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EDITORIAL

It is my privilege to bring before you the new issue of JIVA which brings about the latest updates in Veterinary Medicine and Animal Husbandry activities.

The editorial board has been working with immense dedication for the release of JIVA in the stipulated time. The enthusiasm shown by veterinarians from different fields in contributing their articles to JIVA was encouraging and commendable. Articles on modern diagnostic and surgical techniques in diagnosing clinical cases, current issues and advances in veterinary field are included with a view to expose field veterinarians to newer technology and to update their professional knowledge. Articles received from the veterinarians working in the Animal Husbandry Department shows their expertise in our profession.

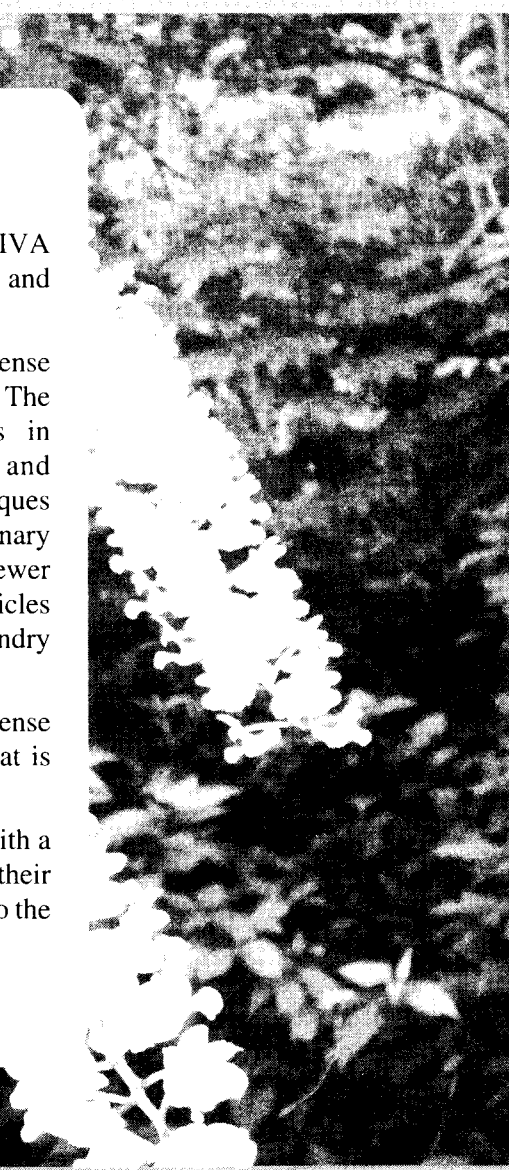
Indian Veterinary Association, Kerala has made an immense effort to make sure that the journal reaches in every hands that is associated with veterinary profession.

I hope that all the veterinarians will accept this issue with a new outlook towards veterinary practice and will continue their support and interest in giving valuable articles and suggestions to the forthcoming issues.

Dr. Laiju M. Philip

Orchid

oxburghii R. Br. (*Rincostylus retusa*)



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EDITOR

Dr. LAIJU M. PHILIP



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JOURNAL OF INDIAN VETERINARY ASSOCIATION, KERALA (JIVA)

Journal of Indian Veterinary Association, Kerala (JIVA), the official organ of Indian Veterinary Association, Kerala is quarterly scientific periodical with international status (ISSN-0975-5195) will bring about the latest updates in Veterinary Medicine and Animal Husbandry Practices. The journal covers almost all topics of Dairying and Animal Husbandry besides special emphasis on Companion Animal Medicine and Surgery, Zoo and Wildlife Medicine, Meat and Feed industry, Diagnostics and Bioinformatics.

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CLINICAL REPORT

<i>Surgical Management of Vaginal Fibroma in a Dog.....</i> M K Narayanan, Prasoon S, Alok Pandey, Rajesh Vishnoi, Dinesh Kumar Maurya, and Chandra Shekhar Kumar	44
<i>Unilateral Cryptorchidism in a Dog</i> Shibu Simon, Daron Joseph, Anupam Abraham, Ayswarya R.Venu, Rejitha Joseph, Anish Rajan, Abhilash A.K.	46
<i>Mycotic Enteritis in Rabbit.....</i> Swapna Susan Abraham, Jacob Alexander, Meera Unwin Antony and H. Viswanathan	48
<i>Uterine Torsion in a Buffalo.....</i> Sandhya.G.Nair,.Rani.K.Oommen, Promod.K.	49
<i>Chemotherapy using Vincristine in Canine Transmissible Venereal Tumour</i> Magnus, P.K. and Lali, F.A.	50

GENERAL ARTICLE

<i>Infrared Thermography in Bovine Reproduction Studies.....</i> G. Ajitkumar.	52
<i>Giardiasis in Cattle and its Zoonotic Importance.....</i> Lucy Sabu.	55
<i>Fertility Management through Immunomodulation ...</i> Gokuldas P.P, Pramod Kumar R, Aravind, S, Sreejith J.R and Rathish R.L	57
<i>Bovine Colostrum and its Multifarious Therapeutic Applications.....</i> T . Rajeev, and P. S. Surya	62
<i>Glowing Genes</i> Muhammed, E.M. and Stephen Mathew	65
<i>Acidosis in Captive Wild Herbivores.....</i> A.V. Shibu.	69
<i>Indigenous Plant Preparations in Ameliorating Nephrotoxicity.....</i> K.J. Bibu and A.D. Joy	73

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POST EXPOSURE ANTI RABIES THERAPY IN DOMESTIC ANIMALS

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Rabies is a viral encephalitis of man and animals. India is endemic for rabies. In addition to the public health hazard, the loss of animals due to this disease also stresses the importance of prevention and control of this disease.

As a part of the rabies control programme, Department of Animal Husbandry and Kerala Agricultural University were conducting anti rabies vaccination camps and seminars for making awareness about rabies among public throughout Kerala. Though, rabies is prevalent in Kerala routine immunoprophylaxis against rabies is not being done in other farm animals like cattle, buffalo and goats. Hence they are most vulnerable to this disease, once they are exposed to rabid animals. In countries where rabies is sporadic, exposed animals and biting animals are killed humanely and disposed immediately and thus the foci of infection is destroyed (Hanlon *et al.*, 2002). Such depopulation of exposed animals for prevention and control of rabies is not possible in our country for ethical, ecological and economical reasons.

Considering the above facts, it is strongly advised to carry out the post exposure anti rabies therapy (PET) in pet and farm animals at the earliest opportunity. The treatment may be discontinued if animal involved (dog or cat) remains healthy throughout an observation period of 10 days. The observation period is valid for dogs and cats only.

Before adopting the PET, as in the case of humans, the exposure has to be classified as follows.

Category I : touching, standing near rabid animals, licks on the skin (conditions where there is no chance of any transmission of rabies virus).

Category II : nibbling of uncovered skin, minor scratches or abrasions without bleeding, licks on broken skin (there is a chance of transmission of virus).

Category III : single or multiple transdermal bites or scratches, bite on head and neck; contamination of mucous membrane with saliva form licks; exposure to bat bites (In India no report of bat transmitted rabies); bite from any wild animals (sure case of transmission).

Basically, post exposure anti rabies therapy (PET) consist of first aid, active and passive immunization against rabies.

Antiviral agents, interferon inducers and massive doses of rabies immunoglobulins have been used to treat human cases, but without any favourable result. Recently, one case of bat transmitted rabies survived following drug induced coma and antiviral treatment, though the same intensive treatment protocol failed subsequently to save the lives of several human patients.

For category I, no treatment is recommended.

For category II, application of first aid and immediate vaccination is recommended.

For category III, application of first aid, and administration of vaccine and rabies immunoglobulin is recommended.

First Aid

WHO TECHNICAL REPORT SERIES 931 mentions that local wound treatment is of very great importance in the management of each and every case of animal bite or exposures to animals. Since the rabies virus enters the human body through a bite or scratch of a rabies infected animal, it is important to remove saliva containing the rabies virus at the site of bite by chemical or physical means.

Washing of the wound must be done irrespective of the time since bite, as the rabies virus can persist and even multiply at the site of bite for a long time. However, care must be taken not to disturb scabs if formed. In addition, tetanus

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prophylaxis, analgesics and anti bacterial treatment/antibiotics has to be given.

Though first aid appears to be simple, if properly done nearly 50 - 60% of the clinical cases of rabies can be avoided. Since ancient times different types of first aids were being done following suspected rabid exposure. Cauterisation with red hot iron, application of hot water, application of chilli powder, turmeric powder, oil etc. are some among them. These methods are not at all recommended as these methods are not conferring any additional advantage over washing with soap and water.

Simple washing of the wound with soap (washing soap/toilet soap/detergent powders) and running water for 15 minutes is the most ideal one. While washing, it is advisable not to use bare hand. Use gloves as far as possible. Scratches /abrasions on hand may lead to the transmission of the virus. Mopping of the wound with clean cloth/sterile cotton and application of antiseptics completes the first aid. Common antiseptics like Savlon (as per the recommended dilution),dettol (as per the recommended dilution),tincture of iodine or aqueous solution of Iodine like povidone iodine or surgical spirit can be applied to the wound.

Even if the animal is presented after few days of bite, it is advised to carry out the first aid .But this very basic step is often undervalued or ignored.

This simple first aid helps to clear as much virus from the site by

- i. Allowing mechanical/physical removal .
- ii. Exposure of the virus to high alkaline pH, helps to inactivate the virus.
- iii. Soap/detergent helps to dissolve the lipid rich (nearly 66%) envelop of the rabies virus, thereby inactivating the virus .
- iv. Application of the antiseptics also helps to inactivate the virus along with other microbes at the site.

So with this simple first aid, both physical removal and chemical inactivation of the virus are done . Moreover this simple method can be adopted by anybody anywhere .

Active Immunization

Rabies virus after entry into the body remains

at the site of entry for a period ,undergo multiplication in the myocytes, then attaches to the nerve ending and travel to the central nervous system and produces encephalitis, which is 100 % fatal in man and animals. So depending on the factors like the site of deposition, distance from the central nervous system, quantum of virus deposited, accessibility to nerve endings, the incubation period varies. It varies from few days to weeks, or even months and years. Exploiting this long incubation period and character of the virus, clinicians can actively immunize the victim and save from the disease even after entry of the virus into the body.

Essen schedule is the one approved by WHO for the human treatment. Only few reports are available in the literature on the application of the same in veterinary practice .

Research works conducted in the Department of Veterinary Epidemiology & Preventive Medicine, College of Veterinary & Animal Sciences, Mannuthy and at Madras Veterinary College ,Chennai proved the effectiveness of active immunization using inactivated tissue culture anti rabies vaccine in cattle, goats and dogs following *Essen schedule*.

Active immunization can be done using any inactivated tissue culture anti rabies vaccine containing an antigenic mass of 2.5iu/dose. If any vaccine contains only 1 iu/dose, multiple vials of the said vaccine has to be used.It was observed that the '*Essen schedule*' (0,3,7,14&28th day) of vaccination is providing protective antibody titre(>0.5iu/ml of serum) in animal victims to save them from this disease.

Basheer *et al.*(1997) reported that post exposure anti rabies therapy following *Essen schedule* and using inactivated tissue culture anti rabies vaccine (*Raksharab*®) protected 100% treated cattle , where in nervous tissue anti rabies vaccine could protect only 84% protection.

Inactivated tissue culture anti rabies vaccine (>2.5iu/dose) and DNA combined tissue culture anti rabies vaccine (>2.5 iu/dose) were found to be giving 100% protective effect in post exposure therapy(PET) in cattle and goats with category II exposure. The PET in *Essen schedule* was giving higher antibody titre than that in animals which were treated using alternative schedule of 0,1,2,3 &4 days of continuous vaccination using either vaccine (James *et al.*,2007; Rishi *et al.*, 2008).

Post exposure therapy (PET) using inactivated

tissue culture anti rabies vaccine (*Nobivac -R*®) was reported to be very effective in dogs. (Manickam *et al.*, 2008).

The vaccination has to be done either deep intramuscularly or subcutaneously. Never deposit the antigen into adipose tissue. As the vascularity to adipose tissue is less, antigen processing and further immune response will not take place leading to poor sero conversion. So, as far as possible avoid fatty area like the thigh /gluteal regions. Administration of vaccines into adipose tissue forms one important reason for the failure of vaccinations. This has been reported as one important reason for the rabies incidence among human beings, in spite of PET. Neck region will be ideal site in cattle, buffalo, goat and pig.

Passive Immunization

Under field conditions, in spite of doing PET, often the bite victims were used to develop the disease. On analysis of each of these cases, it was observed that majority of the rabid dogs bites are in the category III exposure. The field Veterinary doctors are adopting to the post exposure anti rabies therapy mainly by active immunization alone.

In all category III exposure, it is advised to use both vaccine and rabies immunoglobulin (RIG), after doing the first aid. RIG should also be administered in all category II exposures involving immuno-deficient human beings. The readymade rabies antibody inactivates the virus at the site of infection, that is left at the site after the first aid.

RIG for passive immunization should not be injected later than 7 days after the initiation of post-exposure vaccination, as by that time the victim's body might have started the antibody production and further RIG administration interferes with the active immune response and may give a negative result even.

In animals often the bite is seen on face, neck, ears, muzzle and extremities as the attack of rabid animal occurs while they are grazing and their tendency to defend the biting animal by charging. The bite on face, head, neck, ears extremities have to be classified under category III exposure and both active and passive immunization have to be given after the first aid.

Three types of rabies immunoglobulins (RIG's) are available in the market. Human rabies immunoglobulins (HRIG), Equine rabies immunoglobulin (ERIG) and the highly purified equine

F(ab')₂ rabies immunoglobulin products (PERIG).

It is recommended that immunoglobulins be infiltrated into and around the wounds in order to rapidly neutralise rabies virus at the bite sites before it reaches the local nerve endings and the remaining RIG, if any, should be injected intramuscularly (IM) at a site distant from the vaccine injection site using separate syringe and needle. This reduces the viral load and immediately provides neutralising antibodies at the site of exposure before the animal /victim begins producing its own antibodies due the simultaneous active immunization.

HRIG is advised to use in human beings where ERIG is sensitive. It is costly. Its availability is also limited.

In Veterinary practice, we need not use HRIG. As far as veterinary practice is concerned, HRIG being heterologous, need to use only cheaper ERIG products. Even ERIG is heterologous for all species other than equines (bovines, caprines, ovines, canines, felines etc).

HRIG is administered at a dosage of 20 iu/kg body weight with a maximum dose of 1500iu, where as ERIG is administered at a dose rate of 40iu/kg body weight with a maximum of 3000iu. Higher dosage of RIG's interfere with the immune response to the simultaneous active immunization.

In case of younger animals with low body weight, the RIG can be diluted with sterile normal saline and can be infiltrated at the wound site. In such cases the whole quantum of RIG can be infiltrated locally, avoiding parenteral administration.

Suturing of the wound should be avoided as far as possible. The wound should be left opened. Exposure of the wound to sunlight is capable of inactivating the virus. We must opt for all possibilities to inactivate the virus at the bite wound.

Highly purified equine rabies immunoglobulins (pERIG) are efficient, very well tolerated, and more affordable than HRIG. ERIG is treated with enzymes and the Fc (Fraction crystallisable) is cut and removed, only antigen binding sites are provided in this product. The Fc portion is responsible for the hypersensitivity reactions. Still, these products are given in human beings after doing skin tests. In Veterinary practice also whenever we do administration of ERIG's,

better to do skin test, especially in costly dogs and other animals. Skin test can be done by diluting 0.1 ml of the ERIG in one ml of normal saline. From this diluted product 0.1 ml is injected intradermally at neck region or on caudal fold, observing 10 mts for the erythematous reaction at the site. As often it is not possible to do skin test before administering the serum, it is advisable to be equip with adrenaline and steroids to meet any emergency following administration of the serum. Never administer the RIG's intravenously.

In adult cattle and buffaloes, it is not possible to administer the RIG's based on the body weight. In such cases up to a maximum dose of 3000 iu ERIG can be locally infiltrated into the wounds and the balance if any can be administered intramuscularly at distant place of vaccine administration, using a separate syringe and needle.

For rabies exposed animals (mainly dogs and cats) which had previously undergone complete prophylaxis vaccination or post exposure treatment with inactivated tissue culture anti rabies vaccine, intramuscular doses of a tissue culture vaccine on day 0 and 3 days are sufficient. Rabies immune globulin treatment is not necessary in such cases. The same rule apply in dogs/animals vaccinated against rabies and have demonstrated neutralising antibody titres of at least 0.5 iu/ml. But, often reliable history may not be available. Moreover we are not in a position to assess the antibody titer in each case before adopting the treatment in the field level.

Necessity of Category III therapy in Veterinary practice

The scenario changed a lot. More and more costly breeds of animals of various species, particularly dogs started flowing in from abroad. Simultaneously, cheaper products also started flooding the market. So many medical outlets like *Neethi stores* providing vaccines and sera at a nominal price at each and every corner of India. All these factors made it easy for us to adopt the category III PET in veterinary practice.

The main practical limitation for the use of immunoglobulin in veterinary practice was its cost. This fact is true with large animals, but not with small animals (dogs, cats), small ruminants (goat, sheep) and young one of large animals (calves). Most of these animals will be weighing 20 to 50 kg. The ERIG can be administered at a dose of 40 iu per kg body weight.

If we study the present status, we can see that calf/goat/sheep/dog weighing 30 to 35 kg body weight one vial of ERIG (5ml; each ml containing 300 iu) 35 X 40=1200-1400iu; 4-5ml of ERIG), will cater need. The cost per vial of ERIG is around Rs.450/-, the total cost of the vaccine and ERIG, for saving costly animal weighing below 50 kg may not be more than Rs.1000/-. The same will be the case, if we use vaccine and one or two vials of ERIG for infiltration in adult cattle and buffaloes.

ERIG is available in the market in several brands names.

1. *Equirab*® (M/S Bharath Serums and Vaccines Ltd., Maharashtra);
2. *Rabies anti serum* (Haffkine Pharmaceutical Corporation, Maharashtra);
3. *Abhay-RIG*® (M/S. Indian Immunology Ltd., Hyderabad, Andhrapradesh);
4. *VINS-Rabies Anti serum*® (M/s.Vins Products, Hyderabad, Andhrapradesh).

Hence, it is strongly recommended to follow in all cases of Category III rabies exposure, an immunisation with any inactivated tissue culture rabies vaccine (containing 2.5 iu antigen per dose) per Essen schedule, along with ERIG at the dose rate 40 iu/kg body weight, after applying the first aid.

It is also advised to use three vials of the vaccine if the vaccine is containing only 1 iu/dose.

Trends in Post Exposure Antirabies Therapy

Experimental rabies infections were successfully treated using tissue culture anti rabies vaccine and interferon inducers like (poly(I)poly(C) complex poly-L-lysine and carboxymethyl-cellulose) in mice and monkeys. The combination of the interferon inducer plus vaccine could be administered at least 48 hours after the very severe challenge given and provide marked protection. This combination proved to be as effective as hyperimmune serum and vaccine much better than vaccine alone. (Harmon, Janis, 1975; Baer *et al.*, 1979). Much work has to be done in this direction for its field application.

Research works also proved the efficacy of monoclonal antibody against specific epitope of protein of rabies virus. It has been discovered that it inhibits intercellular spread of rabies virus at a cellular level and transcription of mRNA from rabies virus.

infected cells. Post-exposure administration of Mab was able to clear rabies virus from infected cells of the central nervous system (CNS), thereby preventing death from virus-induced encephalomyelitis. Hanlon *et al.* (2002) observed that vaccine alone in category III exposure was unable to provide protection from rabies in dogs. However, vaccine combined with mAb resulted in protection in all treated dogs, revealing potential use of mAb in post exposure therapy against rabies in dogs.

In future, we can expect much more effective treatment combinations of different monoclonal antibodies against proteins of rabies virus, various types of rabies vaccines and interferon inducers in post exposure anti rabies therapy in man and animals.

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CONTROL OF FOOT AND MOUTH DISEASE SOME POINTS TO PONDER

V. Jayaprakasan

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Control of Foot and mouth disease (FMD) involves the containment of the disease, although the ultimate aim should be the total eradication thereof. A systematic approach for an endemic area usually starts with mass vaccination followed by the control of 'sporadic' outbreaks. The status of 'freedom from disease with vaccination' and consequently the status of 'freedom from disease without vaccination' is then awarded to disease-free areas by the Office des Epizooties (OIE). The International Animal Health Code of the OIE defines each of these stages which also entail specific trading implications for live animals and animal products.

Although very important, vaccination is only **one of the several** zoo-sanitary measures essential for the successful control of FMD. Other measures include the control of animal movement, prohibition of import of susceptible animals and animal products from high risk areas, appropriate diagnostic testing of susceptible animals prior to import and the application of quarantine measures as well as the slaughter of infected and susceptible in-contact animals depending on the overall epizootiological situations. The authorities responsible for facilitating an effective control should also have access to specific fields of expertise. These include epidemiology, animal health legislation, risk analysis, logistics, training and education.

Foot and mouth disease virus (FMDV) are believed to exist as a population of quasi-species (a group of viruses with related but non-identical genomes). **In any virus population, continuous mutations and the mutants thus evolved form the basis of their fitness gain and adaptation to changing environments.** A clinical infection of FMD is usually cleared within seven days; however, persistent sub-clinical infection, where reservoirs of virus exist in the pharyngeal region of the esophagus, may occur in sheep and cattle. Persistently infected or carrier animals are defined as those from which live viruses can be recovered even after 28 days of

exposure. It is also known that such persistently infected animals have the capability to pass on the infection to susceptible contacts. The mechanisms of persistent FMDV infection are unknown; however viral mutation leading to antigenic variation has been suggested as one of the key factors.

Though the rate of mutation estimated for FMDV is as high as 10^{-3} per plaque-forming unit (pfu) when compared to DNA viruses (presumably due to lack of proof reading activity in the RNA replicase), it is no higher than mutation rates in other RNA viruses. The region of the VP1 gene which codes for an important immunogenic site on the viral surface shows a high degree of variation. Consequently, nucleotide changes in this region are most likely behind the appearance of new antigenic variants. The genome mutations facilitate the substitution of amino acids, particularly within the capsid proteins. Such changes have been shown to occur during persistent infections in cell culture as well as *in vivo* and were linked to changes in antigenicity, contributing to viral sub-populations escaping clearance by the immune system.

Several research groups have suggested that under the pressure of field outbreaks, natural selection favors such mutants that are not effectively neutralized in the immunized or convalescent animal populations. This **'immune selection'** could be a major cause of FMDV antigenic "drift" and the mutants so generated get amplified by the existence of unvaccinated or partially protected livestock and the unprotected wild animal hosts situated near the livestock production regions (**This could be the most probable cause for outbreaks in the so called vaccinated herds**). Significant antigenic changes are also likely to occur when the virus moves from one host cell type to another- for example, when FMD outbreaks spread from one species to another [from cattle to swine and back to cattle] (**This again strongly points out the need for a compulsory vaccination of all the susceptible hosts in**

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an area). Obviously, the multiplicity of animal hosts contributes to the persistence and variability of FMDV. The number of subtypes is expected to be higher in areas where the virus is indigenous in wild animal populations and in animals transported for breeding, trade or slaughter from endemic areas (**Consider the havoc caused by infected/unprotected animals crossing the border**). Antigenic variations unrelated to immune pressure also occur with many viruses and FMDV is no exception. Wherever these changes are tolerated, **'the more fit variants'** become dominant. Thus the development of viral mutants or quasispecies, can lead to infection with a mixed population of viruses and it is feasible to assume that certain phenotypical changes may also influence the ability of the virus to withstand clearance by the immune system.

Nowadays, molecular methods are widely employed than the conventional serological methods (r-value determination) for characterizing individual outbreak strains of FMDV. Determination of nucleotide sequence and phylogenetic analysis are the most precise and reliable methods used for this all over the world. Molecular epidemiology helps in tracing the route and spread of infectious diseases. In case of an outbreak of FMD, it is essential to make a close match between the vaccine strains and the field strains of virus responsible for the outbreak. It is therefore necessary to continuously monitor the prevailing FMD viruses in an area where the disease is attempted to be controlled by vaccination (**Emphasizes the pressing need to establish facilities for antigenic comparison between vaccine strains and field viruses**).

It is well documented that exposure of vaccinated ruminants to FMDV can protect them against the disease but still permit sub-clinical oropharyngeal infection of a persistent nature. The existence of such vaccinated but persistently infected "carrier" animals creates difficulties in the establishment of a FMD-free status (**This could be yet another explanation for the occurrence of outbreaks in vaccinated herds**). The level of protection conferred by FMD vaccines in primo vaccinated animals primarily depends on the potency of the vaccine and the relatedness of the vaccine strain and the circulating field virus. Routine vaccines used for prophylactic campaigns need to achieve only a minimum of 3 PD₅₀ (PD₅₀ value for the formulated vaccine is calculated based on the extent of protection from clinical manifestation i.e. prevention of generalization of FMDV to the feet) whereas a vaccine to be acceptable as

an 'emergency vaccine', it has to achieve a potency value of 6 PD₅₀ and this is often referred to as a 'high potency' vaccine. The vaccinations with these 'high potency' vaccines, were found to show more inhibitory influence on local virus replication and excretion in the oropharynx than the conventional vaccines, thereby limiting the transmission of disease to other susceptible animals. Thus by increasing the pay load of FMD vaccines, the systemic neutralizing antibody response can be enhanced and clinical and sub-clinical protection rates be improved. A systemic gamma interferon response was also shown to be related to the payload (There is some restriction from Government of India in increasing the pay load of FMD vaccines manufactured in India).

The antigenic diversity of FMD viruses is well known and frequently prompts questions on the selection and potential efficacy of inactivated vaccines employing standard vaccine strains. A common benchmark to assess the probable efficacy of a given vaccine strain in relation to a field isolate is the range of definitions published by the World Reference Laboratory for FMD, based on the r1 value derived from the neutralization of the **field virus** by the sera raised against the **vaccine strain** (Liquid Phase Blocking ELISA is equally good).

The definitions are as follows.

r1 = 0 to 0.19. These values represent a highly **significant serological variation** from the reference vaccine strain. Where possible, it would be advisable to use a vaccine strain with a closer relationship to the field virus. However, in an emergency, a potent vaccine of the type used as reference in the test may provide adequate protection, especially if administered on more than one occasion (This suggests **the effective use of the existing vaccine - but at a higher potency in repeated doses -reconstituted from 'antigen bank' - in the control of outbreaks in vaccinated herds even when the inflicting strain is a different one**).

r1 = 0.2 to 0.39. These values represent an area of concern. They **show significant differences** from the reference strain, but protection may be satisfactory if a **sufficiently potent vaccine** is employed.

r1 = 0.4 to 1.00. These values are not significantly

different from the reference vaccine strain as measured by the particular test system used.

Thus a low rI value gives cause for concern and will often indicate the need for development of a new vaccine strain.

Against this background, a critical question is that, to what extent current vaccine strains could confer protection against the emerging variants. The selection of new strains to be included in FMD vaccines, is usually based on the advice of (inter) national research institutes and in consultation with the World Reference Laboratory for FMD but the final choice for a specific strain is always made by the national authority. There are two reasons to replace an existing vaccine strain:

First, experts, **including the vaccine producers and the reference laboratories** recognize that a significantly different virus has appeared in a region that may/will warrant the development of a new vaccine strain. This recognition is invariably based on a serological assay such as the virus neutralization test but may be supported by sequence analysis of the VP1 protein.

The second reason is concerned with those countries where regular vaccination programs are employed and the epidemiological situations (like the stability of virus, source, closed herd *etc.*) are relatively constant. Such situations support the obvious concept of preparing a vaccine strain from a local field isolate so that the field viruses and vaccine strains are one and the same.

It is opined that antigenic variation does not represent an insurmountable problem in terms of the ability of the manufacturers to keep pace with the commonly observed level of variation in the field. Likewise, the evasion of the vaccine-induced protective immunity by way of the selection of escape mutants does not appear to be a very common phenomenon.

The choice of the most appropriate vaccine strains for use in FMD control programs as well as for storage in 'vaccine antigen reserves' is essential based on the matching of representative field isolates from outbreaks around the world to the available vaccine strains. However, those involved in current control campaigns do not always give this a high priority; and in countries without such effective control programs, little incentives are paid for collection of field samples and their further submission to International Reference Laboratories for the matching process. In short term, specific initiatives targeted collection can provide samples on a periodic basis, but a long-term solution is required for development of FMD control measures. Commercial constraints may crop up on the disclosure of the strains used for vaccine production, or on the supply of reagents needed for the matching tests. Since vaccine matching tests are performed in a relatively small number of laboratories around the world and because neither reagents nor the methods used for vaccine matching are fully harmonized, there is no strict equivalence in results obtained. Alternative approaches using monoclonal antibody panels and/or viral capsid gene sequencing are being developed and could complement the currently employed serological tests. This must also be underpinned by the **strengthening of local Veterinary services and laboratories, and a demand driven production and timely supply of sufficient amounts of high quality vaccine through the establishment of an 'antigen bank'.**

A strong commitment of all the countries at a high political level to harmonize the global, regional, national and state level policies for FMD control is the need of the hour. The eventual eradication of FMD in state, country, region or worldwide could only be achieved if the entire community recognize its contribution as a global public good that will benefit all the races and their future generations.

ENERGY SUPPLEMENTATION ON PRODUCTION PERFORMANCE OF COWS UNDER FIELD CONDITION

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Abstract

Thirty, early lactating cross bred cows (average 40 days in milk) were selected and randomly allotted to the dietary treatments T₁ (control), T₂ and T₃. The animals of T₂ and T₃ were supplemented with 1kg ground maize and 100g of protected fat respectively, over and above the control ration. There was no significant difference (P>0.05) in milk yield, milk composition and yield of 4 per cent fat corrected milk (FCM) between the groups. However, the percentage increase in milk yield was highest in the protected fat supplemented group (T₃). Animals supplemented with protected fat showed post partum heat earlier than the rest of the animals.

Key words: energy supplementation, production performance, early lactating cows, field condition

Introduction

Dairy cows in early lactation do not consume sufficient dietary energy to meet the nutrient requirements for high milk production and are forced to metabolize body reserves leading to a negative energy balance. In Kerala the non availability of good quality fodder and high cost of concentrates and paddy straw have led to a quantitative and qualitative deficiency in the rations of dairy cows, of which energy deficiency is found to be the most critical. This has led to reduced milk production, delayed maturity and poor conception rate in cows. Hence, this study was conducted to assess the effect of supplementing energy in early lactating cows in the form of ground maize and protected fat under field condition.

Materials and methods

Thirty, early lactating crossbred cows (40 days in milk) in a farm in the field were selected and randomly allotted to three dietary groups T₁, T₂ and T₃. The entire animals received routine farm ration comprising a concentrate feed mix in the wet form, the ingredient composition on dry matter basis being, maize 2.4%, maize waste 16%, maize bran 10.2%, beer waste

45%, tapioca starch waste 2.4%, cotton seed cake oiled 2.4%, cotton seed cake deoiled 2.4%, coconut cake 4.3%, rice bran 6%, soya husk 7.2%, salt 0.2% and mineral mixture 1.5%. The quantity of mix given is based on their milk production and 2.6 kg paddy straw /cow/day was given as roughage during the 90 day period of the experiment. The dietary treatments were: T₁-concentrate mix + paddy straw, T₂ - concentrate mix + paddy straw + 1kg ground maize and T₃- concentrate mix + paddy straw +100 g protected fat (EnerFATTM manufactured by Kemin). From the data on daily milk yield and milk composition, 4 per cent FCM yield, fat yield and protein yield were calculated. A digestion trial was carried out collecting 100g dung from four animals of each group allowing a collection period of five days and digestibility of nutrients and total digestible nutrient (TDN) intake of animals calculated. The animals were carefully observed for the signs of behavioral estrus. The cost of feed for producing one kilogram of milk was calculated using the data on total feed consumption and milk yield.

Results

The mean initial milk yield was 13.5, 13.53 and 10.75 kg, while the final yields were 15.35, 14.78 and 12.88 kg respectively for the groups T₁, T₂ and T₃. The milk yield showed 13.7, 9.23 and 19.81 per cent increase respectively from their initial

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values, the percentage increase being highest in the fat supplemented group. However there was no significant difference ($P > 0.05$) in milk yield between the three treatment groups. Schauff and Clark, 1992 and Delahoy *et al.* 2003 also reported similar results.

The TDN intake and requirements of experimental animals of the three treatments T_1 , T_2 and T_3 were 7.06, 7.84, 7.67 kg and 8.8, 8.34, and 7.34 kg respectively. Thus the TDN intake of animals of T_1 was up to the requirement while animals in T_2 and T_3 showed a deficit of 6.0 and 19.86 percent respectively. The digestibility of EE, DM, NDF and ADF was numerically higher for the T_3 ration than that of T_1 and T_2 . Average period for the first post partum heat in days was 89.1, 73.8 and 58.4 respectively, for the three groups. Staples *et al.* 1998 also observed that animals maintained on fat supplemented ration showed post partum heat earlier.

Conclusion

Though there was no significant difference in milk yield, the percentage increase and persistency of yield was greater for the animals of the fat supplemented group. Energy supplementation in the form of protected fat could meet the energy requirement of early lactating animals, improved the digestibility of EE, DM, NDF and ADF and positively influenced the first post partum heat.

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Cover :

Rinco orchid is a glabrous epiphytic orchid. Leaves simple, opposite, long and fleshy; flowers in long paniced spikes, rose colored, fragrant, and last for one month without falling off. Flowering season is only once in a year. Plant sticks on the host tree with its fleshy roots. Plant possesses hanging velemen roots to absorb the atmospheric moisture. Distributed throughout India, growing wild in evergreen forests.

Botanical Name : *Vanda roxburghii* R. Br. (*Rincostylus retusa*.)

Sanskrit : Vrikshakadali, Rasna. Hindi : Rasna

Malayalam : Maravazha, Kurukkan val poovu

Useful parts : Root and Leaves.

Medicinal Properties

Rasna is bitter in taste, pungent in the post digestive effect and has hot potency. It augments the uterine contractions and is a bronchodilator, digestant and blood purifier. It is used in diseases like gout, rheumatic disorders, asthma, abdominal pain, fever, skin diseases, otitis media, nervine weakness and in poison bites.

Medicinal Uses

The roots of Rasna have great medicinal value, externally as well as internally. Externally, the roots mashed in cow's urine, used as a paste, for topical application in skin diseases like eczema, scabies, pruritus etc. Rasna contains an alkaloid, which enters into the composition of several medicated oils, for external application in rheumatism and allied disorders and also diseases of the nervous system. The massage with Rasna oil is beneficial in swollen and painful joint.

Internally, Rasna is a keen stimulant, cholegogue, digestant and a mild laxative. Along with external therapy, rasna is a valuable remedy for inflammation of joints, internally also. It is salutary in seizures as well as facial palsy. In respiratory ailments like cough, asthma, pulmonary edema, pleurisy and tuberculosis, It is a great panacea for oligomenorrhea, amenorrhea and dysmenorrhea; as it augments the uterine contractions. It is also a remedy for secondary syphilis and scorpion bite.

PSEUDOPREGNANCY IN A GOAT

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Introduction

Pseudopregnancy is an anoestrous condition which occurs when the Corpus Luteum persists in the absence of viable conceptus in the uterus with the accumulation of uterine fluid. It is seen in dog, goat, sheep, cat, mare, rabbit, mouse, pig and even in humans. The exact etiology is unknown. In goats it is well known to be a cause of infertility. The uterine fluid is accumulated between the second and fifth month after mating or insemination. The fluid could easily be diagnosed by the trans-abdominal ultrasonographic approach using B-Mode transducer.

Materials and methods

A nulliparous doe with an earlier history of abortion was presented to the Gynaecology Unit, Veterinary College Hospital, Mannuthy for pregnancy diagnosis. The animal was inseminated 155 days before by the local Veterinary Surgeon. The animal had engorged udder and distended abdomen. The clinical parameters of the animal were taken and found to be normal.

The abdomen was palpated and B-Mode ultrasonography was performed for diagnosing the condition. The ultrasonography results were suggestive of pseudopregnancy since there were sacculations due to the coiling and kinking of the uterine horns (fig. 1).

Treatment

To bring about luteolysis, 1.5 ml (0.294 mg) PGF_{2α} analogue Iliren (Tiaprost[®]) was given intramuscularly. After 24 hours cloudburst (release of the fluid) was noted and 12 days after the cloudburst, a second dose of Iliren was given to reduce the recurrence of the condition.

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Result and Discussion

After the treatment with two shots of PG_{2α}, the owner reported that the animal returned to cycle and showed heat signs within a period of 2 weeks. The incidence of false pregnancies is fairly high, particularly in some strains of dairy goats, and incidences of between 3% and 30% have been reported in commercial herds. Due to the persistent corpora lutea, the goat with hydrometra is in anoestrus and the breeders assume that the doe is pregnant. The common synonyms are hydrometra and cloud burst.

Certain breeds seem prone to develop the condition. A persistent corpus luteum following embryonic death with resorption of the embryo. The condition is observed even if the goat is unmated during oestrus. The doe acts as if pregnant, with enlargement of the abdomen and a degree of udder development if not milking. Milking does may show a sharp drop in yield, and this may result in a significant economic loss if the condition is not corrected. Fetal fluids collect in the abdomen (*hydrometra*) and the doe may become enormously distended, although the amount of fluid varies from one to seven litres or more. Before luteolysis occurs, progesterone secretion ceases and the fetal fluids are released termed as **cloudburst**. The expelled fluid (cloudburst) is generally clear, cloudy and mucoid even have a bloody discharge.

When the false pregnancy occurs in a doe that has not been mated, the release of fluid often occurs in less than the normal gestation period, the doe may cycle again and a further false pregnancy may occur if she is not mated. The abdomen decreases to a normal non-pregnant size and bedding appears wet. The diagnosis of the condition is mainly based on the history, clinical signs, enzymatic assays, radiography. Even though all these can be



done the best method resorted to diagnose the condition is B-Mode trans-abdominal ultrasound scanning.

Real-time ultrasound scanning of the right ventrolateral abdominal wall in early false pregnancy, or of either flank later, shows large fluid-

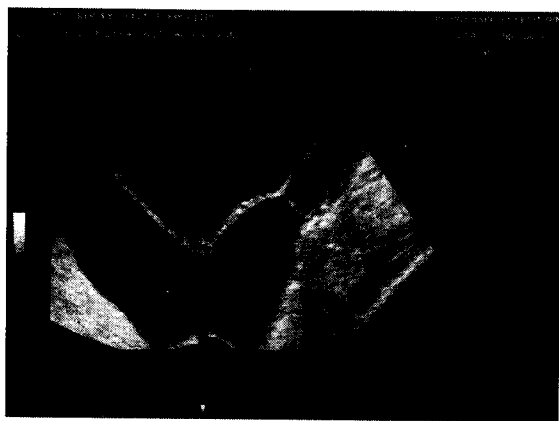


Fig.1 The B-Mode Ultrasonographic picture depicts mobile, echogenic and relatively thin trabeculae (due to coiling & kinking of the uterine horns) and the absence of foetal structures

filled anechoic or hypoechoic compartments with the absence of fetuses or caruncles. White flecks may be seen in the fluid. Scanning should take place at least 40 days after mating to avoid confusion with early pregnancy, and is easier before 70 days.

Abdominal radiography is useful to detect pregnancy. It also provides an accurate alternative when ultrasound equipment is not available. In pseudopregnancy radiograph at 70-90 days of breeding fails to show fetal skeletons in an anoestrus doe with a distended abdomen.

The differential diagnosis of pseudo pregnancy is to be carried out essentially for getting better production from farmers' point to sustain his economy as the goat is considered as "Poor man's cow." The condition should be differentiated from pregnancy, foetal mummification and maceration, pyometra, early embryonic death and even persistent tympany.

(A) Pregnancy: Pregnancy in goats is diagnosed using the clinical trials, lab methods and even the use of highly sophisticated techniques. The pregnancy is diagnosed by methods like palpation, ballotment, vaginal biopsy, ultrasonographic techniques (fig. 2) or even enzymatic assays using RIA which includes Progesterone, Oestrone sulphate, PSP-B, PAG, Placental Lactogen assays.

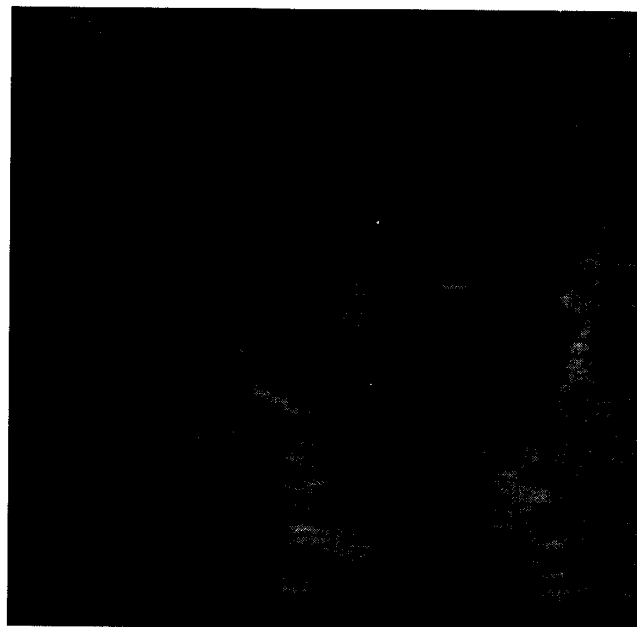


Fig.2 shows the presence of fluid in the uterus along with the placentomes and foetal parts suggestive of the pregnant uterus.

(B) Pyometra: Pyometra is a condition wherein there is accumulation of purulent secretion in the uterine lumen with a persistent Corpus Luteum. It is rare in small ruminants. The real time (B-mode) ultrasonography may reveals that there is a fluid filled uterus lacking placentome with hyper-echogenicity and "snow-storm" or speckled echo texture. The uterine wall will be thick and the uterus will be doughy.

(C) Foetal Mummification: In goats the type of mummification is papyraceous. The mummification may be due to genetic or chromosomal defects, torsion, compression of umbilical cord, placental defects or even any infectious agents. The animal upon examination reveals hyper-echoic areas due to the lack of fluid contrast, fluid free uterus, a hard firm foetus close to the horn of the uterus and absence of placentome. Due to the invasion of infectious agents it

will lead to maceration where in there is foul smelling discharge and presence of bones protruding from cervix.

(D) Foetal Death: Early death of foetus in the genital tract is detected ultrasonographically by the presence of freely floating foetal masses, ribbon like placental membranes lying contralateral to the normal foetus, lack of "crisp" margins of placentomes. The heart beat, foetal movement, amniotic fluid, blood flow through the umbilicus will be absent on examination.

(E) Persistent Tympany: Hydrometra could be associated with tympany in animals. The fluid filled uterus interferes with the eructation reflex leading on to tympany. On per-rectal examination, the uterus will be fluid filled and felt descended into the abdominal cavity. The fremitus, placentome and foetal parts are absent.

After ruling out all these conditions, we may arrive whether the animal is pseudo pregnant or not and the required treatment using $\text{PGF}_{2\alpha}$ is sought for. The treatment should be aimed to return the animal to cycle and to avoid further complications. The efficacy and economy of the treatment regime should be properly resorted to be affordable for the farmer.

Pseudo pregnancy in goats is characterized by a persistent Corpus Luteum. In order to lyse the Corpus Luteum, usually 2 doses of $\text{PGF}_{2\alpha}$ are given and cloud burst (expulsion of the fluid in the uterus) will occur within 1-4 days. After the first dose, a second dose of $\text{PGF}_{2\alpha}$ is administered 12 days after the cloud burst which helps in the remission, cycling of the animal and prevents the recurrence of the condition. Drugs like Tiaprost, Dinoprost and Cloprostenol are found to be effective. The cases that are not responding to PGF_{20} therapy can be treated using 50 IU Oxytocin, BID for 4 days.

Acknowledgement

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CIRRHOSIS IN A DOG

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Introduction

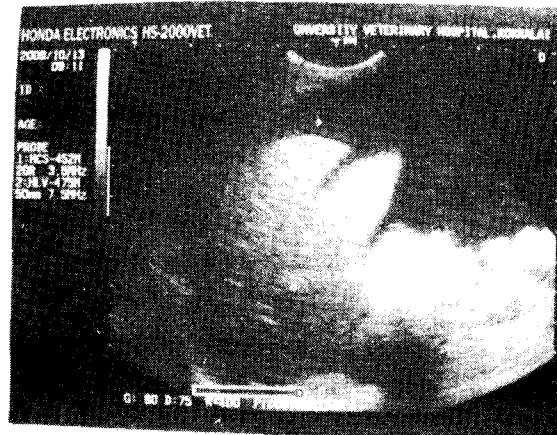
Cirrhosis can be considered as the end stage of many inflammatory hepatic diseases resulting in a series of pathologic events leading to increase in dense fibrous connective tissue in the liver. The entire liver gets affected with disorganization of normal lobular architecture of the hepatic parenchyma with formation of nodules separated from one another by irregular bands of fibrosis. The hepatocellular necrosis of varying etiology results in alternate areas of necrosis and regenerative nodules.

Materials and Methods

A two and a half years old male Rottweiler dog weighing 44 kg was brought to the Veterinary College Hospital, Mannuthy with a complaint of enlargement of abdomen, polydypsia and polyphagia. Abdominocentesis was performed. Whole blood and serum sample were collected for routine laboratory investigations. Faecal sample, wet film and blood smear were examined. Ultrasound scanning was done using DC-6Vet Scanner.

Results

Clinical examination revealed a temperature of 104⁰F and congested mucous membrane. The heart rate and respiratory rate were normal. The circumference of abdomen at the umbilicus was 101 cm. Ascitic fluid collected by abdominocentesis was a transudate. Smear prepared out of ascitic fluid revealed few mesothelial cells and occasional inflammatory cells. There was no malignant or abnormal cells. Ultrasonographic examination of the abdomen revealed the presence of anechoic fluid and fibrin strands. Liver showed increased echogenicity with irregular borders. Other organs were of a normal echo pattern.



Hematological and serum biochemical values include a haemoglobin of 9.3g%, PCV 25.2%, albumin-1.8g/dl, A/G ratio -0.46 and ALT-110 U/L. Based on ultrasonographic findings, hematological, serum biochemical and ascitic fluid examination reports, the condition was diagnosed as cirrhosis. Animal was treated with Lasix[®] @2mg/kg body weight BID, Sylimarin[®] [140mg tab BID], Essentiale L[®] -[1 cap OD], Hermin[®] [100ml intravenously]. Advised a diet low in sodium. The animal responded to the treatment and recovered completely.

DAYS	BODY WEIGHT[Kg]	ABDOMINAL CIRCUMFERENCE[cm]
Day 1	44	101
Day 2	44	101
Day 7	41	96
Day 15	30	71

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Before treatment [fig 2]

On 15th day of treatment [fig 3]

Discussion

Cirrhosis is the result of a series of pathologic events leading to increase in dense fibrous connective tissue in the liver. Clinical signs include vomiting, loss of appetite, ascites, fever, diarrhea, polydipsia and polyuria.

Diagnosis based on clinical signs, haemogram, ultrasound scanning, radiography, electrocardiography, cytology of ascitic fluid, liver biopsy and postmortem lesions. Conditions to be differentiated are Chronic hepatitis or cholangiohepatitis that has not progressed to cirrhosis, chronic obstructive biliary disease, Chronic fibrosing pancreatitis, Hepatic neoplasia, Congenital porto systemic shunts, Metastatic neoplasia or carcinomatosis, Right sided heart failure, Hemolytic anaemia.

There is symptomatic and supportive treatment based on the condition. Removal of primary etiological agent is the first step in therapy. Managing ascites in patients with cirrhosis typically involves limiting dietary sodium intake (less than 2 grams per day) and the use of diuretics.

A diet preferably of easily digestible high quality protein and energy are advised. Fiber in the diet controls hepatic encephalopathy. Dietary fat should be regulated. Provide several smaller portioned meals rather than one or two large meals.

Maintain hydration using crystalloids (lactated ringers' saline or 0.9%NaCl with KCl 20-30mEq/L) or colloids (Haemacel[®]). Supplements like Vit B

complex (2ml/L), Essential amino acids 1 form (Hermin[®]) with supportive therapy. Glucose is given in hypoglycemia. Vit K₁(3mg/Kg BID) to control coagulopathy and anaemia. Gastroprotectives are used to control gastro intestinal ulcerations secondary to portal hypertension (Ranitidine and sucralfate). Anti-oxidants like SAME (S-adenosyl methionine) normalizes liver functions and prevents liver damages and Sylimarin[®] inhibits lipid peroxidation and thus reduces or prevents liver damages. It also promotes growth of new liver cells. Diuretics- spironolactone [1-2mg/kg PO bid], furosemide[1-2mg/kg PO bid], acetazolamide [25mg bid], hydrochlorothiazide [50-75mg IM or SC]. Hepatic encephalopathy is managed with antibiotics to reduce intestinal bacterial load. Eg. Metronidazole[7.5mg/Kg PO tid], ampicillin [20mg/Kg PO tid], neomycin [20mg/Kg PO tid].Lactulose render the ammonia produced in the intestine inabsorbable and increases bowel transit. In fibrosis, steroids like prednisolone (1mg/kgPO od bid) can be advised with caution. To regulate the accumulation of toxic bile acids, ursodeoxycholic acid is used.

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MEDICAL MANAGEMENT OF MULTICENTRIC LYMPHOSARCOMA IN A DOG

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Introduction

Lymphosarcoma is a spontaneously arising, rapidly fatal malignancy of dogs and the most common canine hematopoietic neoplasm. The 4 anatomic forms of lymphosarcoma comprise the following: multicentric lymphosarcoma, which represents 80% to 85% of cases; alimentary (5% to 7%); cranial mediastinal (5%); and cutaneous forms that combine to make up the rest of the cases. Extranodal sites, such as the eye, nasal cavity, testis, central nervous system, and bone, are much less common. This case report describes the multicentric form of the disease.

History

A seven and a half year old, non-descript male dog was presented to the University Veterinary Hospital, Kokklai, Thrissur with a history of listlessness, excessive panting and severe cough. On physical examination, generalized lymphadenopathy was noticed. On palpation, the superficial lymph nodes were found highly enlarged, nodular and hard in consistency. Rest of the physical examination was unremarkable. Lymph node aspirate was taken from the submandibular lymph node and biopsy done. Blood was also collected for routine haematological examination.

Laboratory Findings

Haemogram revealed Total erythrocyte count 4.24 million/cu mm, Total leucocytic count (TLC) 29016/cu mm, while Differential leucocytic count (DLC) indicated neutrophils (59%), eosinophils (5%), lymphocytes (36%), haemoglobin 8.1 g %, Packed cell volume 23.8, Serum calcium 10.70 mg %, Random blood glucose level 59 mg %.

Upon cytological evaluation of lymph node

aspirate taken from the submandibular lymph node and, a monotonous population of large lymphoid cells with high nucleus:cytoplasmic ratio was observed (Fig 1)

Ultrasound Scanning

Ultra sound scanning revealed presence of hyper echoic mass in splenic area and no demarcation between cortex and medulla could be observed in the kidney.

Diagnosis

Based on these lymph node aspiration biopsy and ultrasound scan results, the disease was diagnosed to be Multicentric Canine Lymphosarcoma. The patient was classified according to World Health Organization clinical staging system for Canine lymphosarcoma as Stage IV a.

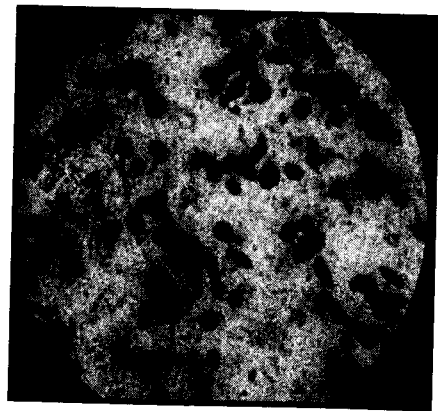


Fig.1:- Lymphnode Aspirate. Monotonous population of large lymphoid cells with high nucleus:cytoplasmic ratio

Treatment

A modified form of the University of Wisconsin-Madison (UW-M) chemotherapy protocol was adopted for treatment (Table 1), along with

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Table 1- Modified UWM Protocol

DRUG	Week 1	Week2	Week3	Week4	Week6	Week7	Week8	Week9
Vincristine@ 0.7mg/m2, i/v	✓			✓			✓	
Cyclophamide@ 200mg/m2, i/v			✓				✓	
Doxorubicin@ 30mg/m2 i/v					✓			✓
Prednisone@ 2.0mg/kg po	✓							
Prednisone@ 1.5mg/kg po		✓						
Prednisone@ 1.0mg/kg po			✓					
Prednisone@ 0.5mg/kg po				✓				
L asparaginase 400units/kg i/m		✓						

supportive therapy using antibiotics, iron supplements and anti-emetics. The animal initially responded very well to the treatment, but at week 6, chemotherapy was interrupted due to fall in haemoglobin level. Blood transfusion was done to counteract anaemia.

Result

The animal showed progressive response initially but succumbed to the disease 8 weeks after initiation of chemotherapy

Discussion

The occurrence of a case of Canine Lymphosarcoma and its treatment was described. Treatment of lymphosarcoma is often rewarding and appropriate chemotherapy can significantly increase survival time. The goals of chemotherapy are to induce a durable clinical remission and to reinduce a remission, if it occurs after one or more relapses. Many protocols are available for treatment and the clinician can choose from among them. The most common presenting clinical sign is generalized painless lymphadenopathy, but other nonspecific signs, such as anorexia, weight loss, vomiting, diarrhoea, ascites, dyspnoea, and fever, are often the only signs of illness. Diagnosis can be made by examining fine needle aspirate of a peripheral lymph node. Differential diagnoses for generalized lymphadenopathy include the following: infectious

causes (such as fungal or rickettsial disease), other cancers (acute and chronic leukaemia), and immune mediated disease (moderate increase in lymph node size). Complete staging involves thoracic radiographs, abdominal radiography and/or ultrasonography, liver, spleen, and bone marrow aspirates.

Summary

A male dog presented with history of listlessness, severe cough, excessive panting and generalized superficial lymphadenopathy was diagnosed as a case of multicentric lymphosarcoma (stage 4b) based on cytological evaluation of LN aspirate. Dog was treated as per Modified UWM protocol, but, after a period of remission animal succumbed to death.

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NODULAR HEPATOCELLULAR CARCINOMA IN A DOG

Usha Narayana Pillai¹, Devi Gopinath², Sameer S², Jomis George²
Suresh Kumar V R², Jones Bobby² and Rechana R²

College of Veterinary and Animal Sciences, Mannuthy

Introduction

Primary hepatic tumours are reported rarely in dogs. Hepatocellular carcinoma (HCC) is the most common primary liver tumour in dogs. Morphologically HCC in dogs can be of three forms namely Massive, Nodular And Diffused. The massive form present as a solitary, large mass confined to one liver lobe. The nodular form represents multifocal disease, with several nodules present in different liver lobes. The diffuse form may present in late stage of tumour development, characterized by multifocal to coalescing nodules in all liver lobes or diffuse effacement of the hepatic parenchyma. Among these the massive form is most common.

There is no firm etiopathogenesis established in dogs. In humans, infection with hepatitis virus B and cirrhosis is the major risk factor for the development of hepatocellular carcinoma. The canine HCC typically having the lowest metastatic rate which is attributed to the more common in massive form of HCC. The use of imaging modality such as ultrasound scanning is the most important diagnostic tool for hepatic neoplasia. The histopathologic studies of liver parenchyma and estimation of serum alpha fetoprotein (AFP) concentration, a major tumor marker of HCC, are also required for the confirmation of hepatocellular carcinoma. The present paper deals with a case of nodular hepatocellular carcinoma in dogs.

Materials and Methods

A four year old male German shepherd dog brought to the Veterinary College Hospital, Mannuthy, with symptoms of anorexia, lethargy, vomiting and polydipsia. Clinical examination revealed elevated temperature (106°F), pulse (108/min) and icteric mucous membrane. Abdominal palpation revealed a cranial abdominal

mass raised the suspicion of hepatomegaly or splenomegaly. Fecal sample and blood smear examinations were carried out to rule out gastro intestinal and hemoprotozoal infection. The urine sample was collected for routine urinalysis. Whole blood and serum samples were collected for estimating complete blood count including platelet, total protein, albumin, globulin, serum bilirubin and serum SGPT. The clotting time was also estimated.

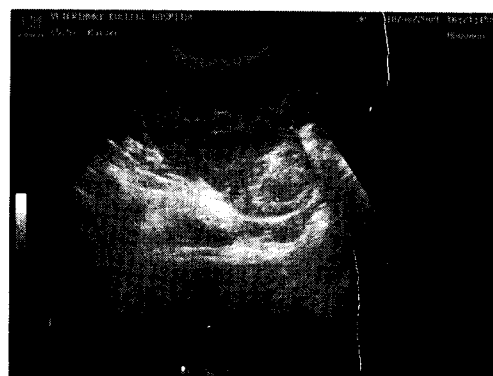
Diagnosis

Urinalysis: Hyposthenuria, Glucosuria and Proteinuria

Hematological examination: low PCV(26%), Leucocytosis (34000/mm³) with Neutrophilia(80%), Lymphopenia (15%), and Monocytosis (4%). Clotting time was elevated (6min).

Serum biochemistry: Hypoproteinemia (5.60 gm%) with Hypoalbuminemia(1.60gm%), Hyperglobulinemia (4gm%). Slight Hyperbilirubinemia (2.1mg/dl) and elevated SGPT level (199u/l).

Ultrasonography: Mixed echogenic pattern with hyperechoic areas of cavitated lesions and anechoic areas in between the liver lobes & nodular masses of varying sizes.



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Alpha-feto protien: A major tumor marker for HCC. It is an Oncofetal glycoprotein. It is active only in fetal tissues. Normal value of Alpha Feto Protein in case of adult dog is <10 ng/ml. The serum alpha feto protein value was 298.6 ng/ml.

Fine needle biopsy of liver : Carried out under aseptic procedures and tissue was subjected to histopathological examination.

Histopathological studies showed that the carcinomatous growth composed of group of large pleomorphic polyhedral or oval cells having hyperchromatic nuclei. There was altered nuclear cytoplasmic ratio and some of the cells have nuclear vacuolation. The cells in some areas showed trabecular pattern. The stroma showed extensive areas of necrosis and degeneration. The histopathological appearance confirmed the condition as hepatocellular carcinoma.



Treatment

Animal was treated with oxytetracycline @ 10mg/kg bodyweight intra-venously, Dextrose 10% and liver extract for 3 days pending the result of histopathology. Since histopathology confirmed as HCC, the gold standard treatment of partial hepatectomy was recommended.

Discussion

Hepatocellular carcinoma occurs in various species including cats, dogs, cows, sheep, pigs and horses. The dog may have a higher incidence of hepatocellular carcinoma than others. The average age of affected dogs is 10 to 11 years, although they have been reported in dogs as young as 4 years of age. Affected cats range from 2 to 18 years of age. In dogs this neoplasm is more frequent in males than in females, but no breed predisposition has been identified. The etiology of spontaneously occurring hepatocellular carcinoma in domestic animal is unknown but chronic infection or chemical ingestion may play a role in tumour development. Morphologically hepatocellular carcinoma could be divided into massive, nodular or diffused

Hypoproteinemia, and altered Albumin:Globulin ratio and elevated ALT level also suggested hepatic involvement. Elevated level of Alpha feto protein (tumor marker) help for early detection of HCC.

The use of imaging modality such as ultrasound scanning is the most important diagnostic tool for hepatic neoplasia. Histopathological studies of liver parenchyma and estimation of serum alpha feto protein concentrate, a major tumor marker of HCC are also required for the confirmation of HCC.

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ARTIFICIAL INSEMINATION IN DOGS

Shibu Simon¹, Visakh Viswam², Harish S.², Sarika K.S.², Lakshmi V.Nair²,
Sneha Augustine² and Soumya Vijayakumar².

College of Veterinary and Animal Sciences , Mannuthy

Introduction

'A dog can be man's most faithful companion'- often we have seen this statement, be it in any movie or even any literature. At present, and even in the on coming future this notion holds and will hold true. Companion animal ownership is gaining momentum in this world where human relationships are becoming increasingly flaccid, so much so that maintaining companion animals has become a part of the lifestyle. This state of affairs can be attributed to the unrelenting relationship that a dog holds with its owner till its last breath. Consequently the role of dog breeders has attained considerable magnitude and owners or clients as they may be called, are not willing to make any compromise regarding the quality of the breed, what ever the expense may be. Hence artificial insemination which can be used to attain and improve desirable characteristics in dogs holds a magnanimous value.

Materials and methods

A female Bull mastiff dog of 2 years and whelped
o n c e

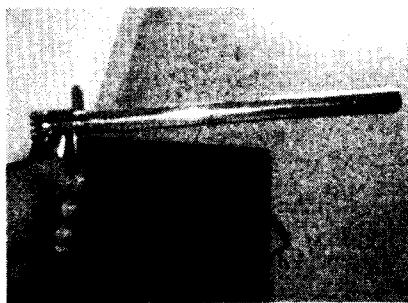


Fig1: The Sigmoidoscope

before was brought to the Veterinary College Hospital, Mannuthy for breeding advice. The owner complained that the animal was having a previous history of abortion on its 55th day of gestation. On vaginal cytology, 40% superficial cells and 60%

anuclear keratinized cells were observed suggesting the animal in oestrus. The owner was advised to cross the animal on the next day after admission. Two days after the owner complained that the animal was not allowing mating. The clinical parameters were recorded and found normal. The cytologic picture revealed 65% anuclear keratinized cells and the rest superficial cells. On sigmoidoscopic examination, vaginal crenulations were noticed suggestive of oestrus (fig.1 & 2). So we decided to perform vaginal method of artificial insemination in the dog after semen collection from a male dog of the same breed.

The semen was collected by digital manipulation and was evaluated for the quality (fig.3). An AI gun with sheath was inserted so as to reach the external os of cervix. After inserting, once the sheath reached anterior vagina the AI gun was removed and a 4 ml syringe with semen was attached to the sheath and insemination was carried out. Feathering was done by stroking the upper and lower walls of vagina to stimulate vaginal contractions so as to enhance semen motility. After finishing the insemination dog was kept in a position

with raised hind limbs for about

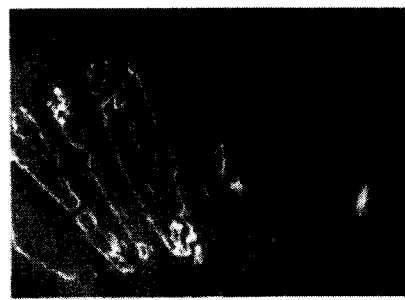


Fig2: Anterior vaginal wall during early oestrus

15 minutes so as to prevent the back flow of semen.

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²Final year BVSc & AH Scholars
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Fig 3: Semen collection by digital manipulation

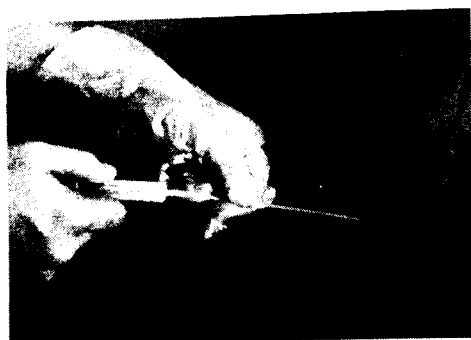


Fig 4: Vaginal insemination

Two days later the animal was presented for a second insemination. Cytologic picture on that day clearly revealed that the animal was in met-oestrus (large intermediate: 60% ; superficial cells: 20% ; parabasal cells 20% and numerous WBCs were present) and the animal was not inseminated.

Result

After a month the animal was brought for the pregnancy diagnosis. Animal had increased its weight from initial 48 kg to 58 kg and enlarged mammary gland. On abdominal palpation, the abdomen was found distended and a hard thick mass at uterine area suggestive of foetal mass was felt. To confirm the pregnancy, performed ultrasonography (fig.5) and gestational sacs were found. The owner was to make a review on 45th day of gestation to take measures to prevent abortion. But the animal was not brought and on enquiry we found that the animal had aborted 10 puppies on 55th day of gestation. However AI done later in another female of the same owner using semen from the same male gave birth to 11 puppies.



Fig 5: Ultrasonographic view of the uterus with gestation sacs at 34 days of gestation

Discussion

Semen collection

Ideally several days of sexual rest should be allowed prior to semen collection and evaluation. Canine semen is most easily collected using digital palpation with a gloved hand (digital manipulation). It can be done preferably in the presence of a bitch in oestrus or a teaser bitch in anoestrus. An artificial pheromone , p-hydroxy benzoic acid can be applied to the vulva and hindquarters of bitch in anoestrus to simulate oestrus. Semen can also be collected using an Artificial Vagina usually made of latex. Optimally , the male and female are brought together in leashes in a quiet room with nonslippery flooring. As dog sniffs at the bitch's vulva or mounts her , the collector quickly moves the prepuce back, behind the bulbus glandis exerting firm pressure behind the bulbus glandis. Once this occurs, the dog will show pelvic thrusting and normal ejaculation.

Factors preventing normal mating

Psychological factors like difference in sexual experience, fear of human disapproval, unfamiliarity with the venue, overfamiliarity, humanization, lack of libido. Diagnosis and therapy of these conditions are difficult. Measurement of circulating testosterone concentrations is not diagnostic because of the large variation throughout the day in normal dog. Administration of hCG may increase testosterone production but is not therapeutic. Administration of testosterone is also not therapeutic and may cause aggression and impaired spermatogenesis due to inhibition of release of



ARTIFICIAL INSEMINATION IN DOGS

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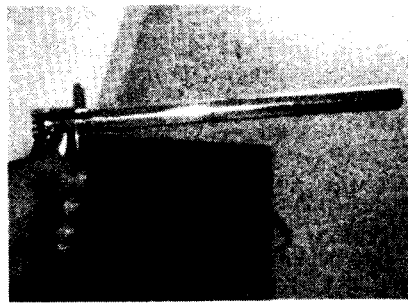


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pituitary gonadotropins. Thyroid replacement therapy may improve libido but such regimes have not been properly evaluated.

Physical factors: Dogs with obvious limb deformities and musculo-skeletal lesions may have problems in achieving a position compatible for intromission; they should not be used for breeding if these conditions are inherited. Subtle lumbar and lumbosacral lesions may prevent dogs from mating, even though there is no evidence of pain in other everyday activities. Dogs with prostatic disease may show signs of pain during or after ejaculation. Dogs with persistent penile frenulum may be asymptomatic but may show pain during excitement, licking of the area or deviation of penis during erection. Treatment is usually surgical correction under light anesthesia. Diseases of the penis like phimosis, deviation of the penis, diphallus etc. may prevent normal mating.

Physical obstruction to mating: Congenital anomalies or changes in the size or shape of the vulva, vestibule or vagina may prevent normal copulation in dog. Male dogs may experience difficulty in achieving intromission of the penis in young bitches with an infantile vulva. Bitches with abnormal sexual differentiation may have abnormal external genitalia. Five major types of abnormalities in embryonic development of the vaginal vault is reported and each will result in narrowing of the tract called vaginal strictures. They include a band of fibrous tissue crossing and narrowing the lumen of vagina, an annular fibrous ring compressing the vaginal lumen, vulval hypoplasia that narrows vulval opening, incomplete fusion of two mullerian ducts causing double vagina and hypoplasia of the vaginal vault. The clinical signs of these conditions include vulval discharge, chronic vulval licking and attracts male, but the bitch will be unwilling or unable to breed. Breeding bitch with these strictures will exhibit normal pro-oestrus, oestrus, mating behaviour, standing and flagging of the tail. But attempts at penetration by the male into the vaginal lumen are associated with the bitch showing signs of pain, moving away from the male or biting at the stud dog or handler. The dogs with retained hymenal remnants may also prevent normal copulatory behaviour.

Insemination is indicated in perceived inability for the male and female to breed. This includes the afore mentioned factors like orthopaedic problems in male as well as in females, major size difference between mates, to avoid venereal diseases like brucellosis, herpes infection, transmissible venereal tumor etc. being transmitted from female to male or vice versa and in males especially Dobermann Pinschers to avoid prostatic bleeding.

Methods of artificial insemination

The two main methods of artificial insemination include vaginal insemination and uterine insemination.

Vaginal insemination: In vaginal insemination first semen is deposited into the vagina using a long inseminating pipette which is later flushed with prostatic fluid or warm physiological saline. Thereafter the vagina is stimulated with fingers which help to initiate vaginal contractions and thus assist in sperm transport. Thereafter the hind quarters are held in raised position for the semen to pool around the cervix. In this case we have followed this method.

Uterine insemination: Here we have 4 main methods namely Foley osiris technique, Norwegian technique, Endoscopic technique, Surgical technique.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary And Animal Sciences, Mannuthy and Professor & Head of Veterinary College Hospital, Mannuthy for providing facilities for the study.

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BENIGN ADENOMA OF NICTITANS GLAND IN A CROSSBRED DOG

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Vijesh Kumar Saini² and Praveena Babu³

College of Veterinery and Animal Sciences , Mannuthy

Introduction

Adenoma is a benign tumor of glandular origin and these growths may overtime progress to become malignant, at which point they are called adenocarcinomas. Harderian glands are found within the eye's orbit which occurs in vertebrates that possess a nictitating membrane. In some animals it acts as an accessory to the lacrimal gland, secreting fluid that eases movement of the nictitating membrane. In canines, harderian gland is otherwise known as nictitans gland. Benign adenoma of nictitans gland is rare in dogs and the present paper describes a case of benign adenoma in a crossbred dog and its successful surgical treatment.

History and clinical findings

A male crossbred dog, aged eight years was presented to University Veterinary Hospital, Kokkalai with the history of abnormal mass protruding from the medial canthus of right eye. The mass was in existence



since two months and the dog was undergoing treatment with antibiotics, anti-inflammatory and analgesic eye

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drops at local hospital.

On examination the dog was apparently healthy and having normal feeding and voiding habits as narrated by the owner. All the clinical parameters were within normal physiological range. A hard friable mass to the size of a quail egg could be observed at the inner canthus medial to the third eye lid of right eye (Fig 1). The cornea of right eye was normal and healthy. Fine needle aspiration cytology of the mass revealed few red blood cells and neutrophils. Since the mass was obstructing the visual field of dog surgical excision was resorted.

Surgical treatment

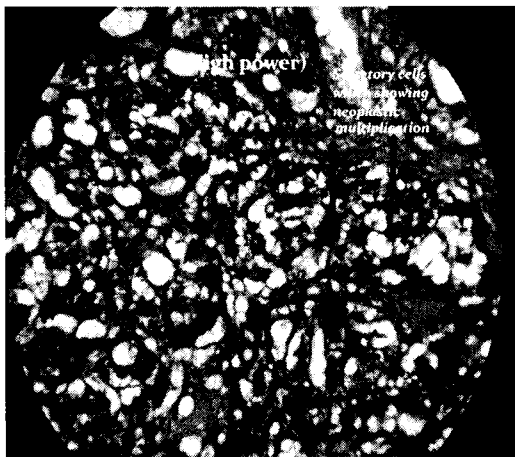
The dog was brought for surgery after overnight fasting. Since the animal aggressive to handle all preoperative preparations were performed after administration of atropine sulphate (Atropin, Neon Labs) 0.045 mg/kg bodyweight and ten minutes later xylazine hydrochloride (Xylaxin, Indian Immunologicals) @ 1.5 mg/ kg bodyweight intramuscular for premedication. Twenty minutes later, ketamine hydrochloride (Kemin, Themis Chemicals) @ 7.5 mg/ kg bodyweight was administered intramuscular to effect anaesthesia. To achieve satisfactory level of muscle relaxation 10 mg of diazepam (Calmpose, Ranbaxy) was administered intravenously.

The dog was restrained on the table with the affected eye above. The mass was cleaned with sterile cotton swab and thoroughly irrigated with sterile warm normal saline followed by administration of antibiotics eye drops. The mass was held with a pair of babcock forceps and everted slightly to expose its base. A small incision was put close to its base at the lower margin of third eyelid to begin ligation of the mass and suturing was completed using plain catgut as continuous



lockstitch along its entire length. A pattern of simultaneous incision of small length and suturing was followed for ligation. The mass was sectioned completely after putting the final suture and the bleeding points were controlled by application of adrenaline. The eye was irrigated with normal saline and infused with chloromycetin ointment.

Post operatively inj of amoxicillin 250mg and cloxacillin 250mg (Megapen, Aristo) and meloxicam (Melonex, Intas Pharmaceuticals) 15mg were administered intravenously. Oral administration of amoxicillin 250mg and cloxacillin 250mg tablets thrice daily were continued for five days. The dog had uneventful recovery.



Histopathological examination of the mass confirmed as neoplastic multiplication of glandular

secretory cells and in some areas infiltration of interstitial space with neoplastic cells indicating a border line malignancy

Discussion

In dogs the different conditions affecting the third eyelid are cherry eye, tumors of harderian gland, eversion of cartilage, follicular conjunctivitis and inflammatory disorders. Among this tumor of harderian gland occur in older animals and their malignant nature is relatively rare. In the present case the condition was encountered in a crossbred dog with benign nature having slight border line malignancy. The treatment of the tumor was conducted surgically and it did not show any recurrence.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy for carrying out the work and according permission to publish the paper

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CLINICAL CONFERENCE CONDUCTED AT COLLEGE OF VETERINARY AND ANIMAL SCIENCES, MANNUTHY

A clinical conference was held at College of Veterinary and Animal Sciences, Mannuthy, the final year (2005 batch) students being the participants. In clinical conference, a group of students take up a case presented in the hospital and extend it beyond the frontiers of just diagnosis and treatment, under the guidance of an expert clinician. They are expected to have a comprehensive approach on the condition, rather a detailed study, the successful culmination of which demands a presentation before the staff, students and subject to evaluation by a panel of professors. Clinical Conference papers were contributed to the JIVA for publication. Students of three batches who presented the following topics emerged victorious.

- I. Pseudopregnancy in a goat
- II. Second Degree Heart Block with mitral valve insufficiency in a dog.
- III. Benign adenoma of nictitans gland in a dog

Dr. Shibu Simon, Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, was entitled "The Best Guide" for the year.

TRANSMISSIBLE VENEREAL TUMOUR IN A BITCH

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Subhash Raj K² and chithra P Arunima²

College of Veterinary and Animal Sciences, Mannuthy

Introduction

Canine breeding nowadays has become a lucrative venture. Successful planned breeding is contributed by both male and female. But there are some disease conditions that affect the females adversely which will bring about great economic loss to the dog breeders. Transmissible venereal tumour (TVT) is relevant in this regard since breeding is not advised in affected animals as it is transmissible from male to female or vice versa. This case report represents the successful treatment of TVT in a Dobermann bitch using Vincristine sulphate.

Case History

A female Dobermann pinscher of age one and a half years old with 23.3 kg body weight was presented to the Small Animal Obstetrics and Gynaecology unit of Veterinary College Hospital, Mannuthy on 25.6.2009 with the complaint of bleeding from the external genitalia for the past fifteen days. On anamnesis it was found that the bitch had no mating history. On further queries it was found that the owner bought the bitch from another kennel three weeks back.

Observations

All the physical parameters were found to be within the normal range. The animal appeared to be alert and active with normal food habits. Body condition of the animal was graded as fair with mild skin lesions on the hind limbs.

On clinico-gynaecological examination with gloved hand, it was observed that there was pooling of serosanguineous, foul smelling discharge at the ventral commissure of vulval lips. On pervaginal examination, a solitary nodule could be felt at the floor of vestibule.

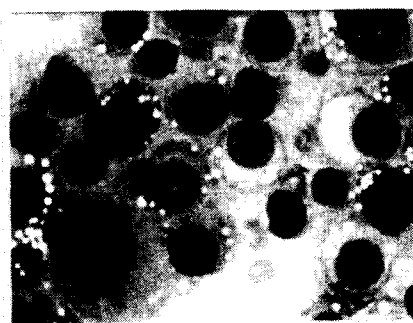


TVT lesion on the floor of vestibule

Sample was taken from the vaginal vault for the exfoliate vaginal cytology (EVC) by the cotton swab method and an impression smear also was taken. The smears thus obtained were stained using Modified Wright Giemsa stain. The area was cleaned with potassium permanganate solution and a local styptic Botroclot solution was instilled to arrest bleeding.

Cytological Finding

Large homogeneous sheets of round to oval cells with increased nuclear cytoplasmic ratio, prominent nucleoli, scant cytoplasm and multiple clear cytoplasmic vacuoles often arranged in chains.



TVT CELLS

¹Assistant Professor

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DIAGNOSIS

The case was diagnosed to be Transmissible Venereal Tumour

Treatment

Chemotherapy using Oncocristin-AQ (Vincristine sulphate) was given after calculating the total dose based on the standard dose rate of 0.025mg/kg. 1 ml of the drug was diluted with 9 ml distilled water and 5.8 ml was administered intravenously with utmost care to prevent infiltration into perivascular or subcutaneous space. The owner was advised to observe the animal for any vomiting or general weakness and asked for a review after one week.

On second visit (2.7.2009), per vaginal examination with the gloved hand revealed slight reduction in the size of tumour mass. Also reduction in the number of tumour cells in EVC was noticed. Since the tumour mass was not completely regressed, Oncocristin injection was repeated on the same day.

The treatment regimen was continued at 1 week interval for the next three visits. In each visit, EVC was done and assessed the regression rate of tumour cells.

On the sixth visit (5.8.2009), on pervaginal examination with gloved hand, the tumour mass was found to be completely regressed and also no tumour cells could be detected in the EVC.



Floor of vestibule after recovery

Clinical Outcome

Since the tumour cells were absent in the smear no treatment was suggested during the last visit. But the owner reported inappetence of the

animal after treatment and hence advised appetite stimulants for one week.

The animal was brought to the clinic on 23.9.2009 for breeding advice. Spotting was observed by the owner for the past five days. On clinico-gynaecological examination with gloved hands the vulval lips were found to be moderately oedematous and turgid to touch. The animal resisted digital manipulation of vulval lips. No flagging of tail was observed.

A sterile swab was taken for EVC. The cell picture was suggestive of early pro-oestrus (50% superficial cells). On second visit (25.9.2009), the EVC picture revealed late pro-oestrus with mild infection. On next visit (29.9.2009), the EVC picture revealed early oestrus (50%superficial and 50%AKC). Hence advised to cross on 30.9.2009, 3.10.2009, 6.10.2009. Also advised folic acid tablets.

Animal was brought for pregnancy diagnosis after 35 days from the last service. On ultra sound scanning no gestational sacs or foetus or foetal skeleton could be observed.

Discussion

TVT is a naturally occurring disease in dogs that usually involves the external genitalia and is typically transmitted at coitus. Stray dogs serve as the reservoir for this problem, which is seen most frequently in temperate climates and large cities

TVT is considered as an example of a naturally occurring allograft. It is assumed that transmission is the result of exfoliated cells from the donor being "seeded" into the damaged genital mucosa of the recipient. The tumour mass may have a cauliflower like shape but also be pedunculated, nodular or papillary. They may be grey or pinkish grey in colour, seen mainly in the external genitalia. The signs of male TVT include preputial swelling, stranguria, phimosis and foul smell. Lesions may also be seen in the extra genital areas like oral cavity, nasal cavity, face etc. In single drug chemotherapy, vincristine sulphate at weekly module at a standard dose rate of 0.025mg/kgBW is the most preferred drug of choice. Doxorubicin @ 30mg/m² intravenously every three weeks can be used as an alternative if there is no response to vincristine sulphate. Also combination chemotherapy using vincristine (0.0125-0.025mg/Kg IV weekly), cyclophosphamide

(1 mg/Kg orally daily or 50 mg/m² orally on even numbered days) and methotrexate(0.3-0.5 mg/kg iv weekly or 2.5 mg/m² orally on odd numbered days is also practiced. Radiation therapy was also reported to be successful in dogs. Surgical excision of the tumour could be considered if tumour is small and accessible.

TVT should be differentially diagnosed from mast cell tumour, histiocytic sarcoma, histiocytoma, canine lymphoma, canine fibroma and vaginal hyperplasia.

Conclusion

Dobermann pinscher presented with transmissible venereal tumour was treated with Vincristine sulphate and the animal had an uneventful recovery.

Acknowledgement

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CHRONIC HEPATITIS IN A RUSSELL'S VIPER (*Daboia russelii*).

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Introduction

The Russell's viper (*Daboia russelii*) is among the most poisonous snakes of India. Considering the internal anatomy, the liver is the largest organ, elongate, single, and encapsulated, spaced between the heart and the stomach. The lungs are tubular, the digestive system is well developed and the genito-urinary organs are paired (Jacobson, 2007). Though Indian snakes are candidates for many organ pathologies, the available reports are scanty.

Hepatitis is inflammation of the liver, focal or diffused, characterized by the presence of inflammatory cells in the organ tissue, irrespective of the cause (McGavin and Zachary, 2007). Hepatitis is common in humans and animals but the reports in Indian reptiles are scarce. A case of chronic hepatitis in a captive Russell's Viper coupled with intestinal helminthosis is described.

Materials and Method

A Russell's Viper was brought from the Zoological Garden, Thrissur to the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, in July 2009, for necropsy (Fig. 1). History revealed that the snake was shifted from Thiruvananthapuram to Thrissur zoo two weeks before death. The animal was off-feed for a week and was found dead that morning. Any previous illness was not known. A detailed necropsy was conducted. Pieces of heart, liver, kidney and intestine (3mmx3mm) were collected in 10% formalin fixative for routine histopathological studies. The intestinal contents were scraped, mixed with a drop of normal saline on a glass slide and any evidence of intestinal parasitism was looked for microscopically.

Results and Conclusions

On post mortem examination, the liver was the most affected organ with patchy yellowish areas on the surface and a thick capsule (Fig. 2). The organ was firm with smooth surfaces and sharp contours. The kidneys showed stray areas of mild congestion. The mucosa of the small intestine revealed mild catarrhal enteritis. The other organs had no pronounced gross lesions.

Histopathological examination of Haematoxylin and Eosin stained liver sections revealed massive infiltration of lobules by chronic inflammatory cells (Fig. 3). The hepatocytes had cytoplasmic vacuolations indicating fatty degeneration. Hepatic parenchyma had marked focal sinusoidal dilatation and areas of hemosiderin deposition. Portal areas evinced fibrous tissue proliferation with focal mononuclear cell infiltration (Fig. 4). The liver capsule had multilayered fibrous tissue proliferation. Renal blood vessels were congested, with occasional fatty degeneration and desquamation of the tubular epithelial cells (Fig. 5). Sections of the intestine showed congestion of the sub mucosal blood vessels with a few infiltrates in the lamina propria.

Microscopically, the intestinal contents revealed a single species helminthosis. On direct examination, thin shelled, oval eggs with a mass of undifferentiated embryo inside, identical in structure to that of Strongyle species could be seen (Fig. 6), about 4-5 per low power field. Interestingly, no adult worms could be appreciated in the mucosal contents.

Chronic hepatitis was attributed to be the cause of death. Helminthosis was assumed to be sub-clinical.

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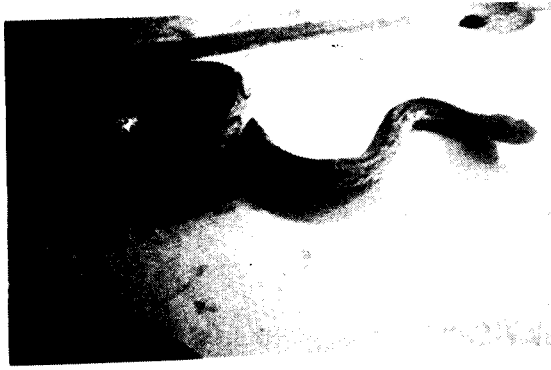


Figure 1 Russell's viper brought for necropsy

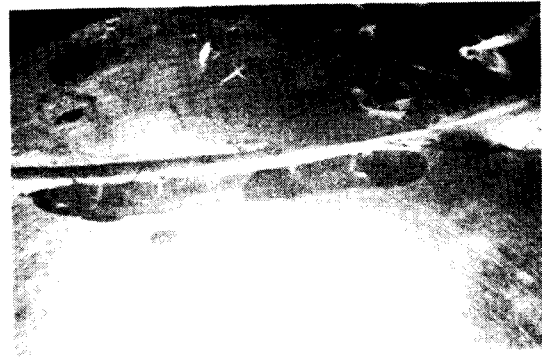


Figure 2 Liver with yellowish areas and thick capsule

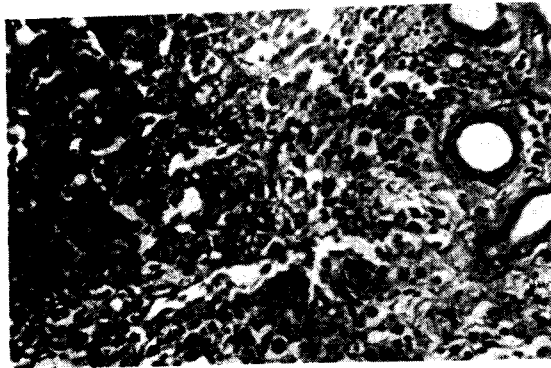


Figure 3 Massive infiltration of liver with chronic inflammatory cells (H&E x 400)

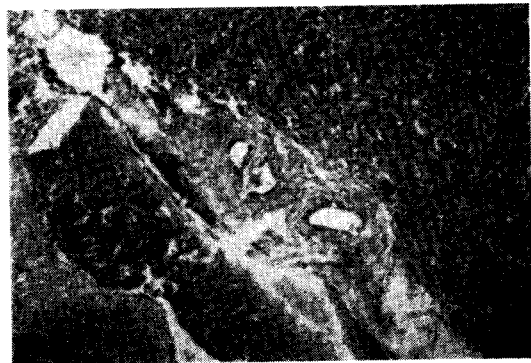


Figure 4 Fibrous tissue proliferation in the portal areas (H&E x 100)

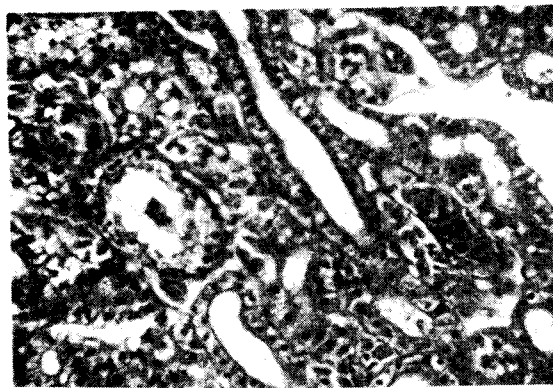


Figure 5 Congestion of renal blood vessels and renal tubules showing fatty degeneration and desquamation (H&E x 400)



Figure 6 Parasitic ova in the intestinal scrapings (x400)

The microscopic changes observed in the liver correspond to those described for chronic hepatitis in other animal species by McGavin and Zachary (2007). Endoparasitism in captive snakes of Kerala has been recorded by Radhakrishnan *et al.*, (2009). Previous reports of organ pathology in the captive snakes of the state were not found.

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SEPTICAEMIC PASTEURELLOSIS IN JAPANESE QUAILS (*Coturnix coturnix japonica*)

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Introduction

Japanese Quails (*Coturnix coturnix japonica*) are reared mainly for meat and egg. Quails are preferred by many farmers compared to chicken because of its short life span (3-4 generations in an year), less expense for feed, less space for rearing. In the present paper death among quails due to septicaemic pasteurellosis caused by *Pasteurella multocida* and its diagnosis and successful treatment with antibiotics is documented.

Materials and methods

Death among quails was reported to Clinical Laboratory, District Veterinary Centre, by a private farmer (rearing about 1000 quails) in Akathethara panchayath from Palakkad district. Five dead birds submitted within two hours of death, formed the material for investigation. Detailed post mortem was conducted and gross lesions were recorded.

Heart blood smear and impression smears from liver and spleen were stained with giemsa. Heart blood, swabs from liver, spleen and kidney were cultured in Brain Heart Infusion Agar (BHIA) with five per cent sheep blood and Mac Conkey's agar and incubated at 37°C under aerobic conditions.

In addition, portions of liver, spleen and intestine were cultured for *Salmonella* by incubating macerated tissues in Selenite F broth at 37°C for 48 hrs. This enrichment was followed by culture on Mac Conkey's agar, Brilliant Green Agar (BGA) and Xylose Lysine Deoxy cholate (XLD) Agar and incubated overnight at 37°C.

Polymerase Chain Reaction using species specific PCR primers was done for confirmation of the isolate obtained.

Results

The most important clinical signs noticed by

the owner were droopiness and sudden death among birds. Gross lesions observed upon necropsy were, pinpoint hemorrhages and necrotic areas in the liver



petechiae in heart, hemorrhages in spleen, trachea, ovary and serosal surface of proventriculus. Catarrhal enteritis and hemorrhages in the mucosa were also observed in the intestine. Almost all the internal organs were showing hemorrhages on the surface.



Heart blood smear and impression smear from liver and spleen upon Giemsa staining revealed presence of bipolar stained bacteria suggestive of *Pasteurella multocida*.

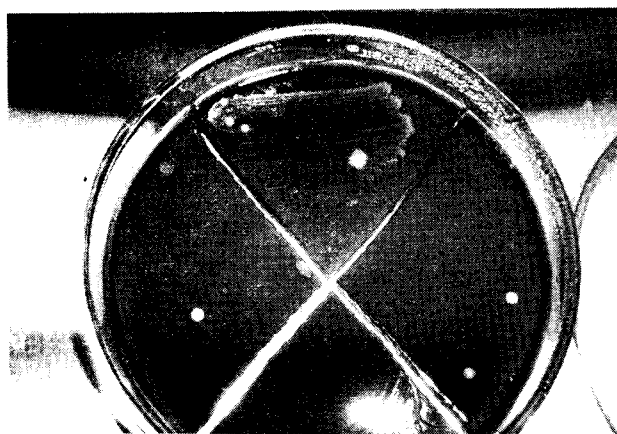
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Bipolar stained bacteria suggestive of *Pasteurella multocida*.

Culture of heart blood, liver and spleen in BHIA with 5 per cent sheep blood yielded growth of *P. multocida* in pure form. Pure cultures revealed mucoid, convex, greyish-white and non-haemolytic colonies. Culture in Mac conkey's agar didn't reveal any growth even after 48 hours of incubation. Enrichment culture of liver, spleen and intestine for *Salmonella* did not reveal any growth even after 48 hrs of incubation.



The isolate of *P. multocida* obtained was Gram-negative, non-motile, cocco bacillary (Fig. 6), grew aerobically and anaerobically, did not grow on Mac Conkey's agar and was non-haemolytic on blood agar. It was catalase and oxidase positive and fermented glucose.

In the second stage biochemical tests, the isolate tested was indole positive, methyl red and Voges-Proskauer negative, urease negative, did not produce H_2S , reduced nitrate, gelatin liquefaction negative, beta-galactosidase activity negative, ornithine

decarboxylase positive and citrate utilization negative. Results from the first and second stage biochemical tests revealed that the isolate was *Pasteurella multocida*.

Antibiotic sensitivity test of the isolate obtained was done as per the standard single disc diffusion method of Bauer *et al.* (1966) using Mueller Hinton's agar. The isolate obtained was sensitive to antibiotics like Enrofloxacin, Ciprofloxacin, Gentamicin, Ampicillin, Chloramphenicol and resistant to Sulpha-TMP.

Amplification of the *P. multocida* isolate obtained from quail by PCR using species-specific primer pairs, KMT1SP6 and KMT1T7 generated product of approximately 460 bp size (Fig. 7).

Based on the post mortem lesions, culture for bacteria and polymerase chain reaction, death of quails was diagnosed as due to septicaemic pasteurellosis.

Discussion

Quails are susceptible to a variety of noninfectious, infectious, and parasitic diseases. Because they are related to chickens and turkeys, many of the diseases in quail are similar to those in poultry. There are reports that pasteurellosis can cause per acute disease in quails with very high mortality. Glisson *et al.* (1999) reported acute mortality due to pasteurellosis in three flocks of Japanese quails with approximately 75,000 birds in each flock. Multifocal small pale areas on liver, spleen and lungs slightly darker in color than normal were noticed as gross lesions. *Pasteurella multocida* serotype 3, 4 was isolated from affected tissues. The treatment was done successfully with chlortetracycline. Goto *et al.* (2001) also reported outbreak of infectious septicemia in a flock of fifty Japanese quails with severe bacteremia observed in all cases. The technique for confirmation was isolation and identification of causative bacteria *i.e Pasteurella multocida* from blood and internal organs.



Primary isolation of *P. multocida* from heart blood and tissues like spleen and liver can be done in five per cent ovine and bovine blood agar. Rimler and Rhoades (1989) have suggested the use of bovine, equine or ovine blood in the media for isolation of *P. multocida*. The blood agar plates, streaked with suspected material can be incubated at 37°C with mild CO₂ tension. These conditions were found to be ideal for the growth of *P. multocida*.

The bacterium is sensitive to commonly used antibiotics like Enrofloxacin, Ciprofloxacin, Gentamicin, Ampicillin, Chloramphenicol *etc* but showed resistance to Sulpha.

Treatment

The disease in quails was successfully treated with oral preparations of ten per cent Enrofloxacin for five days along with supportive therapy using liver stimulants (Liv 52 syrup orally) and vitamin supplements.

Conclusion

Death among a flock of Japanese quails was reported to Clinical Laboratory, DVC, Palakkad for disease investigation. Upon post mortem all the birds were showing lesions suggestive of septicaemic pasteurellosis. Isolation of bacteria was attempted in brain heart infusion agar enriched with 5 % sheep blood. Based on cultural characteristics, primary and secondary biochemical tests and polymerase chain

reaction the isolate was characterized as *Pasteurella multocida*. The disease in ailing birds was successfully treated with antibiotic enrofloxacin supported with liver stimulants and vitamin supplements.

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SECOND DEGREE HEART BLOCK WITH MITRAL VALVE INSUFFICIENCY IN A DOG

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History and Clinical findings

A seven year old male spitz dog was brought to the University Veterinary hospital, kokkalai with the complaint of repeated severe epileptic attacks for the last 3 years. Animal exhibited signs like falling while running, passing of urine and faeces, gets thrown to both sides, leg stiffing, circling, eye bulging, howling, nocturnal coughing and weakness with a gap of 15 days between the episodes. The owner reported that the dog was under phenobarbitone therapy(30 mg tab BID) for three years. Upon clinical examination, respiration and temperature were found normal. Pulse rate was slightly reduced and arrhythmia was noticed.

Electrocardiography revealed isolated P waves without QRS complex indicating second degree heart block (figure 1.1). Colour doppler echocardiography revealed hypokinastia of cusps, indicating valvular insufficiency and increased turbulence at mitral valvular area indicating valvular insufficiency. (figure 2.1). Haematological results showed neutrophilia (82%). Biochemical evaluation of serum revealed lowered thyroid hormone status (30µg/dl).

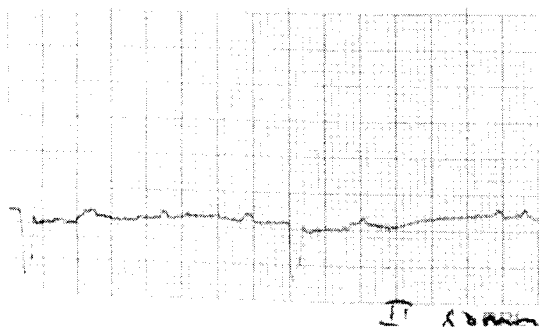


Figure 1.1. Electrocardiogram showing second degree heart block (before treatment)

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Figure 2.1 Colour Doppler Echocardiogram showing increased turbulence at mitral valvular area (before treatment)

Treatment

As a treatment for second degree heart block, single dose of atropine sulphate, was administered at a dose rate 0.02mg/kgBW subcutaneously and owner was advised to repeat the same treatment if there is any recurrence of arrhythmia. Antibiotic therapy with a combination of amoxycillin and clavulanate suspension at a dose rate of 12.5mg/kg BID orally was advised for 5 days. Also the owner was advised to continue phenobarbitone therapy. The owner was asked to present the case for review after two weeks.

Further review showed improvement with an increased pulse rate compared to the previous recording. On electrocardiography, frequency of occurrence of isolated 'p' wave were found to be reduced (figure 1. 2). Echocardiography revealed persistence of hypokinastia with reduced turbulence (figure 2.2). The owner was advised to continue antibiotic for two weeks and to reduce phenobarbitone to half the total dose rate. Since cardiac involvement in the seizures was confirmed, it was advised to start treatment with ACE 1 inhibitor, Enalapril tablet at a dose rate of 0.25mg/kgBW BID for life long therapy. The owner



reported a reduction in frequency of epileptic attacks. Also the reduction in dose of phenobarbitone did not increase the frequency of epileptic attacks.

Figure 2.2 : Colour Doppler Echocardiogram showing reduced turbulence at mitral valvular area(After treatment)

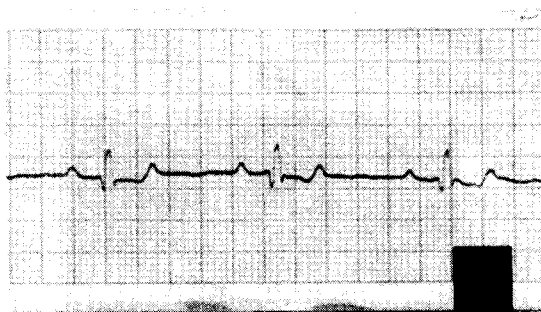


Figure 1.2. Electrocardiogram after treatment

Table 1 : Biochemical values of dog with mitral valvular insufficiency

TEST	OBSERVED VALUE	NORMAL VALUE
T3	30µg/dl	82-138µg/dl
T4	1.40µg/dl	1.0 - 4.7 µg/dL
Serum free T4	0.32ng/dl	0.5-2.7ng/dl
Blood urea nitrogen	36mg/dl	9 - 27 mg/dL
Blood glucose	84mg/dl	65 - 118mg/dl
Serum creatinine	1.3mg/dl	0.5-1.5mg/dl
-Serum uric acid	0.5mg/dl	20-112mg/dl
S. Calcium	10.40mg/dl	9-11.3mg/dl
S. Bilirubin Total	0.2mg/dl	0.0 - 0.4 mg/dL
S. Bilirubin direct	0.1mg/dl	0.06-0.12 mg/dL
S. Protein	7.3g/dl	5.2 - 7.8 gm/dL
S. Albumin	2.7 g/dl	2.6-3.3g/dl
S. Globulin	4.6 g/dl	27-44g/dl
A/G Ratio	0.611	0.59-1.11
SGOT	59 IU/l	23-66IU/L
SGPT	70 IU/l	21-102 IU/L
S.ALP	357 IU/l	20-156IU/L

TEST	OBSERVED VALUE	NORMAL VALUE
RBC	6.84million/mm ³	6-8 million/mm ³
Hb	15.6g/dl	12-18 g/dl
PCV	46.1%	37-55%
MCH	22.5pg	22-24 pg
MCHC	33.8%	30-35%
T.Leucocyte	9800/ mm ³	9000-13000/ mm ³
Nuetrophils	82%	65-70%
Lymphocyte	16%	20-35%
Eosinophil	2%	2.5%
Basophils	Nil	<1
ESR	2mm/hr	5-25mm/hr
Platelet	218000/mm ³	2-5lakh/ mm ³
Total serum cholesterol	219mg/dl	135-270mg/dl

Discussion

Electrocardiography and echocardiography can be used to diagnose second degree heart block with mitral insufficiency with the help of modern diagnostic tool like ECG (Bolton,G.R, 1975.,Nyland,T.G and Matton,J.S, 2002).The convential therapy for epileptic seizure is a prolonged therapy of phenobarbitone. But in the present case,the use of phenobarbitone could not produce any remarkable change. By the use of cardiac drugs, the root cause for syndrome exhibited by dog was relieved to a considerable extend (Amir Zaidi, *et.al.* 2000).Second degree heart block is a condition where the conduction of impulse from SA node to AV node is impaired to a mild degree .As a consequence of this cardiac conduction and circulation to brain is impaired leading to cerebral hypoxia for a short time which is manifested as epileptic seizures (Ettinger,S.J,1995).

Mitral valve regurgitation results in improper closure of left atrioventricular valve leading to increased pressure on left atrium thus impairing the drainage of blood from lung through pulmonary vein into left atrium. The cough observed during the attack may be a consequence of pressure on left bronchus by dilated left atrium or bronchial hyper responsiveness in heart failure (Moore D P, *et.al.* 1993).

Low serum thyroid hormone values observed may be attributed to prolonged phenobarbitone therapy

(Daminet, S., 2003). Also no clinical signs of hypothyroidism were exhibited by the animal in the present study.

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FOREIGN BODY OBSTRUCTION OF SMALL INTESTINE AND ITS SURGICAL MANAGEMENT IN A DOG

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Introduction

Foreign body obstruction of GI tract is a common condition in dogs due to their inquisitive nature. Ingestion of various foreign bodies like bones, choke belts, stones, plastic, toys, fish hook, sewing needle etc were reported in dogs (Mahesh *et al.*, 2008). The common site of GI obstruction in dogs is found to be the jejunal region with about 63% incidence. (Hayer, 2009). Ultrasonography can be used as an effective diagnostic tool in case of radiolucent foreign bodies (Penninck *et al.*, 2003). This paper reports about the ingestion of a raw cashew nut causing obstruction of jejunum in a Basset Hound dog and its successful surgical management.

Case history and Observations

A six month old male Basset Hound dog was presented to the University Veterinary Hospital, Mannuthy, with the complaint of vomiting and off feed for the last five days. The animal was not also defecating for the last two days but urination was normal. Clinical parameters were found to be within the normal range. The animal was found to be dull and depressed. Weight of the animal was recorded to be 11.5 kg. On abdominal palpation, a hard mass could be felt on mid abdominal region. Radiograph of the lateral abdomen revealed thickened intestinal loops (fig. 1) Upon ultrasound scanning, a foreign body which casted a shadow was noticed (fig 2), which confirmed the presence of a foreign body. Haematology revealed leucocytes with neutrophilia and surgical removal of the foreign body was decided.

Treatment

The mid ventral abdominal region was shaved and was prepared for an aseptic surgery. The animal was premedicated with Atropine sulphate @

0.04 mg/kg body weight followed by Xylazine hydrochloride @ 2 mg/kg body weight intramuscularly. After 10 minutes, general anaesthesia was induced with Ketamine Hydrochloride @ 5 mg/Kg body weight intramuscularly. Anaesthesia was maintained by intravenous infusion of a combination of xylazine and ketamine, 1 ml each and diazepam (1 ml) along with dextrose normal saline.

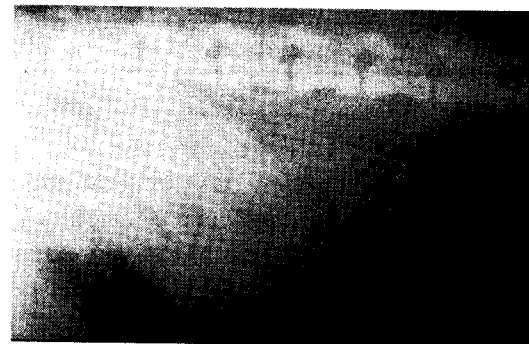


Fig. 1. Radiograph showing thickened abdominal loops`

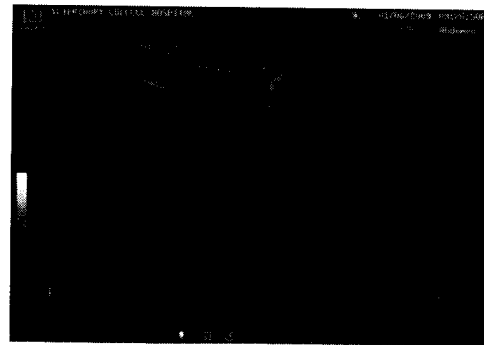


Fig. 2. Foreign body casting a shadow

The site was painted with Tr. Iodine and a 6 cm long incision was made on the skin at the midventral abdomen, just behind umbilicus. Linea alba was exposed and the incision was deepened into the

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peritoneal cavity. The peritoneal cavity was explored and intestinal loop with the foreign body was exteriorized. Loop was then packed with sterile towel. A 2 cm long incision was made on the antimesenteric border of the intestinal loop and the hard mass was exteriorized (Fig.3.) It was found to be raw cashew nut (Fig.4). The site was mopped and incision was sutured in Cushing's pattern followed by Lembert's using Vicryl 2/0. The linea alba was sutured in simple continuous pattern using Vicryl (1/0), followed by suturing of subcutis in subcuticular suture pattern. Finally, the skin was sutured in vertical mattress pattern using nylon. A gauze was sutured over the suture line and soaked with Tr. Benzoin.



Fig.3. Exteriorization of the mass

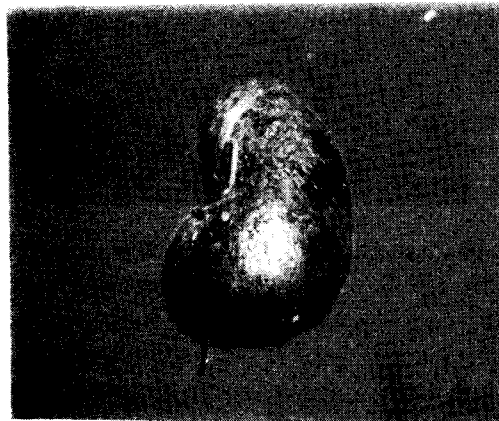


Fig.4. Exteriorized raw cashew nut

Post operatively the animal was administered with 5% dextrose normal saline and Ceftriaxone Sodium 250 mg (Intacef, Intas pharmaceuticals) intravenously. The therapy was continued for six more days. Sutures were removed on the 7th post operative day and the animal made an uneventful recovery.

Discussion

Intestinal foreign bodies pose a constant threat since they cause serious damage to the intestine leading to vomiting and electrolyte imbalance (Uma Rani *et al.*, 2004). Foreign bodies lodged in intestine causes ulceration, haemorrhage, anorexia dehydration, perforation and peritonitis and if untreated leading to death. Radiography is the most ideal imaging technique if the obstructing material is radio dense and often we will have to proceed with contrast radiography if the material is radiolucent. Ultrasound scanning will cast a shadow if the object is dense. In the present case, a raw cashew nut obstructed the jejunum which was diagnosed by radiography and ultrasonography and exteriorised by enterotomy. Enterectomy and then enteroanastomosis will be the sequel if timely intervention is not done.

Simple interrupted or simple continuous suture pattern is the ideal technique for closing the enterotomy wound in order to avoid the chance of development of stenosis of the intestinal lumen. In the present case the enterotomy wound was closed using Vicryl (Polyglactin 910) in inversion suture pattern. It was in agreement with the observation made by (Deveny *et al.*, 1977) and (Broome *et al.*, 2003). Timely diagnosis of the condition by radiography and ultrasonography, surgical management and post operative care led to the uneventful recovery of the animal.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy for granting permission to publish this paper.

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SURGICAL MANAGEMENT OF VAGINAL FIBROMA IN A DOG

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Introduction

The prevalence of cancer in pet animals continues to rise. This prevalence is increasing for a variety of reasons but is at least in part related to animals living to increasingly older ages. Since cancer is generally a disease of the older animal, the price they pay for living longer is an increased likelihood of developing cancer. With this increasing prevalence, veterinarians will be called upon more frequently to diagnose and manage the pet with cancer. Keeping this in mind, the veterinarian should approach the pet with cancer in a positive, compassionate, and knowledgeable manner.

History

A 3½ year old non-descript female dog was presented to University Veterinary Hospital Kokkalai with a history of small growth on the perineal region since 3 months and the owner complained that it was enlarging.

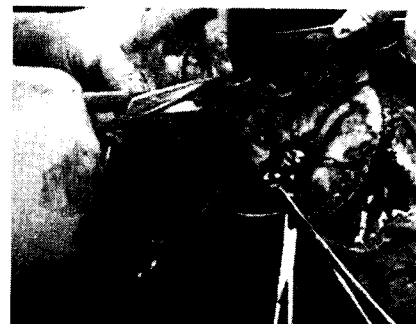
Clinical Signs

A hard mass was palpated at the perineal region, above the vulval lips. The swelling was not painful. The disease was tentatively diagnosed as vaginal tumour and surgical excision of tumour mass was done.



Surgical procedure

After aseptic preparation, anaesthesia was induced and maintained by Atropine, Xylazine and Ketamine. Animal was placed in left lateral recumbency with hind region kept raised. Urethra was catheterized. Applied Tr. Iodine along the line of incision first and the entire surgical area. A vertical incision was made between upper commissure of vulva and anus using B.P. blade no 22. The incision was extended using scissors. The muscles were separated to explore the tumour mass and the mass was separated from subcutaneous tissue. Haemostatic forceps were applied at the base of the tumour mass on vaginal mucous membrane. Excised the tumour mass from vaginal mucosa.



Vaginal mucous membrane was sutured using Catgut No. 2 in simple interrupted suture pattern. Muscles were apposed using Catgut in simple continuous manner. Sub cuticular sutures applied. Skin wound sutured using nylon in simple interrupted pattern. Applied Tr. Benzoin on the incision to control bleeding.

Post operative care:- Antibiotic therapy using Ampicillin - cloxacillin 250mg injection (Megapen®) followed by oral medication was done for 5 days.

Surgical technique

Episiotomy followed by excision of tumour mass.

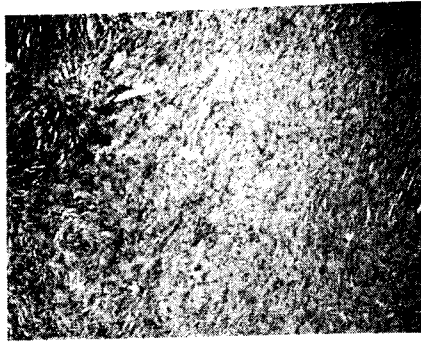
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Diagnostic measures

On histopathological examination interlacing bundles of spindle shaped fibroblasts arranged in all directions is observed and it is an indicative of fibroma. The nuclei of fibroblast were spindle shaped. Blood vessels and variable number of lymphocyte, monocyte and eosinophils were observed. Disease is diagnosed as vaginal fibroma.



Discussion

Fibroma is a benign tumour of fibrous connective tissue. Generally well circumscribed, encapsulated and firmly attached to soft tissues. They are well differentiated cells, localized, grow slowly and never metastasize. Based on consistency there occur two types of fibroma - hard fibroma (Fibroma Duram) and soft fibroma (Fibroma Molle). All breeds of dogs are susceptible to fibroma irrespective of age, but incidence is more in older aged dogs. Vaginal fibroma occurs within vestibule, vulva, vagina and cervix. Diameter of vaginal fibroma varies from 2 to 20 cm. Vaginal fibromas occur in both normal and spayed female.

Differential Diagnosis

Biopsy and histopathological examination helps differentiate fibroma from other neoplasms like leiomyoma, lipoma, malignant tumor, fibrosarcoma, leiomyosarcoma, liposarcoma, mast cell tumor, histiocytic sarcoma and histiocytoma. Vaginal hyperplasia, Vaginal prolapse, Transmissible venereal tumour and clitoral enlargement may be confused with vaginal fibroma.

Diagnosis

Diagnosis is done with the help of clinical signs, biopsy examination, Exfoliative cytology, Fine Needle Aspiration Cytology (FNAC), Radiology, chemical and serological test.

Clinical sign such as slowly growing tumour is the first evidence of fibroma noted. It is fibrinous in consistency usually ulcerate because of infection. Rarely contain areas of hemorrhage and necrosis. Biopsy examination is the most reliable method of diagnosis of any tumor. In exfoliative cytology cells can be collected from tumour and can be stained and diagnose type of tumour. It is mainly for diagnosis of uterine and bronchiogenic cancer. In Fine Needle Aspiration Cytology (FNAC) a needle is inserted into body and a small amount of tissue is sucked out for examination.

Treatment Of Fibroma

Medical management of fibroma includes use of antibiotic ointments, neck collars and bedding to prevent further injury and complications. Surgical excision of the tumour mass is the most effective treatment. Prognosis depends on early diagnosis, success of surgery and malignancy of tumour.

Acknowledgement

Authors are thankful to Dean, College Of Veterinary And Animal Sciences, Mannuthy and Head of University Veterinary Hospital, Kokkalai for providing facilities for the study.

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UNILATERAL CRYPTORCHIDISM IN A DOG

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Introduction

Cryptorchidism has been reported as the most common (13%) disorder of sexual development in dogs. It is found that the condition can be of inherited nature or it can be predisposed. Unilateral cryptorchidism is more common with one testis undescended, but the animal is perfectly normal otherwise, usually, (as it is capable of sperm production in the descended testicle) and can be maintained so if proper management/treatment is opted for. This aims at descend of cryptorchid testis, thereby probably minimizing the probability of developing tumours/neoplasms within/other complications due to cryptorchid testis. Further breeding of the animal should not be considered as there is always a possibility for hereditary nature of the condition.

Case History and Observations

A 110 days old male German shepherd dog was presented at Veterinary Hospital, Mannuthy with the complaint of undescend of one of the testes. The animal was bought by the owner from Coimbatore and later, on examination he found that the animal was a cryptorchid. All the clinical parameters were normal. On examination and palpation, it was observed that right testis has descended into scrotum and the left one still in the inguinal canal. The condition was diagnosed as unilateral cryptorchidism (left sided).

Treatment

Four injections of Human Chorionic Gonadotropin (Inj. CHORULON 1500 IU vial) 750 IU IM were given on the 1st, 2nd, 7th and 8th day.

Result and Discussion

The animal responded promptly to the treatment and the cryptorchid testis descended into

the scrotum by 8th day of treatment. The dog was monitored for a period of 8 months after treatment and was found perfectly normal during that period.

Cryptorchidism is the developmental defect in male dogs in which one or both testicles fail to descend into the scrotum. If the testicle has not descended into the scrotum by a speculated time period (characteristic to each species), there usually is little chance that further descent will occur.

Causes of Cryptorchidism

Deficiency of or insensitivity to hormones (like AMH, androgens especially testosterone, GnRHs and gonadotropins), maldevelopment of gubernaculum and alteration in intra abdominal pressure (due to infections like peritonitis, navel ill, etc.) can cause cryptorchidism.

Position of Abnormal Testis

A testis which is absent from the normal scrotal position can be anywhere along the path of descent. It may be high in the posterior (retroperitoneal) abdomen or inguinal canal. In some cases the testis may be ectopic (in locations like femoral canal, under the skin, opposite scrotum, superficial inguinal pouch, perineum, pubopenile region, etc). Otherwise it may be hypoplastic or dysgenetic. In rare cases, the testis may have vanished after a period of development.

Fate of Cryptorchid Teste

Usually the retained testis will be smaller than the scrotal testis. The diameter of seminiferous tubules of affected testis is reduced by 60%. There will be interstitial cell atrophy or hypertrophy. Cryptorchid testes are incapable of spermatogenesis due to lack of thermoregulation and degeneration of seminiferous epithelium, but are capable of steroidogenesis.

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Bilaterally cryptorchid dog will be azoospermic and unilateral cryptorchid animal will be oligospermic but fertile.

Complications of Cryptorchidism

The cryptorchid testis is predisposed to neoplasia with 4-40 times more chance than of a normal testis. The common tumours encountered are Sertoli cell tumours and seminomas. There is also increased risk of testicular torsion and development of Male feminizing syndrome.

Diagnosis

Include visual inspection, palpation, imaging techniques (Radiography, Ultrasonography, and MRI), hormonal assay (Testosterone, LH, and FSH), Gonadotropin stimulation test and rectal palpation of prostate gland.

Differential Diagnosis

Cryptorchidism should be differentially diagnosed from conditions such as retractile testis, anorchia/agonadism and intersex. Castrated animals can be differentiated by the previously mentioned tests.

Treatment

Hormonal therapy can be tried; but, bilateral castration is the recommended practice. Surgical correction (Orchiopexy) is rarely adopted.

Hormonal Therapy

Gonadotropins: hCG stimulates production of gonadal steroid hormones by stimulating the Leydig cells to produce androgens. The exact mechanism of the increased androgens in testicular descent is not known but may involve effects on the testicular cord or cremaster muscle. Many dosage schedules have been reported, ranging from 3-15 doses. However, hCG appears to be as effective in 3 or 4 doses as with 9 or 10 doses. A dose 500-1000 IU is given intramuscularly four times in a period of two weeks. More than 80% success have been reported when the treatment had begun before the pup attained 16 weeks of age.

Gonadotropin releasing hormones: Agonistic analogues of GnRH such as nafarelin or buserelin stimulate the release of the pituitary gonadotropins, LH and FSH, temporarily increasing gonadal steroidogenesis. Repeated dosing abolishes the

stimulatory effect on the pituitary gland, and twice-daily administration decreases secretion of gonadal steroids by 4 weeks. GnRH is available as a nasal spray but is approved for the treatment of cryptorchidism only in Europe.

Surgical Therapy

Successful surgical placement of the testis in the scrotum is based on the principles originally described by Bevan in 1899. These include adequate mobilization of the testis and spermatic vessels, ligation of the associated hernia sac, and adequate fixation of the testis in a dependent portion of the scrotum. Orchiopexy is mainly done in human beings.

Summary

A dog was presented to Veterinary Hospital, Mannuthy with the complaint of undescend of one of the testis. The animal was examined and the condition was diagnosed as left sided unilateral cryptorchidism. Medical treatment using human chorionic gonadotropin (CHORULON 750 IU IM, 4 injections over a period of two weeks) was adopted. The animal showed response to treatment by the descent of cryptorchid testis on 8th day of treatment. The animal was monitored for a period of 8 months and was found perfectly normal. The owner was advised not to breed the animal by making him aware of the hereditary nature of the condition.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary And Animal Sciences, Mannuthy and Professor & Head of Veterinary College Hospital, Mannuthy for providing facilities for the study.

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MYCOTIC ENTERITIS IN RABBIT

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Introduction

Diarrhoea is a major cause of death in young rabbits. Diet, antibiotic treatment and other factors that create disturbances of Gastro Intestinal microflora may predispose rabbits to intestinal dysbiosis and enteritis (Cheeky, 1987). Many bacterial and mycotic organisms are associated with enteritis in rabbits.

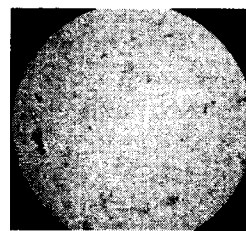
The caecal digestion/processing of food by well balanced population of bacteria and yeast play an important role in the digestion of rabbit. *Ciniclomyces guttulatus* (previously known as *Saccharomyces guttulata*) is the dominant yeast in the caecal flora of rabbit which is considered non pathogenic and supposed as beneficial to the host (Hage, 1945). Caecal dysbiosis occurs when rabbits consume a diet that is too rich in sugars and simple carbohydrates. Excessive amounts of fruits and cereals in the diet cause the yeast overgrowth and imbalance of microflora leading to diarrhea and subsequent problems. Young ones are most often affected and may succumb to the disease.

Case Study

A few dead baby rabbits were brought for post mortem with a history of diarrhoea and tympany among rabbits and death of about 6-8 rabbits over a period of 2-3 days. History revealed that the animals especially young ones were maintained mainly on fruits like banana and cereals like corn assuming as easily digestible foods.

On detailed necropsy, the intestine was found to be dilated, ballooned, and flabby and fluid distended with severe mucosal congestion. However the contents were devoid of any blood tinge. There were signs of dehydration. On microscopical examination of caecal and intestinal contents and faecal pellets of ailing rabbits,

abnormally large number of yeast cells were detected which were identified as *Cyniclomyces guttulatus* based on morphological features.



Considering the very high number of yeast cells in the absence of other organisms, this was interpreted as pathogenic. The high sugar diet fed to the young ones was assumed as the initiating cause. The owner was advised to eliminate the

excess sugar items from the diet and put on a diet strictly limited to leafy green vegetables until recovered. Nystatin @ 22,000 IU/Kg for 3 days orally was also advised. The follow up of the case revealed that the therapy was effective and the remaining rabbits have recovered completely.

This case highlights the role of *Cyniclomyces guttulatus*, a normal commensal of the rabbit caecum, in the development of enteropathy when the diet was unscientific and rich in sugars and simple carbohydrates. Many authors considered this fungus as a contributory or opportunistic pathogen in enteritis complex in rabbits and lack of dietary fiber and high sugar diet were often cited as predisposing causes.

Acknowledgement

The authors are thankful to the Director of Animal Husbandry, Kerala for providing Telepathology discussion facility.

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UTERINE TORSION IN A BUFFALO

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Introduction

Uterine torsion, predominantly a complication of first stage of labor is the most common in cows and occasional in does (Roberts, 1971). It is the single largest cause of maternal dystocia with incidence as high as 67% (Singh and Nanda, 1996) and is a highly stressful reproductive disorder in buffaloes. However, in Kerala reports on uterine torsion in buffaloes are scanty. The present paper is a case report of uterine torsion in buffalo and its successful correction using modified plank method.

History and Clinical Signs

A full term she buffalo aged 8 years and in third parity was presented at Veterinary Hospital, Perumbavoor with the history that the animal showed labor pain 12 to 15 hours earlier which ceased within a few hours, there after no signs of parturition and was anorectic. The animal appeared normal, teats were engorged with milk and pelvic ligaments relaxed. The animal had brisket oedema, occasional cough, rectal temperature was 106°F and slight muco-purulent vaginal discharge was noticed. Pervaginal examination revealed clockwise twist of the anterior vagina with one finger dilatation of cervix but the fetus was not palpable through the twist. The case was diagnosed as post cervical uterine torsion of approximately 180 degrees. It was decided to correct torsion by rolling the animal using plank method with some modifications. Clinical signs like duration of torsion (less than 36 hours), let down of milk and relaxation of pelvic ligaments were taken as criteria for success of treatment.

Treatment

The animal was casted on the right lateral recumbency while both fore and hind limbs were tied separately with long ropes. A plank of about 4m length

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and 20 cm width was placed on left flank of the animal while one end of the plank was resting on the ground. One person stood on the end of the plank resting the ground and a second person stood on the plank at the flank region and the animal was rapidly turned to the right side in the direction of torsion. The man standing at the plank region applied sufficient pressure to fix the uterus while rolling and with two rollings complete detorsion occurred. There was sufficient dilatation of the cervix and a dead fetus was delivered by traction. Immediate post partum treatment comprised of intravenous injection of compound ringer lactate 900ml, and Enrofloxacin 15ml, Diclofenac sodium 20ml and Chlorpheniramine maleate 10ml were given intramuscularly for three consecutive days. Oral therapy included Replanta powder and Involon bolus for two days. The fetal membranes were expelled within 6 hours and the animal recovered uneventfully.

In buffaloes uterine torsion of less than 36 hours of duration can be corrected effectively by rolling the animal using modified plank method under field conditions.

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CHEMOTHERAPY USING VINCRISTINE IN CANINE TRANSMISSIBLE VENEREAL TUMOUR

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Introduction

The transmissible venereal tumour of the dog, also known as canine condyloma, venereal granuloma, infectious sarcoma, infectious lymphosarcoma and contagious venereal tumour, is a neoplasm occurring naturally on genitalia of both male and female dogs (Bloom *et al.*, 1950). It was defined as an undifferentiated round cell neoplasm probably of histocytic origin. The disease is mostly seen in free roaming, sexually active dogs in tropical and subtropical climates.

Case History

A three year old female German Shepherd dog was presented with clinical signs like vulval oedema and serosanguinous discharge from vulva. On per vaginal examination diffuse multiple grey firm nodules were present in the vaginal mucous membrane. Nodules were distributed more on caudal vaginal wall. Some of the nodules were multi-lobulated. There were small ulcerations in the vaginal wall. Animal was actively moving around. Animal was having slightly reduced appetite, while urination animal occasionally exhibited discomfort. These symptoms started three weeks back. Animal has whelped once and it was one year back. Four to five months back on exhibition of oestrous signs animal had been mated with two dogs, but failed to conceive.

Vaginal swabs were used for making smears. Direct impression smear was taken from nodules. Smears were stained with modified Wright-Giemsa stain.

Result

On microscopic examination round cells with large nucleus were observed throughout the smear. Cells were having distinct cytoplasmic borders. Vacuoles were present in the cytoplasm on

the periphery of the cells. There was mild anisocytosis and anisokaryosis. Mitotic figures were present. Cytoplasm was moderate and granular. From the clinical symptoms, anatomic location of lesions and cytological examination disease was confirmed as canine venereal tumor (CTVT).

Treatment

Animal was treated with Vincristin sulphate 0.025 mg/kg intravenously (CYTOCRISTINE 1 mg/ml). Advised to bring the animal after one week. After one week the tumor mass had regressed considerably. A second dose of vincristin was given. On third week the tumor has completely disappeared. So vincristine was not administered. Advised the owner to observe the animal for possible recurrence.

Discussion

Transmissible venereal tumor (TVT) is a coitally transmitted neoplasm of dogs and is common among sexually active dogs (Nak *et al.*, 2005). Tumor is an undifferentiated round cell neoplasm. The tumor can be transplanted as an allograft with in same species as well as to other canids such as foxes, coyotes and jackals (Stettner *et al.*, 2005).

Due to unique nature of sexual contact, the external genitalia of either sex are most commonly affected. Less commonly tumor may also be transmitted to nasal or oral cavities, skin and the rectum by sniffing or licking. More rarely they may be found in other areas, including the lips, oral mucosa and peritoneum or in organs such as tonsils, eye, liver, spleen, kidney, lung and musculature (Park *et al.*, 2006). In this case tumor was present in the vaginal wall alone.

Diagnosis in this case was made based on the anatomical location of the tumor, gross morphology and

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cytological examination.

After transmission to a susceptible host TVT grows progressively for 4-6 months then remain static for a time. Spontaneous regression is observed after several months. In this particular case history suggest that animal had been crossed four months back. This could be responsible for transmission of the disease.

Reported treatments of TVT include surgical excision, radio therapy, immunotherapy and chemotherapy. The last is considered treatment of choice. Antimitotic agents such as vincristine, vinblastin and doxorubicin are the preferred chemotherapeutic agents (Das and Das, 2000). Vincristin is the drug of choice. Use of doxorubicine is reserved for dogs that do not respond to vincristine and is limited due to adverse reactions.

The animal completely recovered after vincristine sulphate chemotherapy weekly once for two weeks. Nak *et al.*, 2005-reported total regression of 31 cases of TVT affected dogs, after two to seven doses of vincristine.

Gonzalez *et al.*, 2000 reported that, after vincristin therapy tumor cells started regression and during regression tumor cell proliferation decreased, apoptosis increased, leucocytes increased (with increased proportion of T lymphocytes), tumor parenchyma collapsed around intratumoral vessels, and fibrosis increased. They suggested that tumour immunity plays a role in tumour regression after modest

chemotherapy.

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INFRARED THERMOGRAPHY IN BOVINE REPRODUCTION STUDIES

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Infrared thermography (IRT) is a noninvasive and safe technique for thermal profile visualization. Every object on earth generates heat radiation in the infrared part of the light spectrum. The intensity and spectrum distribution of it depends on the material's temperature and the radiation properties of its surface layer. Using infrared camera, it is possible to detect this type of radiation. This special type of camera is able to visualize and quantify changes in surface temperature and to convert this infrared radiation into electrical impulses that are seen in colour on a monitor. The visual image of skin of a particular region of an animal can graphically maps the body temperature and is referred to as a thermogram. Advanced diagnostic software provides subsequent mathematical evaluation of the image (Knizkova *et al.*, 2007).

An infrared camera measures and images the emitted infrared radiation from an object. The fact that radiation is a function of object surface temperature makes it possible for the camera to calculate and display this temperature. However, the radiation measured by the camera does not only depend upon the temperature of the object, but is also a function of the emissivity. Radiation also originates from surroundings and is reflected by the object. The radiation from the object and the reflected radiation will also be influenced by the absorption of the atmosphere. To measure temperature accurately, it is therefore necessary to compensate for the effects of a number of different radiation sources. This is done online automatically by the camera. However, the following object parameters must be supplied for the camera: the emissivity of the object, the reflected temperature, the distance between the object and the camera and relative humidity.

Thermal imaging can be a very effective method of highlighting the location of problem areas which then require further exploration. Veterinary infrared thermography is a completely safe, radiation free technology that involves no contact, compression or discomfort. This advanced technology can detect abnormal infrared patterns in soft tissues including nerves, muscles, tendons, ligaments and organs. It can show areas of inflammation, infection, injury and nerve irritation. Infrared digital imaging provides different information to tests such as X-ray or CT scans. It is an excellent adjunct to clinical examination and is complementary to other imaging techniques such as radiology, ultrasonography, and scintigraphy. Thermographic method has found numerous applications in veterinary medicine, primarily for diagnostic purposes.

Applications of infrared thermography in bovine reproduction studies

IRT has been recommended as a good method in helping to study thermoregulation. A major advantage of the method is the fact that it does not require a direct physical contact with the surface monitored, thus allowing remote reading of temperature distribution. Hurnik *et al.* (1985) studied the relationship between differences in body surface temperatures and the oestrus in Holstein-Friesian dairy cows, and a possibility of using this technique to determine the onset of the oestrus. Because inaccuracies were encountered in determining the oestrous cycle during the experiment, the authors did not recommend IRT for routine detection of the oestrus, but it is nevertheless completely adequate in skin temperature studies, or, more precisely, in the studies of body surface temperature changes. Hellebrand *et al.* (2003) concluded that gravidity of heifers in their usual environment (pasture or barn) cannot be determined by

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simple monitoring with a thermal imager, but they did find that the external pudendum temperature follows the core body temperature, and thus IRT can be utilised for oestrus climax determination.

Kozumplik *et al.* (1989) used IRT in the diagnosis of inflammatory processes on the sex organs of breeding bulls. The objective was to obtain an overall thermogram of the gonads of bulls with normal and disturbed fertility, and to assess the possibility of using IRT for the diagnosis of sex organ diseases accompanied with local temperature changes. Thermograms of the scrotum showed that the warmest and the coldest zones lie at the head of the epididymis and a part of the testicles adjoining the tail of the epididymis, respectively. The authors recommend thermography as a useful tool for the identification of initial stages of diseases of sex organs in breeding bulls. Kastelic *et al.* (1995) found that temperature gradients were most pronounced on the scrotal surface, less in the scrotal subcutaneous tissue, and slightly negative (relative to the surface) within the testicular parenchyma. Scrotal surface temperature decreased from the neck of the scrotum to the ventral aspect to scrotum. Conversely, the ventral pole of the testis was slightly warmer than the dorsal pole. The caput of the epididymis was warmer than the adjacent testicular parenchyma, while the cauda was cooler. IRT was also used by Kastelic *et al.* (1996a, 1996b) to study environmental factors that influence the bovine scrotal surface temperature and to study the influence of ejaculation on the scrotal surface temperature in bulls. The authors concluded that representative temperature measurements of the scrotal surface could be taken at any time of the day except at feeding and rising. Moreover, the scrotum should be dry.

Measurements are independent of the ambient temperature provided it is stable. Abrupt changes in the ambient temperature may, however, result in artifacts due to overcompensations. Thermographic measurements showed that spontaneous ejaculations as well as electroejaculations increased the surface temperature of the scrotum. Further, Kastelic *et al.* (1996c, 1997a) found that insulation of the scrotal neck affected scrotal surface temperature, scrotal subcutaneous and intratesticular temperatures, and increasing testicular temperature results in defective spermatozoa, with recovery dependent on the nature and duration of the insult. Kastelic *et al.* (1997b) used

IRT to study the contribution of the scrotum, testes, and testicular artery to scrotal/testicular thermoregulation in bulls at two ambient temperatures. Their results supported the hypothesis that blood within the testicular artery has a similar temperature at the top of the testis compared with the bottom, but subsequently cools before entering the testicular parenchyma.

Gabor *et al.* (1998) determined the effect of GnRH treatment on plasma testosterone concentrations and scrotal surface temperature, the repeatability of different morphologic, thermal and endocrine measures before and after GnRH treatment. They also examined the correlation between the total number of spermatozoa and the proportion of live and motile spermatozoa, using the different morphologic, thermal and endocrine measures before and after GnRH challenge. The authors concluded that GnRH treatment significantly increased plasma testosterone concentrations and usually caused significant increases in scrotal surface temperature measured by IRT. Scrotal circumference and the total number of spermatozoa per ejaculate were highly correlated. Other measurements were less correlated, though with an apparent effect of ambient temperature on measurements of the scrotum and assessment of scrotal surface temperature. Significant regression equations were derived for the total number of spermatozoa and the percentage of motile spermatozoa; plasma testosterone concentrations and scrotal surface temperature gradients, respectively, were the significant independent variables.

Conclusion

The above examples prove conclusively that IRT can produce important information where conventional diagnostic techniques have exhausted their possibilities. But there are some limitations and factors that need to be considered when using IRT. Thermograms must be collected out of direct sunlight and wind drafts. Hair coat should be free of dirt, moisture or foreign material. The effect of weather conditions, circadian and ultradian rhythms, time of

feeding, milking, laying and rumination etc. are also



factors that need to be considered and require further investigation as a part validating IRT. Then infrared thermal measurements can be used very successfully in prediction, detection and diagnosis of diseases, and others applications in livestock science.

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GIARDIOSIS IN CATTLE AND ITS ZONOTIC IMPORTANCE

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Giardiasis caused by *Giardia duodenalis* (Syn. *Giardia lamblia*, *G. intestinalis*) is one of the most commonly identified gastrointestinal pathogens affecting humans and animals in the world. Though humans, dogs, cats and certain wildlife are described as the principal hosts of *Giardia*, infections in ruminants, pigs and horses have been reported from many countries throughout the world. Diarrhea is the predominant sign in calves, lambs and kids. In calves the infection is associated with ill thrift and poor weight gain. It is believed that Leeuwenhoek observed the protozoan for the first time after examining his own diarrheic stool in 1681. But the discovery of the organism is credited to Lambl, who confirmed the parasite's existence in 1851. It was once believed that *Giardia* was not pathogenic, as many infected people are asymptomatic. Now this ubiquitous parasite is well known as a common disease of travelers, children in day care centers and as a frequently identified pathogen in waterborne outbreaks of gastrointestinal illness.

Morphology and Taxonomy

Giardia intestinalis has a trophozoite and cystic stage in its life cycle. Trophozoites are binucleate, pear shaped organisms that possess four pairs of flagella measuring 9-12 μm in length by 5-15 μm in width. The two prominent nuclei are visible under light microscope. The most prominent feature of the trophozoite is its ventral adhesive disc composed largely of tubulin as well as proteins called giardins. The cysts are ovoid in shape and measure approximately 8-12 by 6-8 μm . They are binucleate or quadrinucleate and various internal structures such as the flagella and median bodies can be visualized using light microscopy.

Filice (1952) proposed three species based on morphological characteristics; *Giardia agilis*, described only from amphibians, *G. muris* infecting

rodents, birds and reptiles and *G. duodenalis* affecting a wide variety of mammalian hosts, including humans and also birds. During the past decade, molecular studies have demonstrated that *G. duodenalis* is a genetically diverse species. At present seven assemblages (A to G) have been identified within *G. duodenalis*, some of which have distinct host preferences or limited host range. Assemblages A and B are prevalent in human patients. Assemblages C and D primarily infect dogs. Hoofed livestock is affected by E. Assemblage F in cats and G in rats. Zoonotic *Giardia* assemblage is A.

Transmission

Infection occurs by ingestion of quadrinucleate cysts. Inappropriate handling of food and washing food with contaminated water has been implicated in food borne outbreaks of giardiasis. Feces from animals such as small rodents may contaminate cereal grains or water sources leading to potential human and animal infections.

Clinical signs

Many infected hosts experience no symptoms at all and asymptomatic infections comprise majority of cases. In symptomatic cases, the clinical signs include severe diarrhea, steatorrhea, abdominal cramps, and nausea and weight loss. These symptoms may persist for a few weeks in the case of acute giardiasis or evolve into chronic recurring disease. The variation in clinical signs experienced by the host is likely the result of both host and parasite factors (O'Handley, 1999).

Pathogenesis

The infection results in a number of morphological and physiological changes to the small intestine which culminate in a malabsorptive diarrhea. Trophozoites do not invade the epithelium of the small intestine, but attach themselves to both

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the microvillous and basolateral membranes of the enterocyte. Despite the non-invasive nature of the parasite, once *Giardia* trophozoites have colonized the small intestine of the host, a wide variety of morphological abnormalities can result. Under light microscope severe villous atrophy have been noticed. Using E/M a diffuse shortening of epithelial microvilli appeared characteristic of giardiasis even when villous architecture appears normal. Apart from the morphological alterations of the intestine *Giardia* infection is associated with accelerated gastric emptying and increased intestinal transit. Increased contractility of smooth muscle may contribute to abdominal cramps associated with the disease. Together these morphological and physiological alterations associated with giardiasis result in malabsorption, maldigestion and hyper motility, the consequence being diarrhea. There is some evidence to suggest that *Giardia* may produce toxins.

Zoonosis

A vast variety of mammalian hosts have been reported to be infected with *Giardia* sp. This has raised great concerns regarding the zoonotic potential of the parasite. But recent findings, many employing molecular techniques, provide evidence to support and refute the zoonotic potential of giardiasis. In general, cattle are not considered as an important source for human giardiasis due to the high prevalence of the livestock specific assemblage in E in cattle in Australia, New Zealand and the US. But a recent study in Belgium revealed high prevalence of the zoonotic *Giardia* assemblage A infection in calves.

Giardiasis in Cattle

High prevalence of *Giardia* has been reported in calves in North America and elsewhere. This may pose a public health risk as some of the organisms have been identified as the zoonotic assemblage A. Treatment of the infection may be necessary in calves to prevent economic losses, improve animal health and minimize the parasite's zoonotic potential.

Treatment

Several studies have demonstrated that benzimidazoles (BZ) are effective in treating *Giardia* infections. They act by inhibiting tubulin

which is a major component of the *Giardia* cytoskeleton and in many of its organelles, including its adhesive discs. As a result *Giardia* trophozoites exposed to BZ experience alterations in shape and the disappearance of the ventral adhesive disc prevent them from adhering to cell surfaces. BNZ have been found to be 30-40 fold more potent than metronidazole (Edlind *et al.* 1990). Mebendazole, albendazole and fenbendazole (FBZ) are also very effective against *Giardia* in vitro and are currently used as anthelmintics (Morgan *et al.* 1993). The BZs maintain their anti-*Giardia* activities past 48 h and failure to induce resistance for both albendazole and fenbendazole have been demonstrated.

Fenbendazole

First described in 1974, fenbendazole is a broad spectrum anthelmintic, effective against a wide range of helminth species and is extremely safe for use in cattle. Compared to other BZ anthelmintics, FBZ is more slowly absorbed following oral administration and this may contribute to the drug's efficacy. Absorbed FBZ is also important in maintaining the efficacy of the drug. Fenbendazole was very effective in eliminating the passage of cysts and trophozoites from infected calves. Treatment can result in clinical benefit to calves, potential economic benefit to producers and a decreased threat to public health. Since *Giardia* cysts can survive for one week in feces and seven weeks in soil, reinfection can occur from a contaminated environment shortly after treatment. This emphasizes the need for an integrated control program combining treatment with cleaning and disinfection of the environment at the end of the treatment period to further minimize the risk of reinfection after treatment. Heat or desiccation and disinfection with quaternary ammonium can be used in calf facilities.

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FERTILITY MANAGEMENT THROUGH IMMUNOMODULATION

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Introduction

Improvement of reproductive performance and fertility management are of great significance in the livestock sector. Attempts to increase reproductive performance include stimulation of reproductive hormone secretion, induction of ovulation and superovulation using purified and synthetic hormones, more recently, the use of in vitro fertilization and embryo transfer techniques. These methodologies have provided various degrees of success in increasing reproductive performance in different species. Each of these methodologies, however, is labor intensive and requires sophisticated and expensive technologies for its implementation. Regulation of reproduction by immune intervention is fairly a recent development. Immunomodulation is generally used to describe the pharmacological manipulation of the immune system through application of various vaccines and immunomodulatory substances. Following the discovery of the reproductive hormone system, attempts were made to control reproduction by immunization against key hormones in animals. Recently, vaccines have also found applications in animal reproduction processes. Various immunological approaches can be effectively targeted to manipulate the signaling and recognition systems of reproductive hormones. Immunization by raising antibodies against reproductive hormones has been used to explore potential for augmenting reproductive efficiency in livestock.

Reproductive consequences have been studied following immunization against androgens, oestrogens and progesterone. Immunization against testosterone stimulates ovarian activity but it usually causes abnormal ovarian cycles (Price and Morris, 1987). Vaccination of ewes against androstenedione leads to a

reduction in estrogen levels, and estrogen has a negative-feedback effect on the production of follicle-stimulating hormone. Immunoneutralization of androstenedione leads to the increased production of FSH, and this has the effect of increasing the frequency of multiple ovulations. Thus, immunization against androstenedione increases ovulation rate and fecundity in sheep and can be used as 'fecundity vaccine'. Androgen-based vaccines *viz.* Androvax and Ovastim, are now marketed in various developed countries. The former vaccine boosts lambing up to 20% and is an increasingly valuable tool to manage fertility.

Immunization against Inhibin

Inhibin, a glycoprotein hormone secreted by granulosa cells in the ovary plays an important role in FSH release through negative feedback mechanism and thus regulates onset of oestrus. Administration of steroid free follicular fluid in cattle delays onset of estrus suggesting presence of inhibin. Immunization against inhibin has been suggested with a view that immuno-neutralization of endogenous inhibin would enhance FSH secretion and cause an increase in ovulation rate and litter size. Initially, immunization was done using crude or partially purified fraction of follicular fluid. Later on with the establishment of its amino acid sequence, recombinant vaccine was developed. Along with recombinant subunits, chemically synthesized inhibin fragments can also act as good immunogens. An increase in ovulation rate in cattle using crude ovine follicular fluid (oFF) as immunogen has been observed by Cummins *et al.* (1986) while, in sheep this preparation produced variable and transient increase in ovulation rate.

Kamonpatana *et al.* (1990) reported an increase in pregnancy rate from 11% to 55-72% in buffaloes immunized with inhibin antisera. Active

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immunization against synthetic fragment of ovine, porcine and bovine inhibin α -subunit conjugated to suitable carrier protein has resulted in 2-4 fold increase in ovulation rate. Immunization against α -subunit of recombinant human inhibin in goat produced high antibody titre, 3 times increase in ovulation rate and yield of embryo. Passive immunization avoids a potentially permanent response arising from continuous production of antibodies. Passive immunization using follicular fluid and inhibin antisera also resulted in increase in ovulation rate in guinea pigs. In undernourished cattle, passive immunization against testosterone has been found to have beneficial effects on follicular growth.

Anti-fertility Vaccines

Immuno-contraceptive vaccination is a dynamic area of vaccine research and development in the animal health sector, with a number of products for livestock and companion animals recently brought to the market. Many early attempts gave encouraging results but were variable due to a lack of knowledge of how to consistently elicit an effective neutralizing immune response against self-proteins. There are two goals of reproductive control vaccines that can be simply categorized as Immuno-contraception and Immuno-neutering. Immuno-contraceptive vaccines aim to prevent either fertilization of the oocyte by sperm or implantation of the fertilized egg yet retain sexual behavior patterns and competition in mating; this approach is most suited to the control of feral animal pests and native wildlife. Immuno-neutering vaccines aim to prevent all sexual behaviors in both male and female animals as well as controlling fertility; these outcomes are suitable for companion animals, livestock, and in some instances, feral animal pest control.

Vaccines against reproductive hormones and immuno-neutering

The most scientifically and commercially successful approach for the control of reproduction by vaccination consists of targeting specific hormones involved in sexual development. The best-studied and best-characterized hormone used as a vaccine target, has been Gonadotropin-releasing

Hormone (GnRH). Recent studies have demonstrated potential application of GnRH immunization as an alternative to surgical and chemical sterilization in animals. Because of its simple structure and central controlling role, GnRH was the target of vaccine research soon after its discovery. Since then, there have been many research programs for the development of anti-GnRH vaccines; however, only very few commercial successes have been reported so far. Immunological neutralization of GnRH blocks pituitary secretion of gonadotropins resulting in gonadal quiescence and thus prevents reproductive function, provides contraception, controls estrus behavior in females and sexual and aggressive behaviors in males. GnRH is non immunogenic, therefore require conjugation to antigenic carrier proteins for immunization.

The first commercial vaccine developed against GnRH was *Vaxstrate*, comprising a conjugate of ovalbumin and GnRH peptide, presented in an oil emulsion adjuvant. A more advanced anti-GnRH vaccine, *Improvac*, was developed for use in entire male pigs to control boar taint. To date, it is the most successful of all the reproduction control vaccines. *Improvac* is given as two doses, which is sufficient to induce an anamnestic anti-GnRH antibody response that in turn suppresses GnRH production, levels of gonadotrophins, and testicular function and allows the washout of the taint steroid androstenone and skatole. GnRH vaccine treated boars are leaner and had superior feed conversion efficiency. An anti-GnRH vaccine, *Equity*, has also been developed for use in female horses which is effective in controlling estrus and estrus-related behavior during the breeding season. Additionally, suppression of testosterone via anti-GnRH response leads to a reduction in dihydrotestosterone and indirectly controls prostatic hyperplasia. An anti-GnRH vaccine was also conditionally licensed in the United States for the treatment of benign prostatic hyperplasia in male dogs.

Another anti-GnRH vaccine, *GonaCon* has been shown to be effective in valued wildlife animals. Estrus behavior is undesirable in beef heifers as it will increase the risk of injuries, stress, unwanted pregnancies and risk dark cutting beef. Suppression of estrous behavior through GnRH immunization is possible so that meat quality can be enhanced. PGF α

immunization in prepubertal beef heifers can also suppress estrous behavior.

Gonadotrophin-based Vaccines

FSH and LH play a major role in functional and structural maintenance of gonads. Potential contraceptive effects of immunization against these hormones have been demonstrated. Gonadotrophic hormones are immunogenic and do not necessarily require conjugation. However, improvement in antibody production response has been observed when gonadotrophic hormones are conjugated to carrier proteins. Suppression of ovarian activity following immunization against a bovine LH-ovalbumin conjugate has been reported in cattle. Immunization against hCG doesn't interfere with ovarian function and has extensively been studied to develop contraceptive vaccine. Active immunization against LH and FSH receptors can suppress the corpus luteum function. Because of extensive structural homology of LH-R among species and cross species nonspecificity of antibodies, anti-fertility vaccine based on LH-R may have future application. Anti FSH receptor can also potentiate contraceptive effects.

Vaccines against Gamete Antigens: Immuno-Contraception

For the control of wildlife, the widely held view is that the maintenance of libido and sexual behavior would be optimal to achieve this goal, and hence, the hormone system that drives those behaviors has not been generally targeted. The exception to this is the control of native animal species by an anti-GnRH vaccine. Alternate strategies to control wildlife have been to develop vaccines that prevent the fertilization of the oocyte by sperm or to prevent the implantation of the embryo and allow immunized animals to continue to compete in mating rituals. With this approach, antigens of the gametes (sperm and oocytes) have been widely targeted to prevent fertilization. Surface antigens of gametes have been reported to be potential target for contraceptive vaccines. Immunizations with such antigens either prevent fertilization or reduce survival of embryo in the uterus.

Sperm antigens: While some effect could be expected in vaccinated male animals, the large number of sperm in the male reproductive tract and observed

autoimmune-mediated orchitis have focused efforts instead on vaccinating the female. Fertility levels in vaccinated females are generally reduced from levels around 75 to 80% to 25 to 30% in a range of species including mice baboons and guinea pigs. The potency of the range of antigens is similar, and no one sperm antigen gives an exceptional contraceptive effect. Interestingly, production of monoclonal antibodies against sperm surface antigen can be used for precise diagnosis of immuno-infertility and to indicate the correct therapeutic approach in animals.

Oocyte antigens: Zona pellucida is a glycoprotein membrane which surrounds the mammalian egg. A commercially available vaccine called *SpayVac*, designed based on crude porcine ZP antigen preparation (purified from pig ovaries) has been shown to have efficacy in a number of species. *SpayVac* was supplied to researchers for experimental wildlife population control and should not be considered to be a major commercialized vaccine product. Other vaccines like *ZonaCon* have been proved successful as a contraceptive vaccine in deer and feral horse. Many of the experimental ZP-based vaccines have induced reasonably high levels of efficacy sufficient to engender strong interest in further development and commercialization, with ZP antigens alone or in combination with sperm antigens to increase efficacy.

Despite considerable scientific advances, there has been only very limited commercial success of gamete antigen-based vaccines. This approach has some major difficulties for wild or feral animal populations, including developing a delivery system to mass vaccinate wild animal populations without capture or restraint and being capable of delivering a booster dose, i.e., allow revaccination; ensuring reasonable duration of efficacy and being able to induce and maintain high levels of antibody in the female reproductive tract. Currently, these issues remain unresolved, and it is unlikely that fertility control vaccines will be used in wildlife management programs or commercialized until such technical hurdles are overcome.



Modulation of uterine defense mechanism (UDM)

The uterine defense mechanism involving neutrophils, monocytes and tissue macrophages prevents colonization of invading microbes in the uterus. Methods for enhancing immune function in periparturient cows have the potential for treating uterine infections. Bacterial modulins like *E.coli* LPS and other bacteria free filtrates have been found effective to enhance the uterine defense mechanisms. Lysozymes and bacterial cell free filtrate have also been used as an alternative therapy for endometritis. Bacterial modulins increase PMN cell influx to uterus through IL-1 production and increase phagocytosis. Lysozymes have the property of chemotaxis and modulate synthesis of TNF and IFN- α involved in immune response. Addition of serum, 10% Oyster glycogen or PMN extracts increase the opsonizing capacity, enhance phagocytosis and can be used for treatment uterine infections.

Immunomodulatory Role of Cytokines

Studies on the relationship of cytokines with embryonic implantation and their role in development and maintenance of placenta have provided new valuable data on the mechanisms of immune regulation of reproduction. Currently cytokines are regarded as the primary factors of communication between the endometrium and developing embryo. Trophoblast of sheep and cattle embryo secretes ovine and bovine trophoblastic proteins or interferons (bTTP-1, IFN- δ). These proteins are type of cytokines responsible for maternal recognition pregnancy and embryonic development. Unlike other interferons, IFN- δ is not virus inducible and present immunomodulatory action towards WBC by changing their proliferative responses and cytokine production. This has been intensively exploited and regarded as a potential tool in improving performance and biotechnical processes in ruminant reproduction. These proteins may be used as potential fertility promoters especially in sub-fertile or infertile animals. Such cytokine have been isolated, partially purified and used for early diagnosis of pregnancy and fetal development in cattle and in buffaloes. Recombinant interferon has been shown to have anti-luteolytic and

luteotropic action on porcine CL. High antiviral potency and low cytotoxicity of IFN- δ , increases its use in animal medicine. It is proved useful in limiting early embryonic mortality and increasing reproductive performance in ruminants.

Future Prospects in Immunomodulation of Reproduction

Mucosal vaccines : Several recent studies focus on establishing the molecular basis of mucosal defense mechanism and development of mucosal vaccine. This will obviously contribute to elucidate many important problems concerning reproduction such as fertility regulation and management of venereal diseases. Innate and immunological defense mechanisms occurring at the mucosal surfaces of genital tract has to be exclusively studied to produce effective vaccines against sexually transmitted diseases, treatment of inappropriate immune responses leading to reproductive failure.

Thymosin α -1 is an immunoreactive peptide purified from thymus and its extract has been sequenced and synthesized. It has shown to increase fertilizing capacity of sperm by modulation of membrane protein phosphorylation pattern during capacitation. It has also growth factor like enhancing effect on in vitro development of murine pre-implantation embryos and thus can be exploited for use in the field of embryo transfer technology.

Conclusion

The field of reproductive immunology is making rapid advances towards establishing the immunological and molecular basis of reproduction and development of effective vaccines and use of various immunomodulators. Ovulation rate and reproductive efficiency can be improved through the use of fecundity vaccine. Immunization against androgens and inhibin has shown promising results for increasing the litter size and rate of ovulation. Immunomodulation of Uterine Defense Mechanism is a topic of importance especially in the therapeutic strategies for uterine infections. Use of cytokines has been intensively exploited as a potential tool in improving reproductive performance and may be used as potential fertility promoters especially in sub fertile or infertile animals. Immunomodulation is applicable to solutions for many important problems concerning reproduction such as

fertility regulation and management of reproductive diseases, ultimately improving the reproductive performance of livestock.

Advancement in technology and detailed knowledge of immunology, molecular biology and biochemistry among other basic science disciplines along with introduction of novel ideas in vaccinology have defined new directions for reproductive vaccine development. The promise is that effective application of immunomodulation coupled with biotechnology will increase reproductive efficiency in farm as well as wild animals as new technologies are transferred to the marketplace and adopted by the livestock industries.

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BOVINE COLOSTRUM AND ITS MULTIFARIOUS THERAPEUTIC APPLICATIONS

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Introduction

Colostrum is the first milk produced by the mammary glands of all mammals during the first 2-3 days after birth. Colostrum contains factors that are protective for the neonate and a rich source of immuno-modulatory molecules that positively influence the immune status of the neonate. It is the most potent natural immune booster, protective against wide spectrum of bacteria and viruses and is a rich source of nutrients. It contains proteins, major portion of which is immunoglobulins, non-protein nitrogenous compounds, growth factors, fat, ash, vitamins, minerals etc. Its medicinal importance was described even in ancient Book Ashtanga Hridayam: Sutrasthanam Vol.1 written by Vagbhatacharya. The bactericidal property of milk was recorded earlier, in 19th century. Later the antibiotic, immunogenic and nutritional properties of colostrum were studied and found out that it can be used to treat different diseases such as rheumatoid arthritis, gastro intestinal diseases etc.

Colostrum can be used as a feed supplement for improving body composition, increasing athletic performance and reducing diarrhea in patients with immunodeficiency conditions. Since human colostrum is difficult to obtain in large amounts, bovine colostrum can be considered as a better alternative. Colostrum is an excellent nutritional supplement for children, elderly people and for immuno-compromised individuals with no contraindications.

Preservation of Colostrum

The colostrum can be collected from a hygienically well maintained cows within few hours of calving. The animal should not be exposed to antibiotics, antihelmintics, pesticides etc. The colostrums collected within 24 hrs of calving

contains maximum amount of substances but there after immunoglobulin will be less. The bovine colostrum can be heat treated at 60^o c for up to 120mts without affecting IgG concentration and viscosity. The bacterial load of colostrum can be considerably reduce without damaging the immunoglobulin level by pasteurization at 60^o c for 60 mts). The colostrum can be stored without increasing bacterial load up to 96hrs in refrigeration temperature by adding potassium sorbate. Spray drying can produce dried colostrum in which immunoglobulin quantity and function were preserved. This is a cost effective method for preservation of colostrum.

Major Components in Bovine Colostrum

There are about 90 factors relating to health in colostrum and can be divided into two important components immune system related factors and growth factors.

Bovine colostrum is 4 times richer in immune factors than human colostrum and contains special glycoproteins. The main immune system related factors are immunoglobulins. Immunoglobulins are selectively transported from the serum into the mammary gland, so the first colostrum contains high concentration of immunoglobulins(40-200 mg/ml in bovines). Major immunoglobulin in bovine colostrum is IgG, IgM and IgA also present. IgG1 constitute 75% of colostrum whey, then IgM, IgA and IgG2. IgG in human colostrum is only 2%. The concentration of IgA in colostrum is almost a 100 fold higher than in milk. Bovine colostrum contains immunoglobulins specific to many human pathogens, including *Escherichia coli*, *Cryptosporidium parvum*, *Shigella flexneri*, *Salmonella*, *Staphylococcus* and rota virus (which causes diarrhea in infants).

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Lactoferrin is another immune factor, which is a non-haeme iron binding glycoprotein facilitating iron absorption. It is a prominent component in first line of mammalian host defense and effect is up-regulated in response to inflammatory stimuli. It act as a potent anti-inflammatory protein at local sites of inflammation including respiratory and GI tracts.

The other immune factors include cytokines, lymphokines, proline rich polypeptide, oligosaccharides, glycoproteins and trypsin inhibitors, lysozyme, leucocytes, lactalbumins, α lacto globulin, α 1 antitrypsin, α 2 macro globulins, complement components C3 and C4 etc.

The important growth factors in bovine

colostrum are similar to human colostrum. Colostrum is the only natural source of two major growth factors namely Transforming Growth Factors α and β (TGF α and β) and Insulin like Growth Factor I and II (IGF I and II). The concentration of IGF in bovine colostrum is much more higher (500 μ g/L) than in human colostrum (18 μ g/L). The growth factors have significant muscle and cartilage repair characteristics. They promote wound healing. Colostral growth factors have multiple regenerative effects that extends to all structural body cells. In addition colostrum contains vitamins, minerals, fat etc. Dams health status especially hypocalcaemia, Ketosis, anemia etc. can influence the composition of colostrum

Major components of bovine colostrum

HEALTH FACTOR	COMPONENTS	FUNCTIONS
Immune factors	Immunoglobulins	IgG - Neutralizes toxins and microbes in the lymph and circulatory system IgM - Destroys bacteria IgA - Stimulate increased production of salivary IgA which protects against upper respiratory tract infections.
	Lactoferrin	It has anti-inflammatory and anti-microbial effect. It inhibits reproduction of bacteria by depriving them of iron
	Proline rich polypeptide (PRP)	Hormone regulating the thymus gland. Up-or down-regulate the immune system
	Cytokines	Regulates the intensity and duration of immune response.
	Lymphokines	Mediates immune response.
	Glycoproteins and Trypsin inhibitors	Prevent breakdown of colostrum in gut thus helping its activity against <i>Helicobacter pylori</i> .
	Lactalbumins	Effective against many types of cancer and viruses.
Growth factors	IGF I and IGF II	Anabolic agents helps in the utilization of fat, proteins and sugar by the body.
	TGF α and β	Helps in the proliferation of connective tissue and aids in the formation of bone marrow and cartilage. A potent chemo attractant for neutrophils and stimulate epithelial cell migration at wound site.
	growth factor (PDGF)	tissue, fibroblast, smooth muscle and neuronal regeneration.
	Epithelial growth factor(EGF)	Helps in normal skin growth and cellular tissue repair.
Vitamins	Vitamin A,C, E	Antioxidant properties, neutralizes free radicals and reduces tissue injuries.



Uses of Bovine Colostrum

Cows can be hyper immunized against microorganisms of specific antibodies are required. Hyper immune bovine colostrum derived from cows immunized with rota virus, cryptosporidiosis, *H. pylori*, cholera can prevent infection in neonates.

Topically applied colostrum is effective in eye infections especially it alleviates severe eye dryness and problematic eye lesions.

Human colostrum cells in combination with antibody are able to destroy Herpes simplex virus infected cells (antibody mediated cellular cytotoxicity).

Lactoferrin helps in preventing shigellosis.

Bovine colostrum may prevent upper respiratory tract infection since salivary IgA increases after supplementation with bovine colostrum.

Bovine colostrum helps in prevention of NSAID induced gut damage mainly by various growth factors like TGF α and β which help in the muscle and cartilage repair. It is also used in the treatment of ulcerative colitis, Chemotherapy induced mucositis Surgical and trauma patients and Crohn's disease.

Due to anabolic effects of IGF I and II in bovine colostrum, supplementation leads to increased exercise performance.

In immunodeficiency conditions like AIDS, immunoglobulins from bovine colostrum effectively reduces and prevents bacterial and viral infections.

Bovine colostrum promotes normal cell growth and DNA synthesis, thus helps in chronic wound healing. Topical administration to wounds results in more effective healing.

Pre-treatment with bovine colostrum reduces amount of endotoxins produced after abdominal surgery, partial or total gastrectomy.

The anti oxidant property of vitamins (A, C, D and E) present in the colostrums neutralizes the free radicals and has tissue repair properties. This prevent aging process and can be used as anti ageing agent.

Bovine colostrum can fed to weaning puppies. It can improve fecal quality in puppies subjected to the stress of changing both diet and environment.

Dosage and Administration

Improvement of Athletic performance	60g/day
Immuno deficiency related diarrhoea	10g/day
Prophylaxis/treatment of infections	10g/day
NSAID-induced GI infections	15g/day
Post surgical conditions	14gq.i.d

Product information

Nectera powder by Lupin pharma, each 3g contain 500 mg colostrum (28% IgG), available as 900g tin.

Conclusion

Bovine colostrum has multifaceted therapeutic applications as the specific hyper immune bovine colostrum are effective against *Cryptosporidia*, rota virus, *H. pylori*, *Shigella*, measles etc. apart from this bovine colostrum is used to prevent NSAID induced gut damage, promotes wound healing and repair, fights stress by its antioxidant potential and possess antiageing property. When farmers become aware of these facts, wasting of excess colostrum can be avoided and it will also give an additional income to them.

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GLOWING GENES

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Introduction

Discovery of X rays revolutionized the medical and veterinary science as it made possible to visualize internal structures of body. The rise of Green Fluorescent Protein (GFP) as a fundamental staple of biomedical research has also been termed a revolution since it is utilized to visualize proteins and biochemical processes that take place inside the living cells. Bioluminescence is the production and emission of light by a living organism. Its name is a hybrid word, originating from the Greek *bios* for "living" and the Latin *lumen* light". Bioluminescence is a naturally occurring form of chemiluminescence where energy is released by a chemical reaction in the form of light emission. The first phase of the revolution began when GFP was used to highlight sensory neurons in the nematode *Caenorhabditis elegans*. Then the wild type GFP (wtGFP) was modified to produce variants emitting the blue (BFP), cyan (CFP), and yellow (YFP). Now GFP has become very popular in research. Using GFP we can see proteins inside the living cells, when they are made and where they go. This is done by joining the GFP gene to the gene of the protein of interest, so that when protein is made it will have GFP hanging to it. Since GFP fluoresces one can shine light at the cell and wait for the distinctive green fluorescence associated with GFP to appear. GFP thus allow the monitoring in time and space of an ever-increasing number of phenomena in living cells and organisms like gene expression, protein localization and dynamics, protein-protein interactions, cell division, chromosome replication and organization, intracellular transport pathways, organelle inheritance and biogenesis, to name but a few. In addition, the fluorescence from single GFP molecules has made it feasible to image at a spatial resolution higher than the diffraction limit.

Furthermore, sensors that report pH values, Calcium concentrations and other essential features of the interior of living cells have been engineered from GFP.

Green fluorescent protein exist for more than one hundred and sixty million years in one species of jelly fish *Aequorea victoria*. In 1960 Osamu Shimomura isolated the GFP and determined which part of the GFP was responsible for its fluorescence. Douglas Prasher was the first person to realise the potential of GFP as a tracer molecule. It means when GFP is attached to a specific protein, for example haemoglobin, one would be able to see the green fluorescence of GFP that is attached to the haemoglobin. It would be a bit like attaching a light bulb to the haemoglobin molecule. The method is to attach GFP gene at the end of the haemoglobin gene right before the stop codon. As a result, the cell would produce haemoglobin molecule with GFP attached to it. The GFP gene can be introduced into organisms and maintained in their genome through breeding, injection with a viral vector, or cell transformation. To date, the GFP gene has been introduced and expressed in many bacteria, yeast and other fungi, fish (such as zebra fish called 'glow fish'), plant, fly, and mammalian cells, including human. Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien were awarded the 2008 Nobel Prize in Chemistry for their discovery and development of the green fluorescent protein.

Why GFP works as a tracer?

1. If enough of it made, we can shine light on organism and see where GFP is.
2. GFP is small and is unlikely to hinder the protein to which it is attached.
3. GFP glows without needing any enzyme or cofactors except oxygen.

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When GFP is attached to the promoter of a gene we can identify when a gene is activated. The promoter is a region of DNA located in front of a gene, when the cell needs to make a specific protein the promoter of that gene is activated which in turn activates the gene. By attaching GFP to the promoter GFP would be produced whenever the promoter attached to it is activated. In this way GFP fluorescence could be used to signal activation of GFP tagged promoter.

While Shimomura, Prasher and Chalfie were all instrumental in taking GFP from the jelly fish and showing that it can be used as tracer molecule, it is Roger Tsien who is responsible for much of our understanding of how GFP works and for developing new technologies and mutants of GFP. His group developed mutants that start fluorescing faster than wild type GFP that are brighter and have different colours.

Simplicity makes it all

Many different organisms, ranging from bacteria and fungi to fireflies and fish, are endowed with the ability to emit light, but the bioluminescent systems are not evolutionarily conserved: genes coding for the luciferase proteins (Lase) are not homologous, and the luciferins are also different, falling into many unrelated chemical classes. Biochemically, all known Lase are oxygenases that utilize molecular oxygen to oxidize a substrate (a luciferin; literally the 'light bearing' molecule), with formation of a product molecule in an electronically excited state. The color of the light may differ, even though the same luciferin/Lase system underlies the reaction. Filters or differences in Lase structure are responsible in some cases; in others a secondary emitter associated with a second protein is involved. In the coelenterates a green fluorescent protein, whose chromophore is derived from the primary amino-acid sequence, results in a red shift of the emission. In the bacteria accessory proteins causing either blue- or red-shifts have been isolated from different species; the chromophores are noncovalently bound. Although radiation less energy transfer has been implicated in the excitation of such accessory emitters, this may not be so in all cases. The maturation of the tri-peptide-based chromophore in GFP only requires oxygen and does

not depend on the presence of enzymes or of auxiliary factors.

GFP contain 238 amino acids and the GFP gene is composed of 714 bp (i.e., less than one Kb). GFP has a typical beta barrel structure, consisting of one α -sheet with alpha helix(s) containing the chromophore running through the center. Inward facing side chains of the barrel induce specific cyclization reactions in the peptide Ser65Tyr66Gly67 that lead to chromophore (light producing substance) formation. This process of post-translational modification is referred to as *maturation*. The hydrogen bonding network and electron stacking interactions with these side chains influence the color of wtGFP and its numerous derivatives. The tightly packed nature of the barrel excludes solvent molecules, protecting the chromophore fluorescence from quenching by water. In *A. victoria* a protein called aequorin release blue light upon binding with calcium. This blue light is then totally absorbed by GFP which in turn gives off the green light. The final fluorescent chromophore formed is P-hydroxymethylbenzylideneimidazolinone.

Applications of GFP

Cell biological applications of GFP may be divided into uses as a tag or as an indicator. In tagging applications, the great majority to date, GFP fluorescence merely reflects levels of gene expression or sub cellular localizations caused by targeting domains or host proteins to which GFP is fused. As an indicator, GFP fluorescence can also be modulated post-translationally by its chemical environment and protein-protein interactions.

Reporter Gene, Cell Marker

The first proposed application of GFP was to detect gene expression *in vivo*, especially in the nematode *Caenorhabditis elegans*, whose cuticle hinders access of the substrates required for detecting other reporter genes. GFP was particularly successful in confirming the pattern of expression of the *mei-1* promoter, which drives the formation of tubulin in a limited number of mechano sensory neurons. GFP independence from enzymatic substrates is likewise particularly promising in intact transgenic embryos and animals and for monitoring the effectiveness of gene transfer.

GFP as an Indicator

The rigid shell in GFP surrounding the chromophore enables it to be fluorescent and protects it from photobleaching but also hinders environmental sensitivity. Nevertheless, GFPs that act as indicators of their environment have been created by combinations of random and directed mutagenesis. While most small fluorescent molecules such as FITC (fluorescein isothiocyanate) are strongly phototoxic when used in live cells, GFPs are usually much less harmful when illuminated in living cells. This has triggered the development of highly automated live cell fluorescence microscopy systems which can be used to observe cells over time expressing one or more proteins tagged with fluorescent proteins. For example, GFP had been widely used in labeling the spermatozoa of various organisms for identification purposes as in *Drosophila melanogaster*, where expression of GFP can be used as a marker for a particular characteristic. GFP can also be expressed in different structures enabling morphological distinction. In such cases, the gene for the production of GFP is spliced into the genome of the organism in the region of the DNA which codes for the target proteins, and which is controlled by the same regulatory sequence; that is the gene's regulatory sequence now controls the production of GFP, in addition to the tagged protein (s).

Fusion Tag

The most successful and numerous class of GFP applications has been as a genetic fusion partner to host proteins to monitor their localization and fate. The gene encoding a GFP is fused in frame with the gene encoding the endogenous protein and the resulting chimera expressed in the cell or organism of interest. The ideal result is a fusion protein that maintains the normal functions and localizations of the host protein but is now fluorescent.

Brainbow

In 2007, Jeff Lichtman and Joshua Sanes, researchers at the Harvard Brain Center, have created transgenic mice with fluorescent multicolored neurons. But it is not their colorful splendor that makes these genetically modified mice so amazing. It is their potential to revolutionize neurobiology that excites scientists. The mice created by a genetic strategy termed

"brainbow" will have a similar effect on neuroscience as Google Earth had on cartography. Using a brainbow of colors researchers will now be able to map the neural circuits of the brain. The individually colored neurons will help define the complex tangle of neurons that comprise the brain and nervous system. By creating a wiring diagram of the brain researchers hope to help identify the defective wiring found in neurodegenerative diseases such as Alzheimer's and Parkinson's Disease.

Cancer research

The intent of cancer surgery is to remove malignant tissue together with margins of presumably normal tissue to ensure complete removal of abnormal cells. Estimating margin width during surgery is critical and depends on the surgeon's vision. There have been many developments intended to improve the delineation of tissue margins using morphologic and optical differences between normal and abnormal tissue. A major enhancement of cancer surgical navigation has been reported using the selective fluorescent labeling, in vivo, of malignant tissue. Bright GFP fluorescence clearly illuminates the tumor boundaries and facilitates detection of the smallest disseminated disease lesions. Internal green fluorescent protein fluorescence can intensely illuminate even single cells but requires *GFP* sequence transcription within the cell. All malignant tissues are characterized by the presence of an active telomerase. Introducing and selectively activating the *GFP* gene in malignant tissue in vivo is made possible by the development of OBP-401, a telomerase-dependent, replication competent adenovirus expressing GFP.

Malaria

Malaria is the world's most common and deadly parasitic disease. The World Health Organization estimates that each year 300-500 million cases of malaria occur and more than 1 million people die of malaria. A possible breakthrough in curtailing the spread of malaria carrying mosquitoes was reported with the creation of mosquitoes with green fluorescent testicles. These male mosquito larvae can easily be separated from

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female mosquito larvae. Without green fluorescent gonads it is impossible to separate mosquito larvae based on their sex, and it is very difficult to separate the adults since they fly about and bite (actually only the females bite). Once separated from the females it is trivial to sterilize the males and release them into the environment where they will mate with wild females. Female mosquitoes only mate once in their two-week cycle, so if they chose a sterilized male they will produce no offspring. If a large enough population of sterilized males is released into the wild, population should be eradicated in a fairly short time.

GFP in nature

The purpose of GFP fluorescence in jellyfish is unknown. GFP is co-expressed with aequorin in small granules around the rim of the jellyfish bell. The secondary excitation peak (480nm) of GFP does absorb some of the blue emission of aequorin, giving the bioluminescence a more green hue. The serine 65 residue of the GFP chromophore is responsible for the dual peaked excitation spectra of wild type GFP. Roger Tsien has speculated that varying hydrostatic pressure with depth may affect serine 65's ability to donate hydrogen to the chromophore and shift the ratio of the two excitation peaks. Thus the jellyfish may change the color of its bioluminescence with depth. Unfortunately, a collapse in the population of jellyfish in Friday Harbor, where GFP was originally discovered, has hampered further study of the role of GFP in the jellyfish's natural environment

Conclusion

Synergisms between the suitability of GFPs as precisely targeted intracellular genetic tags, rapid development of imaging techniques and data analysis have boosted use of GFP in biological sciences. In just three years, the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* has vaulted from obscurity to become one of the most widely studied and exploited proteins in biochemistry and cell biology. Its amazing ability to generate a highly visible, efficiently emitting internal fluorophore is both intrinsically fascinating and tremendously valuable. High-resolution crystal structures of GFP offer unprecedented opportunities to understand and manipulate the relation between

protein structure and spectroscopic function. GFP has become well established as a marker of gene expression and protein targeting in intact cells and organisms. Mutagenesis and engineering of GFP into chimeric proteins are opening new vistas in physiological indicators, biosensors, and photochemical memories.

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ACIDOSIS IN CAPTIVE WILD HERBIVORES

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Introduction

The feeding of herbivores animals in captivity has the challenge of diversity in feeding behavior and nutritional requirement. Unlike domesticated animals species, there are few guides as to standards for requirement or for nutritive value of feedstuffs. Nutritive value is a particular problem for herbivores because of variation in composition of plants and the variable abilities of different herbivores to extract energy from carbohydrates.

Ruminal Acidosis in wild animals has been recognized where supplemental feeding is practiced. Carbohydrases are produced by various microorganisms in the guts of herbivores, thus fermentation chambers to harbor microbes are important evolutionary adaptations in the digestive sequence. These organisms consist of anaerobic bacteria, protozoa, and fungi that are adapted to the utilization of available carbohydrates upon which they grow. The main net products of mixed gut fermentation are volatile fatty acids (VFA), principally acetic, propionic, and butyric acids, CO_2 , methane and possibly hydrogen. Herbivores absorb these organic acids from the rumen and (or) cecum for metabolism by tissues.

When carbohydrate supply is increased abruptly by following grain engorgement or during adaptations to high concentrate diets, the supply of total acids and the prevalence of lactate in the mixture increase. Normally, Lactate is present in the digestive tract at only low concentration, but when carbohydrate supply is increased abruptly, lactate can accumulate ruminal concentration to a higher level that leads to metabolic disturbance and acute impaction of rumen.

It is crucial to develop an understanding of factors that put animals at risk of developing different type of ruminal acidosis and how feeding and management practice can help minimize this risk.

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Ruminal Acidosis

Ruminal acidosis refers to a series of conditions that reflect a decrease in pH in the rumen of Herbivores. Rumen lactic acidosis (Syn: Overeating, Grain overload, grain poisoning, acute indigestion, founder) develops in herbivores that have ingested large amounts of unaccustomed feeds rich in ruminally fermentable carbohydrates.

Rumen (Paunch) is a multi-chambered fermentation vat, where digestion of the food occurs through microbial fermentation.

The microbes in the rumen are classified as:

a. Cellulolytic

b. Amylolytic

c. Proteolytic, which preferentially digest structural carbohydrates, nonstructural carbohydrates and proteins respectively. All these bacteria are anaerobes. They are predominantly Gram negative but Gram positive bacteria are also present. In addition to the bacteria, rumen also contains a lot of protozoa and anaerobic fungi.

Importance of pH in Rumen function

For optimal function, the rumen pH is very critical. Ecological conditions within the rumen must be controlled within limits in order to maintain normal function for microbial growth. The cellulolytic organisms grow and function optimally at pH 6 - 7 and deviations of pH substantially higher or lower than this point become inhibitory and dry matter digestibility decreases with decreasing pH. If pH levels drop below 5.5, Ruminal acidosis results.

The rumen pH fluctuates through out the day and also is diet dependant. However, the mean pH for optimum digestion is maintained by:

a. Intake depression

b. Endogenous buffering and



c. Acid absorption.

Each animal has an inherent capacity to buffer and absorb acid that is produced in the rumen as a result of fermentation of carbohydrates. If the ruminal acid production increases, ruminants possess highly developed systems to maintain rumen pH within the physiological range. As ruminal pH begins to drop, the ruminant's first response is to stop eating.

Ruminants produce a large amount of saliva which buffers the ruminal pH because it is rich in sodium, potassium, bicarbonates and phosphates. Unfortunately, saliva production is not triggered by declining ruminal pH, but rather is determined almost entirely by the amount of fiber present in the diet. Saliva is secreted during the chewing activity in response to the amount of fiber in the diet. In addition, the ruminal epithelial cells also contribute towards buffering by secreting carbonate and phosphate ions which buffers acid. The ability of the

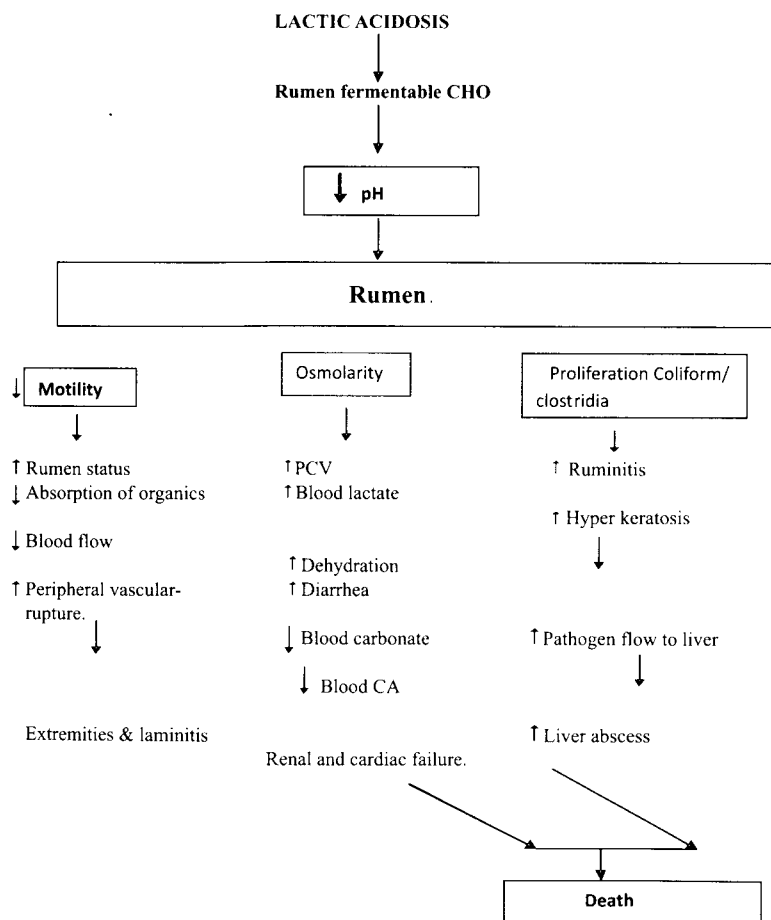
rumen to absorb organic acids contributes greatly to the stability of pH in rumen. Absorption of VFA from rumen occurs passively across the ruminal wall. If the rumen wall is damaged due to chronic ruminitis, absorptive capacity of the cells is impaired and acid accumulates.

Pathogenesis

- ◆ Histamine level increased
- ◆ Ethanol, Methanol, Tyramine, Tryptamine production contribute to CNS depression
- ◆ Thiaminase production may result in development of Polyencephalomalacia.
- ◆ Death of gram ve bacteria can cause endotoxin release.

Factors for acidosis

The risk of acidosis is not the same for all animals. Individual animals exhibit variation in tolerance for Ruminal acidosis. Not much is known about what causes animal variation, but presumably,





it is related to the combined effects of level of feed intake, eating rate, sorting of feed, salivation rate, the inherent ruminal microbial population, previous exposure to acidosis and other aspects of physiology and animal behavior. Ruminal acidosis is the consequence of feeding high grain diets. This may be either intentional (as practiced by dairy farmers to increase milk production) or accidental (animals getting access to excess grain). The condition is accentuated by over eating following periods of feed deprivation. Meal frequency may also play an important role in causation of acidosis. When excessive amounts of feed are provided (free choice intake) acidosis may be expected.

Factors contributing for acidosis

1. Lack of transition ration.
2. Rate of Starch Digestion.
3. Roughage level (Neutral Detergent Fibre) fed.
4. Consumption of large amount of grains that are rapidly fermented.
5. Roughage particle size.
6. Interruption of consumption pattern (Environmental changes like heat, cold and competition)
7. Adaptation to ration.

Starchy cereal grains which are incorporated in feed as a source of Carbohydrates, particularly those that can be rapidly rumen fermentable, produce lactic acid and increase risk of acidosis. In terms of acidosis, the ranking of grains that readily ferment is: wheat>barley>oats>sorghum>maize. This ranking reflects the amount, rumen biodegradability and cellular structure of starch in each grain.

Barley must be processed to maximize its digestibility, but processed barley grain is rapidly and extensively digested in the rumen. In contrast, ruminal digestion of maize corn grain is slower, thus a larger extent of corn starch escapes ruminal digestion and is available for digestion post-ruminally. Because starch digestion within the rumen is greater for barley than corn, cows fed diets containing barley tend to have a ruminal pH that is about 0.2 units lower than for animals fed corn, when diets are formulated to contain the same amount of forage fiber.

Feeds can cause carbohydrate engorgement.

- ◆ Cereal grains
- ◆ Industry by products (brewers grains and sugar)

- ◆ Fruits ,tubers (potato ,sugar beets)
- ◆ Finley ground feeds with large surface area promote rapid fermentation
- ◆ Hay and grass are not highly fermentable due to cellulose and large particle size.
- ◆ Corn silage usually not a problem because much of CHO already reduced to VFAs in ensiling process and also due to large particle size.

What is the minimum quantity of grain required to cause acidosis?

There is a wide variation in the amount of grain necessary to kill an animal by way of acidosis, because tolerance to rations high in starch develops. Acidosis developed by an animal depends on its inherent capacity to buffer and absorb acids.

In experiments with sheep, it has been established that 60 grams of wheat / kg body Wt. is enough to cause death of the animal. However it may be pointed here that it is the sudden increments of grain (Starch) that is of more importance than the amount of grain ingested.

Shortly after the ingestion of large amounts of carbohydrates, the rumen pH begins to fall depending upon the capacity of an individual animal to buffer. The decrease in pH during the first 8 Hrs. after ingestion is due to VFAs and not lactic acid. As fermentation proceeds, production of lactic acid increases and there is a change in the ruminal microflora as they are very sensitive to changes in pH. Ruminal Cellulolysis is totally inhibited at a pH less than 6.0 and dry matter digestibility decreases with decreasing pH. A drastic shift in microbial populations from gram negative predominance to gram positive lactic acid producers results. (*Streptococcus bovis*). Ruminal pH further drops and it is lethal to normal microflora which die, resulting in populations dominated by lactic acid tolerant lactobacilli. Ruminal lactic acid concentration may exceed 300 mmol/L, resulting in increased drawing of water into the GI tract leading to decreased plasma volume, hemoconcentration and circulatory collapse. Production of other toxic factors such as histamine or endotoxins produced by the lysis of the gram negative anaerobes. The wall of the rumen is not protected by the mucus. The cells of



the wall of the rumen are damaged due to the action of the acid, the rumen wall become more permeable, facilitating absorption of acid from rumen into the general circulation resulting in metabolic acidosis. At this stage the blood pH may be as low as 7.0 which causes depletion of alkali serves in the body. Because of the increased vascular permeability and circulatory failure, Acute pulmonary edema develops resulting in respiratory failure. The animal eventually collapses due to toxemia and multi-organ failure. Further, the undigested starch and glucose, if they reaches the intestines, stimulates the resident Clostridial organisms to multiply and produce exotoxins, which are absorbed resulting in enterotoxaemia.

Clinical forms of Ruminal Acidosis

Ruminal acidosis is not one disease, but rather a continuum of degrees of acidosis. Three clinical forms of Acidosis has been recognized:

1. Acute Ruminal Acidosis (Culminates in death if not remedied).
2. Subacute Ruminal Acidosis (SARA)
- Results in Ketosis, Acetonemia, Diarrhea.
3. Chronic Ruminal Acidosis
Results in ruminal ulceration, Parakeratosis of ruminal wall (Thickening of papillae which results in malabsorption), Liver abscess, lameness and overgrowth of hooves).

Clinical signs

- ◆ Abdominal pain
- ◆ Dehydration 6-12%
- ◆ Diarrhea
- ◆ Splashy rumen ,bloat.
- ◆ Depression.
- ◆ Lameness
- ◆ Initially elevated temperature and may be sub normal later.
- ◆ Heart rate-80-140 bpm
- ◆ Elevated respiratory rate (blow off CO₂)
- ◆ Rumens fluid : pH < 5 , Sour odor ,dead protozoa and predominance of gram + ve bacteria .

Necropsy findings in Acute Ruminal Acidosis.

Highly dehydrated carcass with bloody nasal exudation.

- ◆ Generalised congestion of the musculature.
- ◆ Acute pulmonary edema.
- ◆ Congestion of cardiac muscles with epicardial and endocardial haemorrhages.
- ◆ Congestion of the liver.
- ◆ Bulky, grain rich and watery ruminal contents, ruminal epithelium peels off easily, pH of the ruminal contents will be less than 5.0.
- ◆ Haemorrhages in the mucosa of abomasum.
- ◆ Diffuse intestinal congestion with watery contents, and
- ◆ Congestion of the kidneys, are the important necropsy findings in ruminal acidosis.

Conclusion

Ruminal acidosis in captive wild herbivores is a result of abrupt Increased consumption of grains as a supplemental feed by the animal. Risk factor for acidosis is not same for all animals. In acidosis the pH of the ruminal contents goes below 5.5, which is detrimental to normal micro flora of the rumen. The affected animals dies of systemic acidosis and multi-organ failure consequent to absorption of lactic acid into the circulation.

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INDIGENOUS PLANT PREPARATIONS IN AMELIORATING NEPROTOXICITY

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Introduction

Nephrotoxicity is the major side effect of aminoglycosides, especially gentamicin, accounting for 10 to 15 per cent of all cases of acute renal failure. The defective drug excretion adversely affects the different systems of the body. Some of the chemicals which cause damage to the proximal tubule are antibacterial agents such as cephaloridine and aminoglycosides, anticancer drugs such as cisplatin and industrial chemicals such as cadmium, hexavalent chromium, mercury and palladium (Cristofori *et al.*, 2007). Nephrotoxicosis has been reported in 15 per cent of human patients treated with gentamicin. In Veterinary medicine the frequency of this toxicity has not been documented except in dogs. Horses are highly sensitive to nephrotoxicosis as are cats (Lashev and Lasarova, 2001). Since kidneys are the major route of drug excretion, the occurrence of nephrotoxicity is of great concern.

Several Indian medicinal plants have been studied for their pharmacological activity. However, little is known about the nephroprotective effects of Indian herbal medicine. In fact, Indian herbal medicines have a long history of use in the treatment of various renal diseases. Modern day therapy of renal disease includes dietary protein restriction, blood pressure control, angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin receptor blockers (ARBs). In this article, some of the plants which show nephroprotection along with their other pharmacological properties are listed.

Nephroprotective Plants

Hygrophila spinosa

Hygrophila spinosa, T. Anders. (Kokilaksha in Sanskrit and Vayalchulli in Malayalam) Syn.

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²Professor, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy

Asteracantha longifolia, Nees., Syn. *Hygrophila auriculata* (K.Schum.) Heine., Syn. *Hygrophila schulli* (Ham.) (Sasidharan and Sivarajan, 1996) is a well known medicinal plant (family: Acanthaceae) found in paddy fields and marshy areas. The whole plant has medicinal properties and it is being used in ayurveda as a diuretic, aphrodisiac and in the treatment of dropsy, scanty urine and ascites. Bibu (2007) has reported the nephroprotective action of this plant in the gentamicin-induced nephrotoxicity.

Mangifera indica

Mangifera indica Linn. belonging to the family Anacardiaceae has many medicinal properties. The fruit is used as a laxative, diuretic, astringent and the bark is used in the treatment of uterine haemorrhagic ailments (Chopra *et al.*, 1956).

Curcuma longa

Curcumin is a major yellow pigment in turmeric, which is widely used as a spice and colouring agents in several foods, as well as cosmetics and drugs. It represents a class of anticancer, antioxidant which has a strong potency of inhibiting the generation of reactive oxygen species. Curcumin (200 mg/kg body weight in 1 per cent gum acacia) inhibited urinary excretion of N-acetyl- β -glucosaminase, fibronectin and glycosaminoglycan. Curcumin restored renal function by increased glomerular filtration rate and suppressed oxidative stress.

Solanum nigrum

Solanum nigrum L. (Solanaceae), known as 'makoy', is a medicinal plant found throughout India upto 3000 metres in the Western Himalayas. Besides the nephroprotective action, the herb is effective in cirrhosis of liver, as a febrifuge, antidiarrhoeal, in eye diseases and hydrophobia. Chinese medicine employs the juice of the leaves to alleviate pain in



inflammation of the kidney and bladder, and internally for cardialgia.

Tribulus terrestris

Tribulus terrestris L. belonging to family Zygophyllaceae, commonly known as Nerinjil, is a widely distributed plant. It is a herbal remedy used for various medicinal purposes including the treatment of kidney troubles, particularly stones. It has been also useful in treating angina pectoris, hypertension, hypercholesterolemia, colicky pains. It has shown to increase the free serum testosterone and to be effective in the treatment of sexual and erectile dysfunction. It has protective effect on genetic damage and stimulates melanocyte proliferation in the treatment of vitiligo and it has antibacterial, cytotoxic and nematocidal activities. Al-Ali *et al.* (2003) reported that *Tribulus terrestris* had long been used empirically to propel urinary stones. The diuretic and contractile effects of aqueous extract of leaves and fruits of *Tribulus terrestris* (5 g/kg body weight orally) in rats indicated that it has the potential of propelling urinary stones.

Aerva lanata

Aerva lanata commonly known as Cherula in Malayalam is an erect or prostrate herbaceous weed, common throughout the hotter parts of India almost all over the plains upto an altitude of 3000 meters. *Aerva lanata* provided nephroprotection by means of flavonoids which were potent antioxidant and free radical scavengers. The hyperglycemic, anti-inflammatory, antimicrobial, hepatoprotective and cytotoxic activities of this plant are also reported.

Pongamia pinnata

Pongamia pinnata L. Pierre is a medium sized glabrous tree, found throughout India. Different parts of this plant have been recommended as a remedy for various ailments like bronchitis, whooping cough, rheumatic joints and inflammatory and infectious diseases such as leucoderma, leprosy, lumbago and muscular and articular rheumatism. Essa *et al.* (2005) reported that the antihyperammonemic effect of ethanolic extract of leaves of *Pongamia pinnata* (300 mg/kg orally) in

rats could be attributed to its nephroprotective effect by means of detoxifying excess urea and creatinine, free radical scavenging and antioxidant properties.

Boerhavia diffusa

Boerhavia diffusa commonly known as Thazhuthama in Malayalam belonging to the family Nyctaginaceae is a major nephrocurative agent used in the Ayurvedic medicine and in Veterinary practices for many renal disorders. Abraham (1975) reported that the plant *Boerhavia diffusa* as a whole was effective in jaundice, odema, blood pressure and acting as a diuretic in mild doses, it cured asthma and in high doses, acted as an emetic.

Allium sativum

Pedraza-Chaverri *et al.* (2000) stated the protective effect of garlic (2 per cent garlic diet) in rats was associated with the prevention of the decrease of superoxide dismutase, glutathione peroxidase activities and the rise of lipid peroxidation in renal cortex. *In vitro* studies have proved the antibacterial, antiviral, and antifungal activities of garlic. However, these actions are less clear in humans. Garlic is also claimed to help prevent heart disease (including atherosclerosis, high cholesterol, and high blood pressure) and cancer. Animal studies, and some early investigational studies in humans, have suggested possible cardiovascular benefits of garlic. *Allium sativum* has been found to reduce platelet aggregation and hyperlipidemia. Garlic is also alleged to help regulate blood sugar levels.

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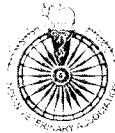
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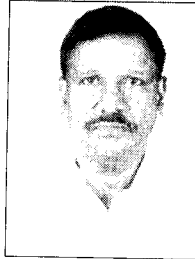


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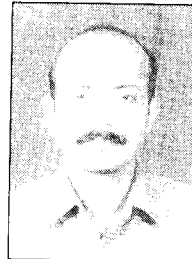
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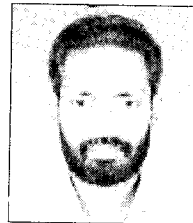
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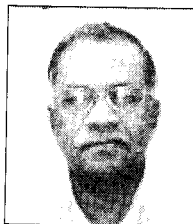
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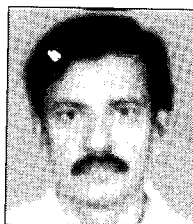
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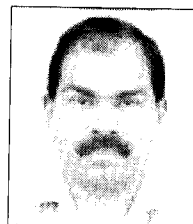
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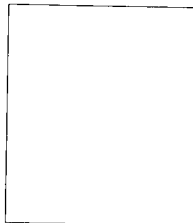
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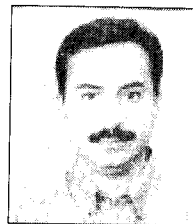
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INSTALLATION OF CEREMONY & INAUGURATION OF ACTIVITIES OF THE ASSOCIATION FOR 2010



The new office bearers of IVA, KVSSA & AHOAK were sworn in, at a glittering function, at Veterinarians' Building, Trivandrum on 09/01/2010. The meeting started with a silent prayer. Dr. Sukumara Pillai, President, AHOAK, welcomed the gathering. Dr. Ramesan V K, President, IVA in his presidential address promised to carry on the good work put in by the previous year's office bearers. Sri. A. SAMPATH, MP was the guest of honour. In his inaugural address, he highlighted the importance of veterinarians in the society and urged the vets to play a proactive role in the society. The new office bearers of IVA & AHOAK were sworn in by Dr A P S Nair and that of KVSSA by Dr. Theodore John. Dr A P S Nair, former President IVA, Dr. C. Sreekumar, President, KVSSA & Dr. Sissy Philip, Secretary, AHOAK felicitated the gathering. Dr. Joby George, Secretary, KVSSA proposed vote of thanks.

STATE EXECUTIVE MEMBERS OF IVA

1. Dr. Bahuleyan. P. (Kollam 9447557515)
2. Dr. K.C. Prasad (Thiruvananthapuram 9447081112)
3. Dr. K.R. Arun Kumar (Trissur 9846075281)
4. Dr. Deepu Philip Mathew (Alappuzha 9447200449)
5. Dr. Alex Abraham (Kottayam 9447808310)
6. Dr. Santhosh S (Kollam 9447734026)
7. Dr. Harikrishnan (Thiruvananthapuram 9447589049)
8. Dr. K.D. Paul (Trissur 9495196470)
9. Dr. Mohammed Haneefa (Palakkad 9447632370)
10. Dr. Suresh T Oranadi (Kozhikkode 944707996)

WORLD VETERINARIAN'S DAY CELEBRATED AT MUNNAR

World Veterinary Day 2010 celebrations was hosted by KVSSA in a befitting manner at Bellmount Resorts, Munnar with just the right mix of seminars, cultural night and a family get together. The function was presided over by Dr C Sreekumar, President KVSSA and was inaugurated by Dr. V.K. Ramesan, State President, IVA Kerala. Mr S Rajendran, MLA, Munnar gave away the prizes for the winners of State level painting competition held in association with this event. Dr G Sukumara Pillai, President, AHOAK and Dr V A Jose, District Animal Husbandry Officer, Idukki felicitated the occasion.



Inauguration by Dr. V.K Ramesan



Dr. Sreekumar. C. presiding over the celebrations

The technical sessions were handled by Dr. Binu Areekal, Assistant Professor, Department of Community Medicine, Medical College, Kottayam and Dr. E. Sreekumar, Scientist, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. An Orchestra was conducted with participation of children and family members of Veterinarians. In the scorching heat of Mr. S. Rajendran handing over the prizes summer and after the tensions of scheme implementations, the entire day was a time for relaxation.



LEAP 2010 - LEADERSHIP TRAINING PROGRAMME



The two day Residential Leadership Training programme for vets was held at Madapparampil River Bank Resorts, Thodupuzha on 15/05/2010 and 16/05/2010. The programme was organized by The Indian Veterinary Association, Kerala in association with the Kerala State Veterinary Council. 36 delegates from all IVA Units participated. Kannur, Kasargod, Trissur and Ernakulam districts had no representation.

The Inaugural function started with a silent prayer. Dr Joseph E Mathew, President, IVA, Idukki welcomed the gathering. Dr V K Ramesan, State President, IVA in his presidential address enlisted the lead role a veterinarian has to play in his day to day work. The programme was inaugurated by Shri Ajith Kumar IAS, Subcollector, Idukki. In his inaugural address, he stressed the importance of ATTITUDE in achieving success. Dr Sissy Philip, State Secretary, AHOAK and Dr Sangeeth Narayanan, Vice President (SZ), KVSSA felicitated the gathering. Dr George Varghese, General Secretary, IVA proposed the vote of thanks.

Sri Ajith Kumar IAS, inaugurating LEAP 2010

The sessions were as follows:

- ❖ Success through Excellence
- ❖ Attitude to success
- ❖ Life skills
- ❖ Building positive self esteem
- ❖ Be a good PRO
- ❖ Delegation & Institution Development
- ❖ IVA History, Role & Bye laws.

The faculty included : (i) Shammeem Rafeek, CEO, Vencedor Management Consultancy (ii) Benny George, MD, Success unlimited (iii) Dr Prakash Chandran, Consultant Psychologist, Amritha Institute (iv) Venugopala Menon, Former District Secretary, Rotary International (v) Dr Sabu Varghese, Research Officer, Planning Board (vi) Dr N NSasi, Registrar, Kerala State Veterinary Council (vii) Dr A P S Nair, former State president IVA, KVSSA & AHOAK. Cultural programmes and an orchestra were arranged on 15/05/2010 evening.

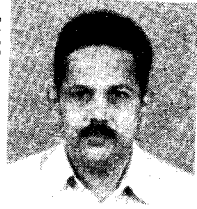
LEAP 2010 was an excellent forum to further strengthen the bonds of friendship and to bask in the world of leisure and learning. It sharpened the skills of all participants to discharge their duties precisely.

ACHIEVEMENTS AND SIGN POST

DR. G. AJITKUMAR BAGGED DR. G. NIRMALAN AWARD

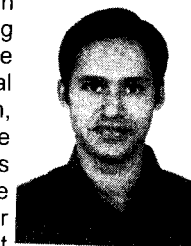
Dr. G. Ajitkumar, Associate Professor, Dept. of Animal Reproduction, COVAS, Mannuthy bagged Dr. G. Nirmalan Award (2009-10) for the Best Research Article published by a veterinarian of the State. His article entitled "Comparative efficacy of bromocriptine, cabergoline and thyroxine in inducing oestrus in bitches" and published in Veterinary Research Communications (2010) 34:6569, DOI 10.1007/s11259-009-9333-1 was

adjudged as the best research article. The award instituted in memory of Dr. G. Nirmalan, Former Dean, COVAS, Mannuthy he is presently pursuing post-doctoral research at University of Calgary, Canada.



JALAS Young Scientist Award to Dr. Harikrishnan V. S.

Dr. Harikrishnan V. S. has been awarded JALAS International Award (Japanese Association for Laboratory Animal Science) as Young Scientist 2009 in Laboratory Animal Science field and has been invited to the 57th annual meeting of JALAS to be held in Kyoto, Japan, in May 2010. The award is offered to one person per year from each Asian Countries by JALAS. He will receive the award in the General Assembly of JALAS and will deliver his work on the "Development of a persistent Interventional Hind Limb Ischemia Model In New Zealand White Rabbits" in the oral session. Dr. Harikrishnan V. S. is presently working at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Bio Medical Technology Wing as Scientist-B.





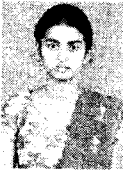
Dr. T.V. ANILKUMAR, Secretary General, IAVP and Scientist, Sree Chitra Institute, has been elected as a Fellow of the Society of Biology (Incorporated by Royal Charter) and attained the status of Chartered Biologist.

INTAS YOUNG SCIENTIST AWARD



Dr. V. R. AMBILY bagged the prestigious Intas Young Scientist Award for her paper entitled "Clinicotherapeutic Studies on Canine Microfilariosis" presented at the 28th annual conference of the Indian Society for Veterinary Medicine held at Hyderabad. Dr. Ambily along with her Guide Dr. Usha Narayana Pillai, Associate Professor, Department of Clinical Medicine conducted an extensive and

pioneering study on canine microfilariosis.



Jeeva John, D/o. Dr. K.R. Johny (Retd. Senior Veterinary Surgeon) secured rank 159 in CBSE Pre medical entrance examination and rank 272 in Kerala state medical entrance examination.

Dr. P.C. Saseendran, Ph.D., Professor and Head of L.P. M. took charge as Dean, College of Veterinary and Animal Sciences, Mannuthy on 1-5-10. He belongs to 1973 batch of B.V.Sc & AH programme. He had acquired M.V.Sc. in LPM in the year 1979. He completed the Ph.D programme from Madras Veterinary College in the year 1995 and his work was on "Monitoring and managing musth in captive Asian elephants". He was nominated by the Govt of Tamil Nadu to the Board of Management of Tamil Nadu



Veterinary and Animal Sciences University. He has served as visiting Scientist of the prestigious Smithsonian Institute, USA. ICAR has awarded him two research projects and Govt of Kerala has entrusted him with a project worth 1.5 crore entitled "Livestock based tribal resettlement project at Aralam, Kannur".

Dr. K. Udayavarman, has taken over as the Director of Department of Museum and Zoos. IVA, Kerala wishes him all success in his endeavour during this tenure.

OBITUARY

Dr. V. RAVINDRAN PILLAI

Retd. Assistant Director, AHD passed away on 14/12/ 2009 at Perunna, Kottayam. He was an Assam Veterinary College graduate. He is survived by his wife (Retd.Teacher), two daughters and one son. May his soul rest in peace.

Dr. THOMAS MANI

Thattumkal, (69) Retd. Joint Director, AHD passed away at Muttambalam, Kottayam. He was an Orissa Veterinary College graduate. The burial was on 17/12/2009 at St. Pauls Church, Nedumavu. He has served as State President, IVA and the Chairman of Building Committee, Kottayam and has made important contribution to the formation of Veterinarian's Building, Trivandrum. He is survived by his wife, three daughters and one son. May his soul rest in peace.

Dr. K.M. MATHEW PARAYIL

Retd. Joint Director, AHD passed away at Muttambalam, Kottayam. The burial was on 13/1/2010 at Puthenpally, Kottayam. He is survived by his wife, one son and two daughters. May his soul rest in peace.

Dr. P.C. MANI PUTHENKANDAM (73),

Retd. Assistant Project Officer, AHD passed away at Kodumpidi, Kottayam. The burial was on 17/1/2010 at Geovalley Church, Elivaly. He is survived by his wife, three sons and one daughter. May his soul rest in peace.

Dr. ARAVINDAKSHAN

1959 batch retired Joint Director, AHD and native of Irinjalakuda passed away on 08-02-2010. May his soul rest in peace

Dr. V. RAVINDRAN

Parikkappally (Kailas), (71) Retd. Deputy Director of Animal Husbandry Department passed away on 27/02/2010 at Vaikom, Kottayam. District. He is survived by his wife and daughter. May his soul rest in peace.

Dr. THAMPI ABRAHAM

(Retd. Joint Director) Puthenpurackal, Kottayam passed away. He is survived by his wife, son and daughter. May his soul rest in peace

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UPCOMING EVENTS

Indian Veterinary Association Kerala is hoisting a State level seminar on **July 6th 2010 at Dass Continental Thrissur** to commemorate the World Zoonoses Day. IVA is honouring Dr. Arthur Vijayan Lal, veterinarian of international repute for his life time achievements in biomedical research. On this occasion, scientists of national and international repute will be presenting papers. All are requested to participate.

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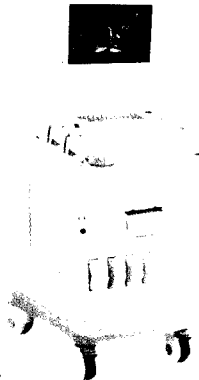
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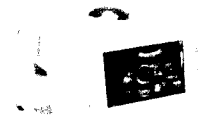
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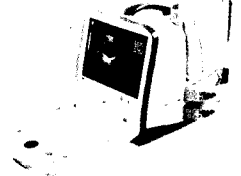
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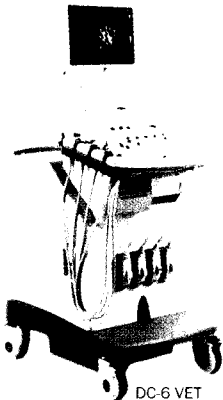
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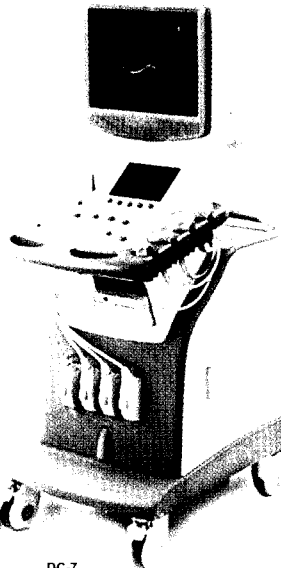
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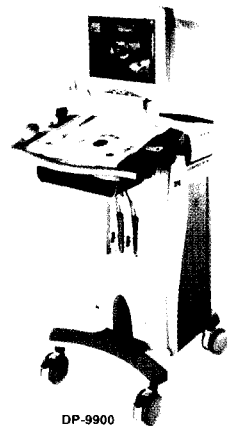
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