. Knowledge about the multifactorial causation of disease
9. Education of people about the disease prevention
10. Genetic improvement of the animal with reference to disease resistance

As farmers, veterinarians and other professionals in Animal Industry are directly dealing with the animals health education is necessary. Awareness program should be undertaken across India for the prevention and control strategy of disease transmission from sheep and goat. Regular surveillance of the animal health on farms/herds should be mandatory.

References

Arricau-Bouvery, N. and Rodolakis, A. (2005). Is Q fever an emerging or re-emerging zoonosis? *Vet. Res.* 36, 327–349

Balkhy, H.H., and Memish Z.A., (2003). Rift Valley Fever an uninvited zoonosis in the Arabian peninsula. *Int. J. Antimicrob. Agents* **21**, 153–157

Bouloy, P.M., Bird, M., Kemp B.H. and Paweska, A. (2010). Rift Valley fever virus (Bunyaviridae, phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Vet. Res.***41**, 61–101.

Gandham P. (2014). Zoonotic diseases with special reference to India, *Inter. J. Basic Appl. Med. Sci.* **4** : 73-87

Goelz J. L. (2002), Sheep Diseases That Can Infect Humans, *Inter. Sheep Lett.* **22**: 8

Rodolakis A. (2014). Zoonoses in goats: How to control them, Small Ruminant Research, G Model RUMIN-4674; No. of Pages 9, http://dx.doi.org/10.1016/j.smallrumres.2014.01.007

Singh, B.B., Sharma, R., Sharma J.K. and Juyal P.D. (2010). Parasitic zoonoses in India: an Overview, *Rev. sci. tech. Off. int. Epiz.* **29**, 629-637

Smits H.L. and Kadri S.M. (2005). Brucellosis in India: a deceptive infectious disease, Ind. J Med. Res. **122**: 375-384

Summary of comments and discussion from the FAO/WHO/OIE electronic conference (2003), FAO, Rome Veterinary public health and control of zoonoses in Developing Countries

Tirumalai P.S. (2013), Listeriosis and Listeria monocytogenes in India, Wudpecker J. Food Tech. **1**: 098 - 103

Wolfe, N.D., Dunavan, C.P. and Diamond, J., (2007). Origins of major human infectious diseases. Nature **447**, 279–283

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Effect on Temperature, Respiration and Pulse Rate before and after administration of Bupivacaine epidurally in Camels

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(received 20/04/2015 - accepted 01/06/2015)

Abstract

The present study was carried out at National Research Centre on Camel (Jorbeer-Bikaner) with the dose rate of 0.10, 0.20, 0.30 and 0.40 mg/kg body weight of bupivacaine, at sacro-coccygeal space. There were non significant changes in the values of rectal temperature, respiration rate, pulse rate, which were observed at different intervals. The values were within the physiological range.

Introduction:

The camel, (*Camelus dromedaries*) is an indispensable and valuable desert animal and directly associated with rural economy and life of India's arid and semiarid zones including extensive western part i.e. Thar desert of Rajasthan state. Camel is used for transport, ploughing, loading, riding and in army too, in sandy areas where other animals and automobiles fail to operate. Successful anaesthesia is essential for restraining and painless surgical interventions without endangering the life of patient from the humanitarian and technical point of view (Hall, 1971) , Epidural anaesthesia is simple, quick, inexpensive and requires no sophisticated equipments (Thurmon et al., 1996). It is a convenient method of producing analgesia and local anaesthesia of tail and perineal structures (Robinson and Natalini, 2002), even up to toe of hind feet in camel. It is essential that there are no adverse effect of epidural anaesthesia on the respiration, puls as well as temperature, at a given dose level. The present study was undertaken to evaluate the effects on rectal temperature, respiratory rate and pulse rate before and after administration of Bupivacaine epidurally in camels.

Materials and Methods

The present experimental work was carried out at National Research Centre on Camel Jorbeer (Bikaner) on 4 apparently healthy male camels. The camels were 6 to 10 years of age weighing between 500 Kg. to 650 Kg. Site of administration of epidural anesthesia was selected at sacrococcygeal space. Inj. Bupivacaine was aseptically administered epidurally at the dose rate of 0.10, 0.20, 0.30 and 0.40 mg/kg body weight. In four camels the drugs were used at 4 dose rates and were injected at 7 days interval. The ambient temperature ranged between 38 and 45^{0}C during the period of study.

Restraint of camels

The camels were restrained in the sitting position (sternal recumbency) for administration of anaesthesia by applying rope halters on the fore and hind limbs to tie over neck and back of the animals, respectively (Fig.1).

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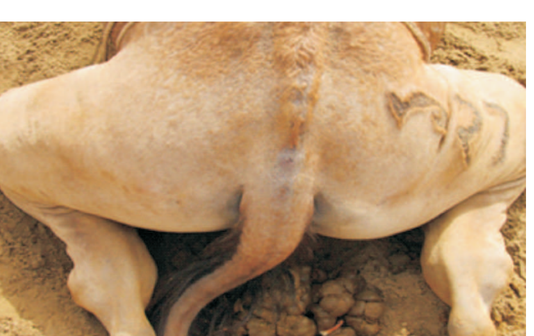

Fig. 1. Restraint on sternal recumbency

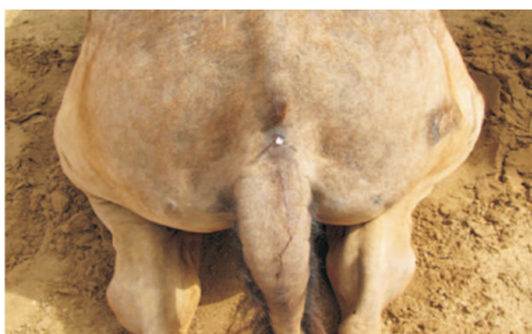

Fig. 2. Site of epidural anaesthesia

Preparation of Site

The proposed site of injection was clipped, shaved and thoroughly washed with soap and water and then scrubbed with savlon 1:3 solution. Tincture of iodine was painted at the scrubbed site. Strict aseptic procedure was adopted during the study. (Fig. 2)

Result and Discussion:

The changes in rectal temperature, pulse rate and respiration rate were found to be non-significant with different dose rates at different time intervals (Table 1 and 2). These findings were in agreement with Skarda and Muir (1981); Gill et al., 1983; Gill et al., 1984; Hussain and Kumar (1988); Rao et al., (1997); Khare and Gahlot (2001). Adetunji et al. (2001) reported non- significant alteration in heart rate, mean arterial pressure, respiration rate and rectal temperature with both epidural lignocaine and bupivacaine in dogs.

Further, in the present study, there was no anaesthetic accident or complication during or after anaesthesia with bupivacaine.

Bibliography:

Adetunji A, Ajadi RA and Opia RE (2002). A comparison of epidural anaesthesia with xylazine, bupivacaine and bupivacine-xylazine mixture in West African dwarf goats. Isreal, J. Vet. Med. **57**: 76-81.

Gill SS, Pandey SK and Chandrapuria VP (1983). Bupivacaine hydrochloride as an epidural anaesthesia in dog. Indian *J. Anim. Sci.* **53**: 998-1001.

Gill SS, Pandey SK and Chandrapuria VP (1984). Bupivacaine hydrochloride as an epidural analgesic in cattle. *Indian Vet. J.,* **61**: 758-761.

Hall LW (1971). Wright's Veterinary Anaesthesia and Analgesia. 7th Edn. Bailere Tindall, London. pp. 01.

Hussain SS and Kumar A (1988). Bupivacaine hydrochloride for epidural anaesthesia in buffaloes. *Indian J. Vet. Surg*., **9**: 12-17.

Khare S and Gahlot TK (2001). Experimental study on subarachnoid anaesthesia in buffalo calves. *Indian Vet. J.* **78**: 438-439.

Macintosh RR and Smith BR (1962). Local analgesia. Abdominal surgery B & S Livingston Limited, Edinburgh and London.

Rao MR, Lakshmipathi GV, Makkena S and Sreenu M (1997). Evaluation of epidural analgesia of 0.5% bupivacaine alone and with different concentration of amyl alcohal in dogs. *Indian J. Vet. Surg.* **18**: 29.

Robinson EP and Natalini CC (2002). Epidural anaesthesia and analgesia in horses. *Vet. Clin. Amer. Equine Pract.* **18** : 61- 82.

Thurmon JC, Tranquilli WJ and Benson GJ (1996). Lumb and Johnes Veterinary Anaesthesia. 3rd ed. Williams and Wilkins, Bltimore. pp. 479-514.

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Table 1 : Effect of different dose levels (0.10 and 0.20 mg/kg) of bupivacaine on temperature, pulse and respiration at different time intervals.

Recording Time Time intervals	Bupivacaine dose rates (mg/kg body weight)					
	0.10			0.20		
	Temp. °F	Pulse no/min	Respiration no/min	Temp. °F	Pulse no/min	Respiration no/min
Before administration of anaesthesia	99.85 ± 0.28[a]	29.00 ± 0.40[a]	9.50 ± 0.28[a]	99.20 ± 0.27[a]	29.75 ± 0.47[a]	10.75 ± 0.25[a]
After onset of anaesthesia	99.80 ± 0.27[a]	29.00 ± 0.70[a]	9.50 ± 0.64[a]	99.35 ± 0.22[ab]	29.75 ± 0.47[a]	10.75 ± 0.25[a]
During anaesthesia	99.90 ± 0.17[a]	29.25 ± 0.47[a]	9.00 ± 0.57[a]	99.15 ± 0.09[a]	29.75 ± 0.75[a]	10.75 ± 0.47[a]
On start of recovery	99.85 ± 0.28[a]	29.50 ± 0.28[a]	9.25 ± 0.47[a]	99.30 ± 0.19[ab]	30.25 ± 0.47[a]	10.75 ± 0.47[a]
On completion of recovery	100.00 ± 0.38[b]	29.00 ± 0.40[a]	9.25 ± 0.25[a]	99.60 ± 0.11[b]	30.00 ± 0.40[a]	10.75 ± 0.47[a]

Table 2 : Effect of different dose levels (0.30 and 0.40 mg/kg) of bupivacaine on temperature, pulse and respiration at different time intervals.

Recording Time Time intervals	Bupivacaine dose rates (mg/kg body weight)					
	0.30			0.40		
	Temp. °F	Pulse no/min	Respiration no/min	Temp. °F	Pulse no/min	Respiration no/min
Before administration of anaesthesia	99.20 ± 0.27[a]	29.50 ± 0.28[a]	10.50 ± 0.28[a]	99.50 ± 0.28[a]	99.50 ± 0.288[a]	9.75 ± 0.47[a]
After onset of anaesthesia	99.30 ± 0.23[a]	30.00 ± 0.00[a]	10.75 ± 0.47[a]	99.55 ± 0.25[a]	99.55 ± 0.250[a]	10.25 ± 0.25[a]
During anaesthesia	99.35 ± 0.15[a]	30.00 ± 0.40[a]	10.75 ± 0.47[a]	99.75 ± 0.09[a]	99.75 ± 0.095[a]	10.25 ± 0.47[a]
On start of recovery	99.65 ± 0.05[a]	29.50 ± 0.50[a]	10.75 ± 0.25[a]	99.75 ± 0.05[a]	99.75 ± 0.050[a]	10.25 ± 0.47[a]
On completion of recovery	100.50 ± 0.10[a]	29.50 ± 0.28[a]	10.75 ± 0.25[a]	100.70 ± 0.10[b]	100.70 ± 0.100[b]	10.25 ± 0.25[a]

Similar superscripts indicate non-significant difference.

ICAR signs MoU for advancing livestock production, health and welfare, research and Veterinary education in India

New Delhi - February 20, 2015 - Indian council of Agril. Research (ICAR) and International Center of University of Edinburgh (Edinburgh, United Kingdom), signed a Memorandum of Understanding at a workshop held on 16th/17th February 2015, to establish and strengthen a long and fruitful relationship to benefit livestock production in India.

These knowledge exchange activities are planned to initiate research collaborations to use new technologies to improve livestock resistance to diseases, enhance sustainable livestock productivity and improve livestock welfare. The MoU further shall consider how through the international partnership, Indian Veterinary and Animal Science education and training can be strengthened to provide well qualified and skilled researchers and Veterinarians needed to serve the changing needs of the livestock sector within India.

Source : ICAR reporter

Camel milk introduced for customers

Bikaner - January, 2015 - The National Research Center on Camel (NRCC) took initiative in establishing a "Camel Milk Promotion Point" in Bicaner city to create awareness among general public regarding functional health food benefits of Camel milk in human health problems like diabetes (type 1) autism, hypertension, milk allergy in children, non-alcoholic liver disorders, TB, asthma etc.

Another important aspect of this initiative is to project camel milk sale as an economic activity for the camel herders, who are not using camel milk to gain commercial benefits. It is expected that this activity of NRCC shall promote the camel herders to unite and form self -help groups who can decide taking a 'Camel Dairy' as a vocation.

The NRCC shall further educate camel milk producers to understand the concept of clean milk production and provide training on pasteurization and storage of camel milk to ensure availability of clean camel milk to the customers.

Source : NRCC, Bikaner

ICAR - Central Institute for Research on Cattle
Meerut Cantt. - 250 001 (UP)

Realizing the demanding need of global Dairy industry, the Central Institute for Research on Cattle, Meerut, the premier research organization of ICAR, was established and is working to develop its R&D programmes for development of new products and practices for the benefit of millions of farmers by improving cattle productivity and thereby to better serve the nation in terms of food and nutritional security, and economic prosperity. It was originally started as Project Directorate on Cattle (PDC), a nodal institution to monitor, co-ordinate and support all research and development projects for cattle improvement on 3rd November 1987 at Military Farms School & Research Centre, Meerut, by upgrading the status of All-India Coordinated Research Project (AICRP) on Cattle. For further intensification and coverage of wide spectrum of cattle research and development, the existing Directorate has been upgraded to Central Institute for Research on Cattle (CIRC) in 2014. Since its establishment, the CIRC has worked progressively to attain its vision through coordinated breeding programmes and supply of elite male germplasm to organizations and livestock farmers. The various activities of CIRC are now organized to meet the basic objectives viz.,

1. To undertake research in the field of cattle breeding, feeding, management and reproduction to enhance productivity and profitability.
2. To plan, coordinate and monitor the

research projects on cattle.

3. To serve as national data repository and provide consultancy for cattle production and reproduction.

Research Achievements by the Institute

Initially, the institute was conceived to take advantage of the achievements made in the AICRP and other crossbreeding experiments at organized farms and under field conditions for evolution of a national milch breed (Frieswal Project) from a reasonably large crossbred base (Sahiwal x Holstein-Friesian), which was a limiting factor under AICRP on Cattle. The Frieswal project envisages evolving a National Milch Breed "Frieswal", a Holstein- Sahiwal cross, yielding 4000 kg of milk with 4% butter fat in a mature lactation of 300 days. Under the project, 300-days and total lactation milk yield of Frieswal cows increased from 3223 and 3274 kg in 2006 to 3262.90 and 3307.09 kg in 2013-14. The herd strength of Frieswal females also increased from 2305 in 1989 to 18047 including 10293 adult cows, 5987 young stocks and 1767 calves at the end of 31st December 2013. The number of elite cows also increased to 951 compared to 685 in 2005. The age at first calving (AFC) has reduced from 1120 days in 1995 to 979 days in 2013-14. The Semen Freezing Laboratory of the Institute is well equipped with latest gadgets and has ISO 9001:2008 certification. During the year 2013-14, a total of 245900 doses were frozen.

Keeping in view the importance of indigenous breeds, known for their adaptability and disease resistance qualities, Indigenous Breeds Project (IBP) was undertaken in collaboration with the SAUs, State Govt. and Non Government Organizations. The project was initiated by establishing germplasm units and a number of associated herds. The programme for genetic improvement of indigenous breeds of cattle were started with Hariana and Ongole, breeds of cattle and at later stages Hariana and Ongole were withdrawn and Sahiwal, Gir and Kankrej were added. The sire evaluation in Hariana breed had been carried out on the basis of estimated breeding value not only from daughter's first lactation milk yield, but also from male progeny's draughtability parameters. A total of 73 Ongole bulls in nine sets were inducted in the program. Three thousand eight hundred and eight daughters were produced due to eight sets in Ongole breed. The bulls' up to the fifth set have been evaluated for their genetic merit based on first lactation milk yield of their daughters. Draft studies of Ongole bulls using single harness plough with digital dynamometer revealed that draught power varied from 0.64 to 0.84 H.P. among the bulls. Improvement programme for three new indigenous breeds (Gir, Kankrej and Sahiwal) started in 2009 and two sets of bulls have been inducted so far.

Field Progeny Testing (FPT) project was undertaken to bring about improvement in crossbred cattle at farmers' herd. The aim of this project is to progeny test Frieswal and other Holstein - Friesian crossbred bulls under field conditions at four different agro-climatic locations in India, viz. Ludhiana (GADVASU), Mannuthy (KAU), Urulikanchan (BAIF) and Pantnagar (GBPUAT). A total of 239 bulls in 11 sets at GADVASU, 223 bulls in 12 sets at KVASU and 215 bull in 10 sets at BAIF Unit and 25 bulls in 3 sets at GBPUAT were inducted so far. The per cent increase in the first lactation milk yield at GADVASU, KVASU and BAIF were 37.1, 39 and 5.4% respectively. The decrease in AFC in the field units in the same order were 13.6, 9.86 and 6.6% respectively .

Short term research projects on different aspects of cattle production were also carried out in the Institute. Morphometric characterization of Frieswal cattle has been compiled. The various

sire evaluation methods were compared for estimation of breeding values of Frieswal bulls. The comparative performance evaluation of Frieswal bulls in farm and field conditions were also carried out. Evaluation of Frieswal herd for performance traits, conception rate, survival pattern, herd structure and expected herd life were also carried out. Molecular marker studies in relation with production, reproduction, resistance to FMD and mastitis and thermo tolerance were also taken up.

Besides various breeding programmes, the institute is working on nutritional, management and reproductive interventions for optimizing productivity of crossbred cattle. Nutritional studies for optimizing the feeding efficiency of Frieswal bull calves revealed higher growth on the diet formulated based on rumen undegradable protein (RUP) requirements. Oral feeding of combined preparation of Estrogen and progesterone along with Agrimin forte@40 g/day for 10 days emerged as most economic and convenient method to overcome the problem of anoestrus in Frieswal heifers. Spermiogram of Frieswal bulls in relation to season and age, seminal attributes and inheritance of seminal quality and sperm abnormality in Frieswal bulls have been established. Age related changes in body size and gonadal development in growing Frieswal bulls were also evaluated. Effect of different planes of nutrition and mineral supplementation on nutrient utilization and semen quality of Frieswal bulls and the effect of type of prepuce on semen production performance was also evaluated.

The CIRC has also established linkages with various other ICAR institutes, Military farms Agricultural and Veterinary universities, Gaushalas and NGOs. The extension and developmental activities are routinely carried out through participation and organization of various Kisan Gosthis, Kisan Melas, Consultancies and infertility camps.

Future Directions:

During the coming decades, the cattle production scenario and system in the country is going to be considerably transformed and would be potentially much different from the existing as a result of altered economic and population determined considerations.

The major future programmes which CIRC would be undertaking in the next 25 years are enlisted below:

1. Genetic up-gradation of huge non-descript cattle population to recognized breeds
2. Establishment and strengthening of bull mother units for major breeds
3. Bringing about 70% of entire cattle population under genetic improvement programme using AI, reliable field data recording (establishment of effective breed societies) and embryo transfer
4. Genetic improvement of indigenous breeds employing phenomics (precise performance recording), Genomics (assessing genomic values for economic traits using DNA markers) and bioinformatics.
5. Genetic improvement of large crossbred population generated over decades.
6. Progressive increase in population of high producing indigenous cattle at the cost of non-descript and crossbreds.
7. Multiplication of high producing animals using emerging reproductive biotechnologies (MOET, IVM, IVF, cloning, commercial embryo production & transfer)
8. Development of Sexed semen technology and its application.
9. Genetic alteration of animals for obtaining tailor made milk of therapeutic use.

10. Management of fertility, reproduction and health of cattle for higher productivity.
11. Meeting the nutritional requirement of high producing cattle population
12. Draught power -an important consideration in cattle research.
13. Alternate use of cattle byproducts for its increased and holistic economic viability.
14. Embarking upon climatic stress and regulation of GHG emissions
15. Development of functional inter- linkages between varied stakeholders for technology development, dissemination and adoption and quality HRD development

A way forward

The prime emphasis of the institute will be on the evaluation of indigenous and crossbred bulls to supply the male germplasm to cater the breeding requirement of the country for increasing the milk production. This will be accomplished by developing the phenotypic data bases on production, reproduction and functional traits of cattle both at farm and field levels and the integration of phenomics with the genomics for selection of superior bulls. The information of full sib females developed through MOET will be used as alternate model for the genetic evaluation of young bulls.The recent advances in the areas of genomics, proteomics, nutrigenetics, metagenomics, nutrigenomics and bioinformatics will be integrated to develop an appropriate module for the genetic improvement of milk productivity of Indian cattle. The whole genome based animal selection strategies; supply of sexed semen, application of MOET, transgene technology, cloning, stem cell research, nanotechnology etc. will be explored for increasing the productivity and economic utility of the cattle. The reproductive efficiency of the cattle will be improved through multidisciplinary research integrating genetic, nutritional, managemental and biotechnological approaches for reducing the reproductive disorders and increasing the productive life of the cattle. The climatic stress arising due to the large cattle population of the country will be reduced to appreciable extent by decreasing the low productive non-descript population. Balanced feeding, selection of animals for reduced methane production, use of phytochemical or other chemicals having methane inhibiting property will be priority research areas for reducing methane emission from cattle. Metagenomics and nutrigenomics studies will be undertaken to alter the rumen metabolism for reduced methane production. The Institute will also endeavor on development of breed specific feeding standards for indigenous cattle and to develop total mixed ration effectively utilizing the available feed resources in collaboration with sister research Institutes. The CIRC would play a crucial role in developing the technologies related to cattle production to meet the milk production demand of the country by 2050 and their effective dissemination utilizing the ICT based extension techniques. The institute will also be a key player in the socio-economic upliftment of cattle owners by infusing superior germplasm in the farmer's herd, formation of breed societies, improving the economy of the cattle production by effective utilization of resources, production of quality clean milk and production of byproducts etc. Research efforts will also be directed towards exploring scientific bases of age old cultural and religious traditions associated with cow for centuries.

In short, the institute will take a leading role by attaining the targets, leading to the accomplishment of the vision "Improvement of cattle for high productivity and profitability".

"Good Bye"

Dr. P. W. Borgaonkar

Member, Editorial Board, Blue Cross Book

Dr. P. W. Borgaonkar retires from MSD Animal Health services from August 2015, subsequent to his glorious career of more than 40 years, starting from BAIF and then in Hoechst Rousell Vet, Intervet (India) and MSD Animal Health.

Starting as Cattle Development Officer in BAIF, Dr. Borgaonkar was soon inducted in a team of dedicated workers, who were entrusted with the task of establishment of BAIF's prestigious FMD vaccine production project, a first of its kind in India, wholly under the management of Indian Scientists. Under BAIF, Dr. Borgaonkar had an advanced training in Virology and Parasitology at INRA (Institute National de la Recherche Agro nomique) in France. Back from the training, Dr. Borgaonkar was placed in Drug Research and Development Department of BAIF, wherein, he was instrumental in developing various animal health products and feed supplements. His initiatives in this department, certainly furthered the vision and mission of BAIF to provide complete health cover to livestock, particularly the crossbreds.

Subsequent to acquisition of Animal Health Division of BAIF by Hoechst Rousell Vet, Dr. Borgaonkar became Production Manager (Poultry Vaccines). His involvement in operating freeze driers, programming of freeze drying cycles, aseptic freeze drying filling etc. earned for Hoechst Rousell Vet WHO, GMP, ISO 9001, ISO 14001 and OHSAS 18001 certifications.

The international merger betweek Hoechst Rousell Vet and Intervet International in 2000, made Dr. Borgaonkar Senior Technical Manager (Customer Service Department) in Intervet (India). His main responsibility was to support poultry farmers in prevention and control of poultry diseases.

Dr. Borgaonkar's vast experience in Poultry Sector earned for him an international assignment in Saudi Arbia with Al-Watania Poultry Project, which had a 5 lacks chick placement per day. Back to Intervet INDIA, Dr. Borgaonkar has been working as Technical Support Manager and Field Trials Co-ordinator in Veterinary Service Department, the assignment related to planning of field trials of the present pharma products to generate the performance data. He has also been associated with renewal and updating of Intervet (India) 's website.

The relaunching of 'Blue Cross Book' is another feather in Dr. Borgaonkar's cap. This professional publication was relaunched after a gap of few years. The release of 'Blue Cross Book' restarted with its 25th volume and with it, all the earlier 24 volumes were published in the cd version. As a Member of Editorial Board, Dr. Borgaonkar has played a pivotal role in publishing the subsequent editions of this professional publication, uninterruptedly.

The entire family of MSD Animal Health, the contributors and readers of the Blue Cross Book, wish Dr. Borgaonkar and his family a happy, healthy and delightful post-retirement life.

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.

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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.

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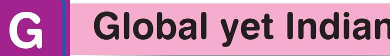

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	Instant	Sustained
Energy supplementation	Simple sugar	Gluconeogenic precursors
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Essential nutrients to ease transition period stress	Microbial protein	Inactive dry yeast culture

Instant & Sustained

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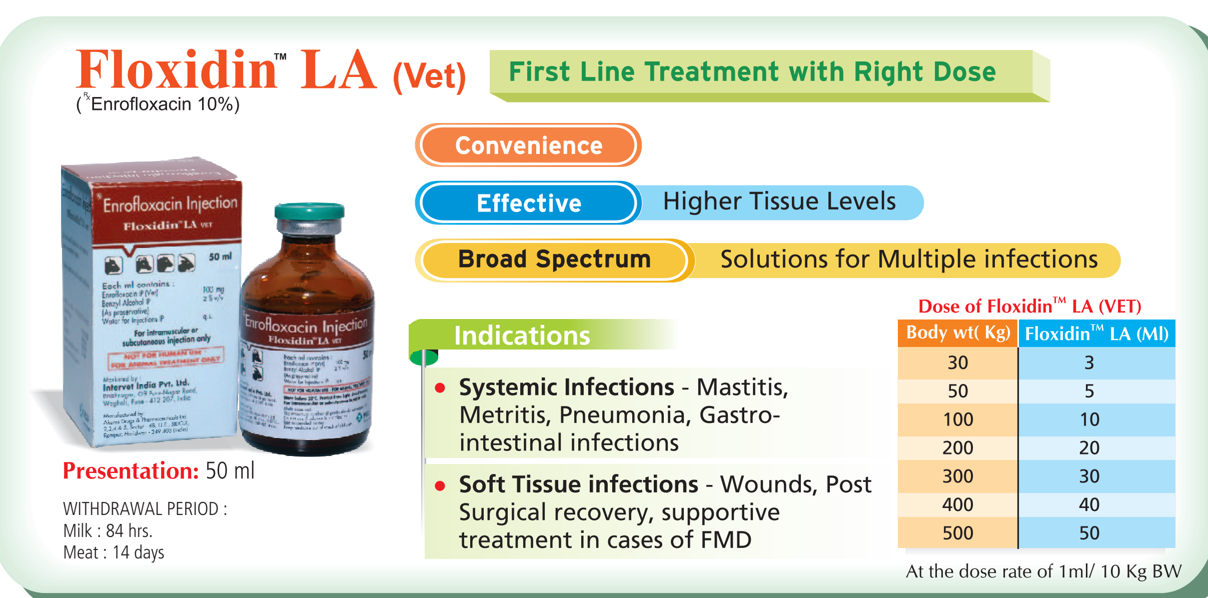
Precaution :

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

Dranch (1) 500 ml — Calving — After parturition

Dranch (2) 500 ml — 48-72 hours after parturition



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

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Each (0.4 ml) dose Contains Brodetella bronchiseptica strain B-C_2 - $\geq 10^{8.0}$ CFU and canine para influenza virus stain Cornell $\geq 10^{3.0}$ $TCID_{50}$

INDICATIONS

Active immunization of dogs against Kennel Cough.

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- Low 0.4 ml dose
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- Can be used with or without applicator

PRESENTATION

One box contains 5 vials of dose and 5 vials of diluent along with one applicator

Ovilis® PPR

COMPOSITION

Freeze dried vaccine after reconstitution with diluent Contains Live attenuated PPR virus NLT 2.5 $TCID_{50}$ per single dose (1 ml).

INDICATIONS

For the active immunization of sheep and goats of 4 months and above age against PPR disease.

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1 ml per animal by subcutaneous route.

PRESENTATION

Vials of 100/50/25 doses.

HORMONES

Receptal® VET.

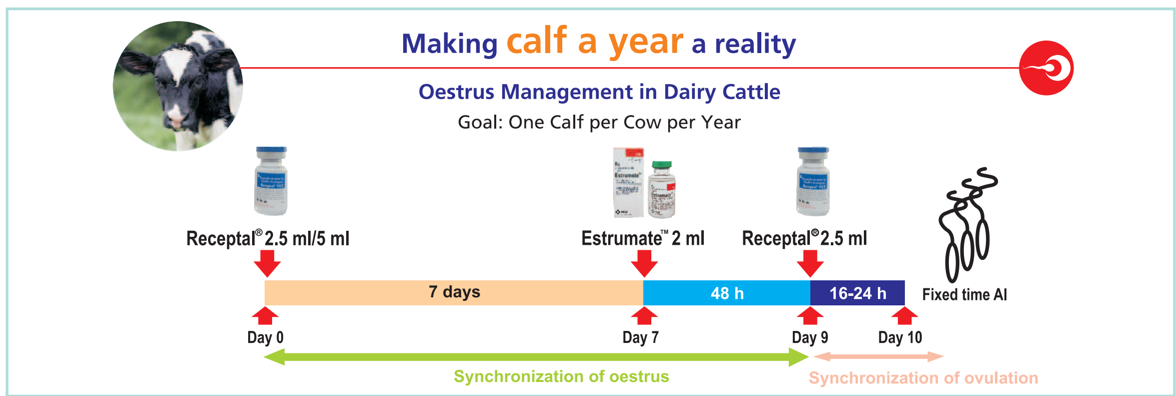
COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Buserelin acetate 0.0042 mg equivalent to 0.004 mg buserelin.	• True anoestrus	5 ml, IM	Vial of 10 ml and 2.5 ml
	• Improvement of conception rate (at the time of AI)	2.5 ml, IM	
	• Ovarian cyst (Follicular), Irregular oestrus, Nymphomania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

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	WITHDRAWAL PERIOD
	• Induction of ovulation (mares)	1500-3000 IU, IM or IV, 24 hours before AI/mating	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



ANTI-INFECTIVE

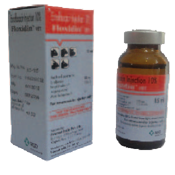
COBACTAN® 2.5%

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) • Acute *E. coli* mastitis with signs of systemic involvement 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 Milk : 1 day Meat : 5 days

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 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 WITHDRAWAL PERIOD Milk : 3.5 days Meat : 14 days

Rx 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.

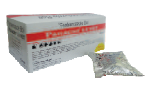
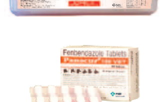
COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Deltamethrin I.P. 12.5mg	To control the ectoparasites in cattle, sheep, goats, horses, camels, dogs & farm houses.	Spray or dip : Ticks : 2 ml/lit Mites : 4 ml/lit Flies : 2 ml/lit Lice : 1 ml/lit	Aluminium container of 5 ml, 15ml, 50 ml, 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

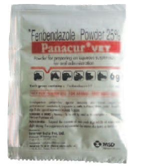
COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each ml contains : Amitraz I.P. (Vet) 125 mg	1. For prevention & control of ectoparasitic infestation like ticks, mites, lice & keds in cattle, sheep, goat, camel & pig. 2. Taktic kills tick, mite and lice. 3. Taktic kills organochlorine, organophosphate & 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 : 7 hrs after applications Meat : 1 day for Cattle & Goats & 7 days for Pigs & Sheep

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 table 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 2 bolus x 15 strip -1.5 gm Box of 10 bolus-3 gm Box of 10's x 5-15 mg tablet WITHDRAWAL PERIOD Milk : 0 (Zero) day Meat : 8 days

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 : 0 (Zero) day Meat : 8 days

PARASITE CONTROL

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 gastrointestinal 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 : 0 (Zero) day Meat : 8 days

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	INDICATIONS	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	INDICATIONS	DOSAGE	PRESENTATION
Each KG contains a nutritional value of (When packed): Cobalt 150 mg, Copper 2200 mg, Iodine 325 mg, Iron 2500 mg, Magnesium 6000 mg, Manganese 2200 mg, Potassium 100 mg, Sodium 8 mg, Sulphur 1%, Zinc 9000 mg, Vit A 1200000 IU, Vit D3 120000 IU, Vit K 200 mg, Vit E 500 IU, Calcium 225 g, Phosphorus 90 g, Niacinamide 1000 mg, Biotin 2% 500 mg, Bioactive chromium 65 mg	• 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

SUPPORTIVES

Rumicare® (Vet)

Normalises milk production by restoring ruminal activity.

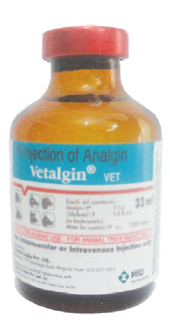
COMPOSITION	INDICATIONS	DOSAGE	PRESENTATION
Each gm powder contains : Calcium Propionate 480.00 mg Methionine 40.00 mg Picrorhiza Dry Extract 2.00 mg Cobalt Gluconate 0.32 mg Vitamin B6 IP 0.32 mg Dextrose Anhydrous IP 428.00 mg	Bloat, digestive disorders caused by decreased activity of reticulum & rumen 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 : 5 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	Available in 20 ml vial (10 Doses)

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 4×10^{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).

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 strain of: Leptospira interrogan serotype canicola strain Ca-12-000-(1500 units) Leptosprira interrogan serotype icterohaemorrhagiae stain 820k-(1000 units)	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 $\geq$ 3 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.

COMPANION ANIMAL

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 and lice at weekly intervals. Taktic to be used as dip or spray	Glass bottle of 25 ml with plastic measuring cup

San Coat®

CONTENTS	INDICATIONS	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

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 $1/50^{th}$ 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)

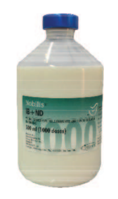
Nobilis® Reo inac

COMPOSITION	INDICATIONS	DOSE & ROUTE	PRESENTATION
Each dose contains : Inactivated Reovirus strains 1733 and 2408, inducing ≥ 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 ≥ 14.5 log_2 VN units/dose, Inactivated Newcastle disease virus (Strain Clone 30) inducing ≥ 4 log_2 HI units per 1/50th of a dose or containing ≥ 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 ≥ 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 ≥ 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 ≥ 4.0 log_2 VN units/dose, IB virus (Strain D249G) inducing ≥ 4.0 log_2 VN units/dose, Inactivated Newcastle Disease virus (Strain Clone 30) inducing ≥ 4.0 log_2 HI units per 1/50th dose or containing ≥ 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 ≥ 6.0 log_2 HI units, Inactivated Injections Bursal Disease virus (Strain D78) inducing ≥ 14.5 log_2 VN units, Inactivated Newcastle Disease Virus (Strain Clone 30) inducing ≥ 4 log_2 HI units per 1/50th of a dose or Containing ≥ 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

The Science of Healthier Animals™

INTRODUCING

CANINE PLUS »

An advanced formula of 30 synergistic nutrients to support health in dogs.

CARDIO STRENGTH »

Unique formulation with L taurine, L Carnithine, DMG, Coenzyme Q10, Folate, Mg, EPA, GLA, Mg, K and Se to support specific cardiac function and ensure healthy heart.

DERMA STRENGTH »

Highly advanced formula with MSM, DMG, Methionine, Cysteine, Ascorbic acid, Proline, essential fatty acids, Zinc citrate, Hyaluronic acid, Vitamin B3, Vitamin A, and Perilla seed to support skin and coat condition.

GLYCOFLEX »

Balanced formula with Glucosamine, Perna canaliculus, MSM, DMG and Manganese to support joint heath in working , athletic and exercising dogs at all ages .

RENAL ESSENTIALS »

Highly superior formula with unique ingredients like Astragalus root powder, Rehmannia(chinese herb), Nettle(Urtica dioica), Cordyceps Sinensis extract to support specific kidney function and healthy and functional kidney.

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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