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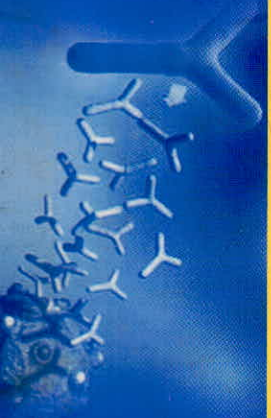

XV Annual Convention & National Symposium

XV Annual Convention of ISVIB, CCSHAU, Hisar • Feb. 26-28, 2009 •

Recent Approaches in Veterinary Immunology and Biotechnology for Animal Health and Production

February 26 – 28, 2009

Department of Veterinary Microbiology and Department of Animal Biotechnology
Chaudhary Charan Singh Haryana Agricultural University
Hisar-125004 (Haryana) INDIA

BOOK OF ABSTRACTS

Indian Society for Veterinary Immunology and Biotechnology

XV Annual Convention & National Symposium

on

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Date
26 February, 2009, 2.00 - 5.00 PM

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CURRENT SCENARIO OF EQUINE HERPES VIRAL INFECTIONS IN INDIA

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Introduction:

The Equine Herpes Virus - 1(EHV-1) is known to cause respiratory infections, stillbirths, foal mortality and neurological disease. EHV-1 is responsible for heavy economic losses to the equine industry worldwide by causing storm of abortions. Two types of herpes viruses are of major concern in equines. These are equine herpes virus-1 (EHV-1) and EHV-4. These viruses are reported to cause abortions, generally EHV-1 affects the genital tract and EHV-4 causes respiratory disease. Equine herpes virus-3 causes coital exanthema (a venereal diseases) in equines.

EHV-1 infection emerged in India during 1972-1976 and infertility, abortion, neonatal foal mortality, paralysis and respiratory syndromes were reported from various parts of the country. However, the first report of EHV-1 induced abortions was by Sharma *et al.* (1965) on the basis of histopathology of aborted foetuses. Subsequently, virus was isolated and characterized by Jain *et al.* (1976). Abortion storm due to EHV-1 was reported by Uppal *et al.* (1991) in an organized equine breeding farm in 1989, where 15% (54/360) mares aborted. Abortions were spontaneous with no warning signs. Petechial haemorrhages on the nasal, pharyngeal and tracheal mucosae, as well as the surface of the heart, spleen, lungs and nodular hepatic necrosis were common in aborted fetuses. Virus is also associated with encephalitis and paralysis (Jain and Batra, 1979; Batra *et al.*, 1982; Shankar and Yadav, 1986), neonatal foal mortality (Jain and Ram, 1980; Rattan *et al.*, 1998) and respiratory syndrome (Tewari *et al.*, 1992). Tewari and Prasad (1983) and Singh *et al.* (1995) studied comparative diagnosis by different tests. Abortions in mares were also recorded during 1996 and 1997 (Uppal et al. 1991: Singh et al 1998)

Virus isolation:

EHV-1 was isolated from 15 aborted fetuses in 1989 abortion storm (Singh et al.1991). Virion size ranges from 180 to 198 nm. Mature virion consists of core, capsid, tegument and envelop. Capsids are icosahedral, composed of 150 hexameric and 12 pentameric capsomere whose size is approximately 100 nm.

Seroprevalence and Monitoring of EHV-1 antibodies in thorough bred horses:

A systematic programme was launched to study the status of EHV-1 related infertility and abortion in thoroughbred mares in 11 states. For comparative studies, sera from apparently healthy horses and donkeys were also included. Seroprevalence and monitoring was conducted between 1989 and 1997, employing micro-virus neutralization (VN) and complement fixation (CF) tests on 2573 serum samples (Singh *et al.* 1999).CF test was performed using 4 units of tissue culture infected antigens and complement respectively. CF antibody titre was expressed as the reciprocal of the highest dilution of the serum inducing hemolysis to 50% or less. Of the 2573 serum samples, 828 originated from aborted mares and remainder from apparently healthy horses (1695) and donkeys (50). Distribution of positive seroreactors was 18.2% (15/ 828) in aborted mares, 11.5% (197/ 16950 in apparently healthy horses and 2% (1/50) in donkeys. Seropositive horses were found in all the 11 states. However, the rates were higher in 3 southern states (15.1%; 123 / 813), followed by 4 northern states (13.5%; 206 / 1530) and two states tested in western region (6.5%; 9 / 138). Seroprevalence in eastern and central regions was 17.6% (9/15) and 4.8% (2/41), respectively (Singh et al., 1998).

Indirect enzyme- linked immunosorbent asay (ELISA) was standardized. ELISA results were compared with VN and CF tests in micro-titre system. Of 255 sera, 178 (69.8%) samples read positive in all 3 tests. However, 77 (30.19%) gave variable results (Singh et al.., 1994, 1995).

Seroprevalence and Monitoring of EHV-1 antibodies in indigenous equids:

In another national survey of EHV-1 infection amongst indigenous equidae in India, a total of 6134 serum samples were tested from 2002 to March 2008. Of these, 4.45% (273/6134) were found seropositive. Seropositivity against EHV-1 infection in equines varied from year to year (2% to 7.2%). Distribution of positive seroreactors equines were 7.2% (12/166) from Karnataka while all equines tested from Andhra Pradesh andTamil Nadu were negative. Accordingly seropositivity of equines against EHV-1 infection from western states varied from 0.9% to 3.6%. It was interesting that seropositivty against EHV-1 amongst equidae from Jammu & Kashmir and Uttaranchal were as high as 9.4% and 8.4% res pectively. Similarly serposivity against EHV-1 amongst horses were also reported from different states of Cental and Eastern region. All samples of horses tested from Bihar and Meghalaya were negative for EHV-1 antibodies.

Abortion due to EHV-1 infection in mares:

A total of seven EHV-1 abortion out break in mares were recorded by this centre from the period 1990 to 2008. All seven out breaks were reported in thoroughbred mares.The first abortion outbreak due to EHV-1 infection in pregnant mares was recorded in1990 in pregnant mares in Haryana. In this outbreak 53 mares aborted within 15 days of EHV-1 outbreak. The second and third out break of EHV-1 abortions were again recorded from Haryana in 1996. Fourth and fifth out break of EHV-1 abortion were recorded from Rajasthan and Delhi in1998. Sixth and seventh EHV-1 abortion outbreaks were recorded again from Delhi.

Comparative pathology and tissue tropism of indigenous strains (H-90 and R-98)

The comparative pathology and tissue tropism of indigenous strains (H-90 and R-98) was studied in mice model. For studying the respiratory affection and abortigenic potential 8 - 10 weeks old pregnant BALB/c mice at 13-14 days of gestation were inoculated intranasally with Hisar-90 ($10^{7.4}$ $TCID_{50}$/ mice), Rajasthan-98 strain ($10^{7.0}$ $TCID_{50}$/ mice). Both the strains led to the affections of respiratory system characterized by dyspnoea, ruffled fur and crouching in corners. Microscopically, lesions in lungs were characterized by presence of intranuclear inclusion bodies, infiltration of neutrophils and lymphocytes, ballooning, rounding and hyperpalsia of epithelial cells of bronchii in early stages followed by necrosis of parenchymal tissue. Lesions in the placenta varied in two strains markedly. Rajasthan-98 strain led to abortions and vaginal discharge and produced severe congestion of sinusoids, chorionic plate necrosis and necrosis in trophoblastic tissue (Virmani *et al.*, 2008). For assessing the neurogenic affinity of the two strains (H-90 and R-98) of EHV-1, suckling mice at 2-3 days of age were inoculated intracerebrally with Hisar-90, Rajasthan-98 strain. Hisar-90 strain failed to produce any lesions where as Rajasthan-98 strain produced severe clinical signs and lesions *viz.* diffuse proliferation of glial cells, degeneration of neurons along with areas of liquefactive necrosis, hyperplasia of endothelium and presence of viral antigen as indicated by IIFT (Virmani *et al.*, 2005).

Polymerase chain reaction (PCR) for EHV-1 diagnosis

Aborted fetal samples (50), were diagnosed for the presence of EHV-1 by PCR(Gupta *et. al.*, 1996)

Development of monoclonal antibodies against EHV-1

Thirteen hybrid cell lines of mouse myeloma cell lines SP_2/ O and spleen cells of BALB/c mice producing monoclonal antibodies (Mabs) against abortigenic Hisar-90-7 EHV-1 strain were developed. Purified Mabs were isotyped and characterized by ELISA, fluorescent antibody test (FAT), CFT and VNT. The isotype results showed Ig M (2) and Ig G (11). Different Ig G sub types were Ig G1, 1; Ig G 2a, 4; Ig G 2b, 5 and Ig G 3,1. Amongst the panel of Mabs developed, neutralizing, non-neutralizing and CF Mabs were 3, 7 and 1 in number respectively (Singh *et al* 2001). Amongst all the Mabs tested in Western blotting, 10 Mabs reacted specifically to 140 kD EHV-1 polypeptide target antigen. Three Mabs did not react in Western blotting.

Antigenic differentiation of EHV-1 isolates using Mabs

From the panel of 13 Mabs produced., 9 hybridoma cell lines secreting Mabs were used for antigenic

differentiation of EHV-1 isolates. ELISA and VN tests detected antigenic (epitope) differences in three EHV-1 isolates (Tohana-96-2,Raj-98 and Delhi-98),out of six isolates (five Indian;1 reference strain) tested with these Mabs. Reference strain and Jind–96 (Indian) gave positive results with all the Mabs similar to immunizing strain (Hisar-90-7). Two viral isolates (Tohana-96-2 and Raj-98) were not neutralized with N-Mab 1H6 in VNT. Epitopes 5, 4 and 1nos., on Tohana-96-2, Delhi-98 and Raj-98 respectively were not identified using non- neutralizing monoclonal antibodies (non N –Mabs) by ELISA.

Escape of neutralization of virus isolates by neutralizing Mab (N-Mab) 1H6 and negative results in ELISA with several non Mabs indicated the emergence of antigenically different EHV-1 strains compared to that of reference strains and immunizing Indian strain (Hisar-90-7) (Singh et.al., 2002)

Differentiation of Indian isolates of equine herpes virus (EHV-1) by DNA fingerprinting.

Genome of 5 local isolates of EHV-1 (Hisar-90-7,Jind-96,Tohana-96-2, Delhi-98 and Raj-98) and 1 reference strain (592) were digested with different restriction endonuclease (RE) enzymes to study the genetic variation among different isolates. Virus grown in cell culture were purified by sucrose density centrifugation and DNA was extracted from partially purified virus isolates by phenol-chloroform extraction followed by ethanol precipitation. DNA of each virus was digested separately with EcoR1, BglII, BamH1, HindIII, KpnI and XbaI at 37°C overnight. On electrophoresis of digested DNA in 0.8% agarose gel, Raj-98 could be differentiated from other viral isolates using BamHI and KpnI. While 1 extra band was detected in Raj-98 at position 14 on digestion with BamHI, a band at position 11 was missing on digestion with KpnI in comparison to other isolates. Reference strain (592) differed from other viruses in RE profile of HindIII (one band lacking) and XbaI (2 band missing). No variation in any of the viral isolates was observed on RE digestion with EcoRI and BglII. Jind-96, Tohana-96-2 and Delhi-98 isolates were indistinguishable from each other on digestion with any of the six restriction enzymes in this study. This study indicates that more than one genetically variant isolates of EHV-1 are circulating in equines of Northern India (Singh et al., 2002 a)

Development of a Neutralizing Monoclonal Antibody-based Blocking ELISA for Detection of EHV-1 Antibodies

A single dilution, sensitive and specific monoclonal antibody-based blocking enzyme-linked immunosorbent assay (B-ELISA) was developed as an alternative to the cumbersome virus neutralization test (VNT) for detection of equine herpes virus-1 (EHV-1) antibodies. Neutralizing monoclonal antibodies (1H6 and 9C6) raised against EHV-1 (Hisar-90-7 strain) and sera from 70 horses (30 known negative and 40 known positive for EHV-1 antibodies by virus neutralization test) were used for standardization of B-ELISA. Using a single serum dilution of 1:250 in B-ELISA, 100% specificity was obtained with both monoclonal antibodies (Mabs) in comparison to virus neutralization test (VNT). Similarly, the sensitivity of the B-ELISA was 92.5% and 100% with 1H6 and 9C6 Mabs, respectively. Of 231 field sera, 144 samples were negative for EHV-1 antibodies by both VNT and B-ELISA and 81 positive by both tests. Two samples negative by VNT were found positive in B-ELISA. On the other hand 4 weakly positive samples in VNT (VN antibody titre 0.9-1.2 $\log_{10}$) were negative in B-ELISA. The Mab (9C6) based B-ELISA was found suitable alternative to VNT for screening large number of field sera and enabled confirmatory EHV-1 serodiagnosis (Singh et.al., 2004).

Development of Equiherpes B-ELISA Kit

The refined kit was named as Equiherpes B-ELISA Kit. For validation of kit a total 2108 horse serum samples (884 samples, freeze dried form and 1224 samples, Equiherpes B-ELISA Kit) were tested. Serum samples tested using Equiherpes B-ELISA Kit was from 17 States and three different statuses of horses. These serum samples were also tested by conventional micro-VNT. The agreement between results of two tests on 1224 serum samples was 85.86%.

Assessment of Protective Immune Response of Inactivated EHV-1 Vaccine

To assess immunogenic potential of inactivated equine herpes virus-1 (EHV-1) vaccine in mice model, female BALB/c (group1) were vaccinated intraperitoneally twice, 21 days apart while mice of group 2 were sham-inoculated and served as control. After immunization,

antibodies against EHV-1 were detected on day 14, 21 and 28 day by virus neutralization test and enzyme-linked immunosorbent assay; but level of cell mediated immunity response was unsatisfactory. After mating and confirming pregnancy, these mice of group 1 and 2 were challenged by intranasal instillation of $10^{7.0}$ $TCID_{50}$/25 µl of EHV-1 virus (Raj-98 strain) at day 14 of gestation. Non-vaccinated and non-challenged mice (group 3) served as control. Following challenge, clinical signs, *viz.* dyspnoea, crouching in corners, vaginal discharge, abortion, and mortality increased significantly ($P<0.05$) up to 7 days post-challenge in non-vaccinated (group 2) mice compared to vaccinated mice (group 1), where dyspnoea, crouching in corners and mortality were not observed. After challenge, vaccinated mice showed less number (3/10) of abortions as compared to 4 out of 6 in non-vaccinated mice. Reduced virus titre recovery and score for herpesvirus-specific pathology in maternal lungs were observed in vaccinated dams. Indirect immunoperoxidase staining of lung tissues of immunized dam demonstrated less viral antigen than in unimmunized dams. These results confirm that inactivated EHV-1 vaccine afforded good humoral immune response and partial protection in pregnant BALB/c mice (Singh *et.al.*, 2004)

Development of vaccine against Equine Herpes virus-1 infection:

Presently, Indian equine breeders are using a killed vaccine produced commercially by incorporating army 183 strain of EHV-1. This centre has developed EHV-1 killed vaccine incorporating immunogenic Indian strain of equine herpes virus-1 and to study its immunological response in mares. On primary immunization of horses, vaccine generated good responses as measured by complement fixing (CF) and virus neutralizing (VN) and responses were comparable with the commercial vaccines. Potency testing of the candidate vaccine was done in immunized pregnant mares after challenging with Raj-98 strain and immunized mares withstood the challenge. Vaccine was found very effective in field trials on organized farms also and results were comparable with commercially available vaccines.

Equine herpesvirus-3 (EHV-3):

There is only one report of Venereal infection due to EHV-3 infection in mares from Karnataka. EHV-3 was isolated from clinical cases and confirmed.

Equine herpes virus-4:

Equine herpes virus-4 has been isolated from respiratory cases of the infected foals. Kit for differentiation of EHV-1 & 4 is being developed by this centre. High seropositivity (up to 82%) due to EHV-4 infections is being observed in equines in our study at this centre.

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ANIMAL DISEASE DIAGNOSTICS – RECENT TRENDS

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The importance of animal health in developing countries, controlling and eradicating where animal disease will have direct and major impacts on food security and poverty alleviation. The effective control of major animal diseases will have a positive impact in many areas of concern to society. Due to intensification trends in animal production systems in the developing countries there will be more animal health problems and diseases of intensification.

Animal health can be improved with new technology methods of diagnosis, prevention and control of animal diseases.

The disease control program are essential if eradication of animal and poultry disease and the prevention of the introduction or outbreak of foreign or domestic disease is to be successful.

Valid tests should be developed to properly detect disease that poses a risk to human health. Some animal health disease that require specific attention are :

Avian Influenza
Blue tongue
Brucellosis
Goat Pox
Johne's Disease
PPR
Tuberculosis

There should be an in-vitro testing procedure that is rapid, accurate and cost efficient. The recent developments of biotechnology are significantly contributing to the development of novel powerful diagnostic assays, such as various real time PCR and isothermal amplification techniques, micro arrays, protein detection by nucleic acid amplification, recombinant proteins, synthetic proteins, biosensors and many other approaches to detect the pathogens and the immune response after infection.

The recent development helps in the implementation of DIVA strategy (differentiates infected from vaccinated animals). The diagnostic kits should be made to work in the field to make decision about the exposure of animals during a disease outbreak.

The experience of the author towards the management of certain viral diseases of small ruminants are described below.

PESTE DES PETITS RUMINANTS (PPR)

Peste des Petits is a severe, fast spreading, highly contagious and infectious viral disease of sheep and goats. It is characterized by sudden onset of depression, fever, discharges from eyes and nose, sores in mouth, disturbed breathing, cough, foul smelling diarrhoea and death. It was first described in West Africa during 1942. In India it was first reported in 1989 *(Shaila et al,* 1989). In a study conducted by Central Institute for Research on Goats, Mukdhoom, more than 591 epidemics of PPR in goats have been reported from 15 states in India from 1991 to 1998. Fatality rates of 3.1 to 51.5% were recorded in PPR outbreaks.

PPR is caused by the morbilli virus group of paramyxo virus family. It is closely related to the rinder pest virus of cattle and buffaloes, the measles virus of humans, the distember virus of dogs and some wild camivores and the morbilli virus of aquatic mammals.

The transmission and spread of virus is mainly through the discharge from eyes, nose and mouth as well as the loose feces. Fine infective droplets are released into the air from secretions and excretions particularly when affected animals cough and sneeze. Close contact is the most important way of transmitting the disease; it is suspected that infectious materials can also contaminate water and feed troughs and bedding turning them into additional source of infection. Clinical signs appear an on average of two to six days *(incubation period)* after natural infection with virus. There is a sudden onset of fever with rectal temperature of 40°C to 41°C. Affected animals are markedly depressed and appear sleepy. Their hair stands erect giving them a bloated appearance, especially the short haired breeds. Soon

after this stage a clear watery discharge starts to issue from the eyes, nose, and mouth later becoming thick and yellow as a result of secondary bacterial infection. One to two days after fever has set in, the mucus membranes of the mouth and eyes become very reddened. Then epithelial necrosis causes small pinpoint grayish areas to appear on the gums, dental pad, palate, lips, inner aspects of the cheeks and upper surface of the tongue. These areas increase in number and size and join together.

The lining of the mouth becomes pale and coated with dying cells and in some cases; the normal membrane may be completely obscured by a thick cheesy material. Gentle rubbing across the gum and palate with a finger may yield a foul smelling material containing shreds of epithelial tissue. Similar changes may also be seen in the mucous membranes of the nose, the vulva and the vagina. The lips tend to swell and crack and become covered with scabs. Diarrhoea appears about 2-3 days after the onset of fever although in early or mild cases it may not be obvious. The feces are initially soft and watery foul smelling and may contain blood streaks and pieces of dead gut tissues.

Affected animals show difficult and noisy breathing marked by extension of the head and neck, dilation of the nostrils, protrusion of the tongue and soft painful coughs. A common feature of later stages of the disease is the formation of small nodular lesions in the skin on the outside of the lips around the muzzle.

PPR diagnosis can be made from epidemiological survey and clinical features of the particular animals. In post mortem examination, the mouth reveals that erosions on gums, soft and hard palates tongue and cheeks. Creamy yellow exudates and congestion is seen on nasal cavity. Lymph nodes of lungs and intestines are soften and swollen. Haemorrhages along with fold of lining, joining together as green or black in stale carcasses are seen in large intestine.

PPR can be differentiated from other related disease such as foot and mouth disease, rinder pest, blue tongue, contagious caprine pleuropnemonia, contagious ecthyma, coccidiosis and pasteurellosis etc. Laboratory examination such as counter immuno electro phorosis (CIEP), enzyme linked immuno sorbent assay (ELISA), agar gel immuno diffusion (AGID) are used to detect PPR virus by using samples such as tears swab, gum debris, lymph nodes from lungs (mediastinal) and intestine (mesenteric), portions of spleen and lungs, blood serum for anti body detection and unclotted blood for virus isolation. Treatment and Control of PPR outbreaks is mainly on quarantine of the affected animals with ring vaccination and prophylactic immunization in high risk area population. Antibiotics and chemotherapy are largely used to control the secondary bacterial and protozoal infections. Large doses of fluid and electrolytes will be found beneficial to counteract excessive dehydration.

GOAT POX

Goat pox, which is listed in list A diseases of the OIE (World animal health, 1997) is a highly contagious viral disease of goats characterized by fever, ocular and nasal discharges. It was first reported by Hasen from Norway in !879 (Rafyi and Ramyar, 1959). Goat pox is currently prevalent throughout South East Asia, Indian sub continent and North and Central Africa. In India , an out break of goat pox was first reported in the year 1936 (Imperial Institute of Veterinary Research, 1936-37) The first authentic report of the disease among the goats in India was made by Lall *et al.,*(1947). Since then the disease has been reported from many (States of India like Haryana, U.P., Orissa and West Bengal and recently in Madhya Pradesh (Joshi *et al., al.,* 1999) parts of the world.

The causative organism is goat poxvirus (GPV), which are enveloped double stranded DNA viruses, classified in the genus Capripoxvirus of the family Poxviridae (Murphy et al., 1995). Goat pox affects goats of all ages, sex and breeds but the disease is more common and severe in younger animals, lactating females and older animals.

The present report describes an outbreak of goat pox in an organized farm. The clinical, bacteriological, virological and pathological changes observed in the affected animals and carcasses are presented.

Source of animals

A total number of 120 goats were kept for breeding purposes in an organized farm of Mechery, Salem district. The animals belong to Tellicherry and Salem black breeds and were in the age group of 2 months to 5 ½ of years belonging to both sexes. Incidentally, the farm also maintained - number of sheep belonging to various breeds. The animals were attended by separate

personals and stall fed with concentrates, *libitum* fodder and good quality water.

Outbreak : Morbidity among kids and adults started during the month of may. A total of forty animals were exhibiting the symptoms and at the time of investigation two animals were reported to be terminally ill.

Sample collection : Scabs, blood smears, blood samples, sera samples, ocular swabs and faecal samples were collected. Spleen, mesenteric lymph nodes, nodules from the lungs, liver, kidney, intestine were collected for bacteriological and virological examination in respective transport medium and for histopathological examination in 10% neutral buffered formalin. The samples were processed for bacteriology culturing of blood agar, McConkey agar and manitol salt agar. The specimen was screened for pox antigen by Agar gel immunodiffusion (AGID) test and Counter Immuno-electrophoresis (CIE), with TRIS buffer. Lung tissue specimens were also screened for PCR for demonstration of capripox DNA. The specimens were also screened for PPRV, ORF and bluetongue to rule our these infections.

Results and discussion :

Outbreak: A total of forty animals showed clinical manifestation of pox in varying degrees (morbidity rate 41% and mortality ratewas 14 %) the present out break occurred during the month of may 2005 which is summer in Tamil Nadu. Affected animals showed a rise in body temperature between 105-106 °F. The animals showed hard papules giving rise to nodules ("Stone pox") concentrated particularly head, neck, ears, axis, groin, perineum external mucous membrane eyes/ prepuce, vulva, udder, anus and nostrils. The animals also showed rhinitis and conjuctivitis, which became mucopurulant. The papules on the face changed to scabs over a period of 5-10 days. The disease was more severe in kids than adults, which showed loss of appetite, reluctance to move, acute respiratory distress depression and emaciation

Out of the twenty five animals that died due to goat pox, twenty two belonged to Tellicherry breed, two belonged to cross breed of Tellicherry and Salem black and one to Salem black breed. The case fatality ratio was 60%. The nodules on the body, ear, involved all the layer of the skin and the subcutaneous tissue. The lesions on the lung were severe, extensive, focal and externally and uniformly distributed through out the lungs.

Haematology:

The blood samples collected from the affected animals contained haemoglobin 4.8 to 8.6 gm%, packed ell volume 14% to 25%, total erythrocyte count 2.4 to 6.5 x 10^6 / cu.m.m. and total leukocyte count 4.5 to 8.1 x 10^3 / cu.m.m.

Gross pathology:

The lesions of goat pox in this outbreak were not restricted to the skin alone, but were found extensively spread to the internal organs, especially the respiratory tract and the gastrointestinal tract. The generalization of the disease was more commonly seen in the kids than adults. The skin lesions involved the full depth of the epidermis and dermis and they coalesced in severe cases. Postmortem lesions included tracheal congestion, pox lesions in the lungs, enlarged spleen and lymph nodes, increased quantity of blood tinged pleural fluid and hydropericardium. In the lungs the extensive pox lesions were white to purple in colour, which ranged from 0.5 – 1.5 cm in diameter and was seen focal and uniformly distributed throughout the lungs

Laboratory diagnosis:

The laboratory diagnosis was carried out by AGID test with polyclonal antisera raised against capripox virus. On overnight incubation, a thick precipitate line appeared with the capripox viral antibody and suspended tissue (extract) ground material added well. Whereas, no line appeared with the BTV antibodies. The test were repeated by CIE where in a sharp precipitation line appeared between the suspected antigen and known antibody wells. The CIE test was performed with the TRIS buffer. The ocular swabs and lymphocytic antigen were negative for PPRV by HA test.

Virus isolation was attempted in BHK 21 cell line. By the third passage, CPE was observed in 60 hours post infection, which included vacuolation of nucleus, retraction of cells, nuclear fragmentation and presence of intracytoplasmic acidophilic inclusion bodies,

surrounded by halo. This was followed by loss of cell sheet on 5th day post infection.

The lung tissue samples were processed for polymerase chain reaction (PCR) as per the method used by Ireland and Binepal (1998) using the forward primer 5' TTT CCT GAT TTT TCT TAC TAT 3' (21mer) and reverse primer 3' AAA TTA TAT ACG TAA ATA AC 5' (20mer). The amplification size of the PCR product was 192 bp which corresponded with the positive standard for GPV .

Molecular characterization

For the gene encoding —— a product of — six could be obtained.

Histopathology

Microscopically the affected skin revealed congestion, hydropic degeneration, oedema, hyperplasia, vasculitis and coagulation necrosis in the epidermis and dermis, with mononuclear cell infiltration in the areas of lesions and chromatin margination of the nuclei of infected cell. Intracytoplasmic oeosinophilic inclusion bodies were present in the infected cells of the dermis. The lung tissue was characterised by congestion, haemorrhage, oedema, focal areas of proliferation with necrosis and lobular atelectasis. Further more, the necrotic foci in the lungs revealed oeosinophilic inclusion bodies, which were seen in between the cell debris and exudates. Depletion of lymphocyte population in paracortical regions and absence of germinal centres in spleen were also observed.

Clinical Management

All infected goats were quarantined and placed in a well-ventilated enclosure and fed a balanced diet. To relieve respiratory signs, the nostrils were cleaned and washed with a weak solution of Potassium permanganate (1:10,000). Antibiotic ointment was applied topically to the skin lesions.

Prevention and Control

Attempt to protect goats with sheep pox virus vaccine are usually unsuccessful. Hence, it is recommended that homologous single vaccine prepared from a strain of capri pox virus can be effectively used in controlling the goat pox (Kitching *et al., al.,* 1987).

Since the virus remains viable in the premises for as long as 6 months disinfection by formalin (1%) or phenol (2%) was recommended.

BLUE TONGUE

Blue Tongue Virus (BTV) belongs to the Orbi virus group of the family Reoviridae. It is an arthropod transmitted viral infection of domestic and wild ruminants. Sheep is the most susceptible for the disease which is characterized by high fever, congestion, edema and hemorrhage. Among ruminants sheep develop very severe disease while milk form of the disease has also been reported in cattle.

Economic losses are mainly due to poor quality of wool and meat and also due to the death of animal. The presence of BTV is in livestock is a barrier in trade and free international movement of livestock and germplasm. This emphasis the importance of the disease in a country where BTV infection is endemic like many countries, India is also endemic zone for BTV infection.

The epizootiology of the virus depends on a complex interaction of host, vector, climate and virus itself. The vector involved in transmission of the disease in culicoides sp.

The field diagnosis of BT is usually made by clinical signs, characterized by pyrexia, selling of muzzle, oral lesions, coronitis, stiffness of limbs and in sum case odema of head and neck. The field diagnosis of BT is difficult because of similarity in symptoms and signs with other diseases like sheep pox, foot and mouth, contagious ecthyma, ulcerative dermatitis, photosensitization and pneumonia.

Sero surveillance

Serological surveys of sheep for the detection of BTV antibody of by agar gel immuno diffusion has been well documented.

Serological test used for the detection of group specific antibody was agar gel precipitation test (AGPT) for this, antigen from infected chicken embryos and cell cultures were being used. Recently non-infectious soluble antigen derived from infected cell culture where used in AGPT. The AGPT detects the IgG against the bluetongue group specific antigen polypeptide p7.

The AGPT has several advantages over the other tests in that it is simple, sensitive and economical.

Sera sample were collected from local breeds of sheep

suspected to have been affected with BTV. During the period under report a total of 225 sera samples were screened by AGID. Out of which a total of 62 samples were positive indicating a positive percentage of 36.2%. The positive sera samples wee also screened by counter immuno electrophoresis and Rocket Immuno Electrophoresis. Among these 3 test Rocket Immuno Electrophoresis test is found to be more sensitive than counter immuno electrophoresis.

Out of 62 sero positive samples 6 gene positive precipitation line with the referral BTV antiserum itself indicating the possible presence of BTV antigen in the sera samples.

The coexistence of virus and neutralizing antibodies in the blood is a common phenomena of BTV infection. This seem to indicate a firm adherence of virus on the erythrocytes membrane on localization with in or outside of the membrane to protect itself from the neutralizing effect of the antibodies.

Competitive ELISA

Competitive ELISA in which specific antibodies may be present in the test serum competes with a BTV specific monoclonal antibody of the test kit for the attachment to BT viral antigen which is coated on the surface of the plate wells. The test serum at monoclonal antibody are added simultaneously, the plates are held for two hours at room temperature and any unreacted antibodies or removed by the surface of the wells. Anti mouse immunoglobulin labeled with enzyme horseradish peroxidase is added to the wells to combined with if present more immunoglobulin of the BTV specific monoclonal antibody. The presence of the monoclonal antibodies in the well is measured in the last phase of the assay by adding the enzyme substrate hydrogen peroxide in the conjunction with a chromogen, which quickly imparts colour to the solution if peroxidase conjugated antimouse, immunoglobulin is present and attached to the monoclonal antibody. The development of a colour reaction indicates the presence or atleast a low level of BTV specific serum antibody. The absence of colour or a weaker intensity of colour indicates that BTV specific antigen is present in the test serum and has inhibited to the attachment of the monoclonal antibody. The colour reaction in each well is measured photo metrically and the optical density of the solution determined. The degree to which a test serum inhibits the attachment of the monoclonal antibody is used to determined whether or not the serum is positive or negative for antibody to bluetongue virus.

The C-ELISA was found to be more sensitive in the surveillance of BTV antibody.

Isolation of BT Virus

The isolation and identification of the virus in the blood of BT affected animals confirmed the presence of BTV. Isolation of BTV from the blood cell can be accomplished by inoculation of clinical material in embryonated chicken eggs and cell culture.

ECE propagation

Successful propagation of BTV in fertile hen's eggs was well reported. The infection of 8 day old embryos by the yolk sac route produced consistently higher yields when inoculated at 33.5°C.

The intravenous inoculation of embryonating chicken eggs for the isolation and identification of BTV was found to be an improvement over the yolk sac route of isolation. Nine to eleven day old embryos were inoculated with BTV intravascularly. Embryo usually died on the 2nd or 3rd day after inoculation; the embryo showed a characteristic hemorrhagic and edematous lesions.

Cell culture system

Inspite of high sensitivity of ECE, invitro cell culture system have also been found to be effective for isolation. Among the cell culture system, BHK_{21} has been commonly used for the adaptation of the BT virus.

The CPE has been observed in BHK_{21} cell line from 364 post infection (PI). The infected cell become swollen and ill defined, later these changes spread to entire sheet. The affected cells have shown granularity and under gone variable degrees of shrinkage. Later most of the cells got detached from the glass. Rounding of cells syncytia and grant cells formation and aggregation in to group of cells have also been observed.

The cell lines from insects Source offer a way of growing BTV to comparatively high titre and have the potential has isolation system. Attempts have been made in this study to use Aedes albopictures cell line from NFATCC, to propogate BTV isolates.

Vector for BT virus

Culicoides constitutes the major vector of importance in BTV transmission. There are more than 100 species of culicoides. During the period under report with the use of an improvised 'light trap' insects were collected and culicoides were identified at generic level.

Conclusion

The results prove beyond doubt that BT disease is assuming enromass significance in the animal health scene of the country. Hence, on a long term basis study should be initiated covering all aspects of bluetongue virus infection including epedimology, diagnostic, vectors, pathogenesis and occurrence of disease in other species using sentinel study. The immediate need is to produce BTV diagnostic reagent that could cater to the needs of the field staff. To begin with AGID kits can be produced for sero surveillance on BTV. In this study cELISA kit imported from Veterinary Diagnostic Laboratory, USA was found to be very useful for identification of BTV antibody.

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MOLECULAR EPIDEMIOLOGICAL INVESTIGATION OF A FLEA-BORNE RICKETTSIAL OUTBREAK IN THE WESTERN HIMALAYAN REGION BASED ON GLTA AND OMPB GENES

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The order Rickettsiales is represented by diverse groups of Gram negative, cocco-bacilli, fastidious intracellular bacteria, adapted to exist within the arthropod hosts but are frequently capable of infecting vertebrates including human. We investigated a human outbreak in a village situated in the sub-Himalayan region of Himachal Pradesh. Samples of ticks, fleas and dust mites were collected from households with confirmed cases of human rickettsiosis with Weil-Felix test. Three blood samples from convalescent human patients were also collected. Epidemiological data from 43 individuals showed 65.12% incidences of disease among females and 41.86% among children those stayed at home for longer time and having earthen (kuccha) houses with flea and rats/mice infestations. Haemolymph test on ticks showed no suspected Rickettsia spp. All collected samples were analysed by gltA and ompB based PCR tests. The expected size PCR products were obtained only from rat flea (Ceratophyllus fasciatus) samples and these amplified DNA fragments were directly sequenced. The BLAST search and phylogenetic analysis (using PHYLIP) of obtained nucleotide sequences of gltA gene showed that the fleas were harbouring a Rickettsia sp. similar to Rickettsia spp. SE313, RF2125 and cf1and5 strains reported from fleas of Egypt, Myanmar, Thailand and USA. However, ompB genes sequences analysis of detected strain showed it to be closely related to Rickettsia akari and Rickettsia australis belonging to spotted fever group. These results highlight the public health importance of such newly discovered Rickettsia spp/strains. Association of such flea-borne Rickettsia species in the investigated outbreak was evident from the epidemiological data also.

COMPARATIVE EVALUATION OF THREE ANTIGENS OF BURKHOLDERIA MALLEI FOR DIAGNOSIS OF GLANDERS IN EQUINES

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Glanders, a highly contagious, fatal, re-emerging zoonotic disease of solipeds, is caused by *Burkholderia mallei*. The organism is considered as potential bio-weapon. Since, available sero-diagnostics either lack sensitivity and specificity or are complex, labour-intensive and time-consuming like the golden standard complement fixation test (CFT). Problem of anti-complementary activity in donkey and mule serum makes CFT difficult to be used in field. Thus, the present investigation was undertaken to evaluate three antigens, commercial CFT antigen, antigens A* and B* (purified proteins of about 30 and 10 kD, respectively) for their diagnostic efficacy. Of the 1500 equine serum samples tested by conventional CFT, 1394 were negative while 106 (positive for glanders) showed variable titre of antibodies. Antigens A and B were not found suitable for CFT. In indirect ELISA, 532 negative and 106 positive samples tested using CFT antigen could differentiate the two and difference in the ODs was significant. Using antigen A, positive (41/63) and negative (371/500) samples could be distinguished, however, the difference in the ODs of positive and negative samples was not significant. Antigen B exhibited a significant difference in the ODs of positives and negatives. Antigen B may be used in indirect ELISA as screening tool with high sensitivity and specificity.

SHORT TERM CRYOPRESERVATION OF VERO CELL LINE

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This study describes the observation for the preparation of short term cryopreserved cell lines. These procedures ensured the availability of reproducible vero cell lines for use at different times in the laboratory. DMEM nutrient medium with 10% new born calf serum and 10% DMSO was used for vero cell cryopreservation. Vero cells regularly cultivated in DMEM with 10% NBCS, was cryopreserved in the standard freezing medium (DMEM containing 10% NBCS and 10% DMSO). The two different temperature (-20°C and -70°C) were used for cryopreservation. The cells were washed once with fresh growth medium before cryopreservation and resuspended in cold (4°C) freezing medium at the density $1x10^6$ cells/ml. The cell suspension was frozen through slow cooling method (1°C/min) in 1°C cooler containing isopropanol. Immediately 1°C cryocooler was transferred to -20°C and at ultra low freezer (-70°C).

The cryopreserved cells were revived at weekly interval and observations were taken with respect to cell growth and viability. Vials were removed from cryocooler For the revival of the preserved cells and immersed immediately in 39°C water bath and agitate gently for a minute till thawing. The cell suspension was directly plated into the cell culture flask containing complete growth medium. Maintenance medium was replaced after 24 hrs of plating. No differences were observed in Vero cell lines with respect to their attachment, growth and viability for initial 2 weeks at both the temperature, however degenerative change such as increased cell death and abnormal cells were observed after 2 weeks in cell preserved at -20 °C, where as such changes were not seen even up to 10 weeks in the cells preserved at -70°C. Hence, this study outlines the essentials steps for the short term freezing and storage of vero cell line with respect to temperature and duration of cryopreservation.

ADAPTATION OF HYDROPERICARDIUM SYNDROME VIRUS IN CHICKEN EMBRYOS AND VERO CELL LINE

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Hydropericardium syndrome (HPS) is an emerging disease of poultry, primarily of broiler chickens. The disease is caused by fowl adeno virus (FAV) serotype-4 belonging to family *Adenoviridae*. In present study, the HPS virus was adapted in developing chicken embryos via allantoic cavity, chorioallantoic membrane (CAM) and yolk sac routes. The HPSV infected liver tissue suaspension (20%, W/V) inoculated via allantoic cavity route showed stunting of embryos and haemorrhages with mortality upto 20%. The inoculated chicken embryos, inoculated via CAM route showed haemorrhagic changes in embryos along with dwarfism and mortality upto 60%. The CAM showed oedema, congestion, thickening and distinct pocks. The petechial haemorrhages in liver and hydropericardium in embryos were common. The yolk sac route inoculated chicken embryos showed stunting and severe haemorrhagic changes with mortality upto 80%. CAM harvested at first passage, allantoic fluid obtained at 3rd passage from allantoic cavity route inoculated embryos and allantoic fluid harvested from yolk sac route inoculated embryos showed precipitin band in AGPT indicated lower adaptability of HPSV via allantoic cavity route compared to the yolk sac and CAM route. The VERO cell line inoculated with HPSV infected liver homogenate showed aggregation, rounding, shortening and swelling of cells at third passage level. At fourth and fifth passage level, aggregation or clumping, massive detachment and elongation of remaining cells were noticed. The changes observed in developing chicken embryos via different routes of inoculation and in Vero cell line indicated adaptability of HPSV in these biological systems.

MIXED INFECTION IN A DONKEY

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A five-month-old male donkey was presented to the out patient medical unit large animal clinic of Madras Veterinary College Teaching Hospital with history of anorexia, dullness and watery diarrhoea with foul smelling. *Strongyle* spp eggs were detected on faecal examination and the bacteriological culture of the sample revealed *Salmonella* spp and the diagnosis was confirmed as mixed infection. Antibiotic sensitivity test, indicated that Salmonella isolates were sensitive to Ceftriaxone and tetracycline. This type of rare mixed infection with strongylosis and salmonellosis was successfully treated with antibiotics, anthelmintic and fluids.

APPLICATION OF PCR FOR CONFIRMING BRUCELLA MELITENSIS INFECTION IN SHEEP FLOCK

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A multi pronged approach in confirmatory diagnosis of brucellosis in sheep flocks with history of reproductive disorder *viz.*, infertility, abortion, repeat breeding and retained placenta was adopted. Among RBPT, STAT and I-ELISA employed to screen 527 sheep, I-ELISA detected higher percentage positives. The clinical material *viz.*, blood, serum and vaginal secretions were collected from 33 seropositive and 6 seronegative sheep for PCR. However, only vaginal secretion was used for isolation. *Brucella melitensis* was isolated in 6 of 33 seropositive and none from seronegative sheep. All the isolates were confirmed *B. melitensis* by biochemical tests and genus and species specific PCR. The conserved nature of aforementioned gene sequence was confirmed by RE analysis. The dendrogram analysis indicated the possibility of circulation of more than one strain of *B. melitensis*. The clinical materials were subjected for *Brucella* DNA detection by PCR. Of the 33 sero-positive sheep, the desired amplicon of 223bp using *bcsp*31 primer pair was obtained in 26 vaginal, 2 blood and none in serum samples.
In conclusion, PCR was successfully employed for direct detection of *Brucella* DNA in vaginal secretion, the most suitable specimen for PCR. Further, long period required for conventional identification of *Brucella* species could be avoided by employing *B.melitensis* specific PCR. This practically oriented rapid approach is of immense importance as it is specific and enables to institute the control strategies at the earliest.

MOLECULAR DETECTION OF B.ABORTUS FROM NON-CLINICAL CASES OF APPARENTLY HEALTHY BUFFALOES

Vivekananda[2], Rajeswari Shome[1], S. Isloor[2], K.Prabhudas[1], V.V.S. Suryanarayana[3], D. Rathnamma[2] and Devaiani[1].

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The susceptibility of individual animals depends mainly on its genetic make up and the influence of its environment. It is evident with the detection of variation in susceptibility to few parasitic and infectious agents in some of the indigenous animals. The low incidence of infection reported in buffaloes was attributed to its genetic make up. Brucellosis is one of those diseases showed low incidence in buffaloes. Though indigenous buffaloes were comparatively resistance to *Brucella* infection, the possibility of occurrence of brucellosis in apparently healthy buffaloes could not be ignored. As a routine, a total of 97 Murrah buffaloes brought from Gujarat were screened by AB-ELISA, RBPT and STAT for anti *Brucella* antibody. These were not vaccinated against brucellosis. A six *Brucella abortus*

isolates were obtained from vaginal secretion from six seropositive buffaloes. All the isolates were further confirmed by PCR using *Brucella* genus specific primer pairs *viz.*, *bcsp* 31, *omp*2a and *omp*2b, and species specific primer pairs. The phylogenetic tree drawn from *omp*2a nucleotide sequence indicated the circulation of only one strain in a herd as all the isolates were placed in one cluster. Therefore, while implementing brucellosis control / eradication strategies, importance should also to be given to indigenous buffaloes.

VAGINAL SECRETION AS A BEST SUITABLE CLINICAL SAMPLE FOR IDENTIFICATION OF BRUCELLA INFECTION IN CATTLE BY PCR

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The detection of *Brucella* infection using PCR in blood, serum and vaginal secretion from four herds having history of abortion, infertility, retained placentae etc., were compared to in vitro isolation. Samples were obtained from 56 seropositive and 12 seronegative cattle detected by AB-ELISA. Among 56 seropositive animals, the PCR using *bcsp* 31 primer pairs identified 27 *Brucella* positive cattle in vaginal secretions, 11 in serum and 8 in blood. The *Brucella* organisms were not found in seronegative animals. Further, *Brucella* organisms were also isolated in vaginal secretion from 18 of 56 seropositive animals. All the animals that were positive by isolation were also found to be positive for *Brucella* DNA in vaginal secretion by PCR. The PCR method showed hundred per cent agreement with isolation which is considered as gold standard for identification of *Brucella* infected herds. In this study, our findings indicated the vaginal secretion as a best suitable clinical sample for identification of *Brucella* infection in cattle by PCR using *bcsp* 31 primer pair. Application of this approach avoids long period required for conventional identification of *Brucella* species.

EVALUATION OF HYPER-IMMUNE SERA OF INFECTIOUS BRONCHITIS FOR ELISA

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The enzyme-linked immunosorbent assay (ELISA) was elaborated for the diagnosis of Infectious Bronchitis disease of poultry by polyclonal rabbit sera. Rabbits were immunized by intramuscularly inoculation with 500ìg of the M41 vaccine IBV strain in 0.5 ml vaccine diluents with equal volume of Freund's complete adjuvant. After three weeks, subsequently three boosters of 200 µg vaccine were given intramuscularly at weekly interval with incomplete Freund's adjuvant (IFA). The serum was collected after seven days of final booster. Characterization of IBV antibodies raised in rabbit was done by Western Blotting assay. Known positive sample with negative control were electrophoresed on 10% SDS-PAGE and transferred to nitrocellulose membrane. The diluted polyclonal rabbit serum (1:200) was used as primary antibody and probed with goat anti-rabbit IgG-HRP as secondary antibody (1:1000 dilutions). Finally diaminobenzidine (DAB) was used to develop the color. This identified sera subsequently used for IB detection though ELISA. The serum was standardized for optimum concentration of antigen and amount of serum using checkerboard titration (CBTs) at 405 nm in an ELISA reader. ELISA titer was calculated as reciprocal of serum dilution showing double the OD405 than the negative sample. Therefore study shows that polyclonal sera can be used for efficient and rapid ELISA detection of IB.

PREVALENCE AND CHARACTERI-ZATION OF SHIGA TOXIGENIC ESCHERICHIA COLI FROM LAMBS

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Shiga toxigenic *Escherichia coli* (STEC) are commonly recovered from faeces of food animals and pose threat to human and livestock. The present study is based on prevalence and characterization of Shiga toxigenic *E. coli* on the basis of virulence genes from lambs with and without diarrhoea in Jammu, India. A total of 50 *E. coli* isolates belonging to 13 different serogroups were isolated from 40 diarrhoeic and 31 healthy lambs. All these 50 isolates were screened for the presence of stx_1, stx_2, *eae* and *hlyA* genes using multiplex polymerase chain reaction (m-PCR). A total of 15 (30 %) (9 diarrhoeic and 6 healthy) and *E. coli* strains were designated as Shiga toxgenic *E. coli*. Out of a total of 15 STEC isolated from both diarrhoeic and healthy lambs, 4 (26.7 %) carried stx_1 gene and 4 (26.7 %) harboured stx_2 gene. Seven (46.7 %) isolates were positive for *eae* gene and 11 (73.3 %) were *hlyA* positive. Antibiotic sensitivity tests revealed that 12 (80 %) STEC isolates were resistant to antimicrobials and rest 3 (20 %) were sensitive. All STEC are further characterized by the production of turbid zones of hemolysis on Soyabean casein digest agar supplemented with 5 % sheep erythrocytes and 10mM $CaCl_2$.

POLYMERASE CHAIN REACTION DETECTION AND SEQUENCING OF MYCOPLASMA SYNOVIAE ISOLATED FROM CHICKEN

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The DNA of the *Mycoplasma synoviae* (MS) isolated from chicken using Frey's Mycoplasma media was extracted using commercially available DNA extraction kits. The species specific Polymerase Chain Reaction (PCR) was standardized for the detection of MS from poultry. The DNA was subjected for PCR to amplify 16S rRNA gene of MS with published primer sequence as per Marois *et al.* (2000). The samples yielded amplicon size of 207 bp specific for MS. The PCR products of two local isolates identified as KVAFSU MS R1, KVAFSU MS R4, and MS reference strain (MS-427) and MS colored antigen were sequenced. Their 16S rRNA gene partial nucleotide sequences were analyzed using BLAST program. The local isolates showed a homology of 81 and 79 per cent with already published sequences. The comparison of the sequences amongst, two field isolates, MS reference strain (MS-427) and MS colored antigen showed similarities as well as a few nucleotide differences. The phylogenetic nucleotide sequence analysis revealed that, both the local field isolates, MS reference strain fell into same group but different from the group, the MS colored antigen belonged to.

DETECTION OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS FROM MILK SAMPLES OF DAIRY ANIMALS BY IS900 PCR

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Abstract: *Mycobacterium paratuberculosis* (Map), an acid-fast bacillus, causes Johne's disease (JD) in ruminants. The disease is characterized by chronic granulomatous enteritis, diarrhea and weakness. The Present study was aimed at detection of the organism from milk. Screening of dairy animals from Ludhiana district was done on basis of clinical signs (diarrhea and chronic weakness) and Ziehl Neelsen staining of fecal samples. Milk samples from thirteen suspected animals were collected and centrifuged. The fat and pellet of each sample was pooled and processed for DNA extraction and IS900 PCR, after 0.9% hexadecylpyridinium chloride (HPC) treatment. ZN staining revealed 12 animals as positive for acid fast bacilli whereas IS900 PCR detected 30.8% (4) animals positive for Map. Therefore, milk can be a source for transmission of Map bacilli. The results indicated that IS900 PCR can be used for screening of milk for Map; however the method needs to be evaluated for subclinical cases.

A STEP FORWARD FOR THE DIAGNOSIS OF CONTAGIOUS AGALACTIA

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Contagious agalactia is a reportable disease of sheep and goats belonging to the list 'B' of office of international epizootics (OIE) and has been reported from most of the regions and countries around the world. The disease originally included mastitis, arthritis and kerato conjunctivitis with the classical etiology of *M. agalactiae* which accounts for 90% outbreaks of contagious agalactia syndrome in goats and almost 100% in sheep. Thus *M. agalactiae* is an important organism which causes severe economic losses to sheep and goat breeders all over the world. India has a large population of sheep and goat therefore, it is always important to diagnose the presence of *M. agalactiae* in flock. As *M. agalactiae* and *M.bovis* both are immunogenically related to each other, it is necessary to identify the specific immunogenic protein which can be used for the development of a diagnostic test of contagious agalactia without cross reacting to *M.bovis*. Keeping this in view, the sonicated and whole cell proteins of two different isolates of *M. agalactiae* and one of *M.bovis* were analysed on the basis of SDS-PAGE. The separated proteins of all the three strains were assessed for their cross reactivity against the polyclonal antisera raised in rabbit against the *M. agalactiae* and *M.bovis* in western blotting. On the basis of these, the protein of 58.8 kDa of *M. agalactiae* was found specific and selective. It showed immunogenicity to *M. agalactiae* polyclonal hyperimmune sera without giving cross reactivity to *M.bovis* polyclonal hyperimmune sera. Thus it can be used for the development of selective diagnostic test for contagious agalactia.

STRAIN DIFFERENTIATION OF SALMONELLA ISOLATES OF POULTRY BY AP-PCR

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Salmonellosis has acquired a special importance for developing countries due to extended livestock farming. The importance of *Salmonella* organism as potential pathogens of man and domestic animals need no emphasis. The acquisition of more information of microbiology and epidemiology of *Salmonella* in humans and animals would investigate relevant measures to be taken for *Salmonella* control. Among *Salmonella* serovars, *S.* Typhimurium, *S.* Heidelberg, *S.* Bareilly and *S.* Virchow are more important to human and animal

health. The objectives of the present study were to standardize AP-PCR for molecular typing of *Salmonella*. Thirty strains of *Salmonella* belonging to four serovars viz. *S.* Typhimurium, *S.* Galiema, *S.* Virchow and *S.* Heidelberg were used in the present study. The finding of this study and earlier workers clearly indicates that AP-PCR is an efficient tool for molecular typing in *Salmonella*. It indicates a high level of discrimination ability of AP-PCR. This can be used for molecular and epidemiological studies of Salmonellosis outbreak and vaccination failures. In this study, four serovars were used and it was observed that with this technique sometimes common profiles were generated in between the isolates of different serovars which may sometimes produce ambiguity in the result, so this technique should be supplemented with serotyping and DNA based methods which can differentiate the *Salmonella* at serovar level.

DEVELOPMENT OF RT-PCR FOR SPECIFIC DIAGNOSIS OF JAPANESE ENCEPHALITIS IN EQUINES

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Japanese encephalitis virus (JEV), a member of the family *Flaviviridae* is an important mosquito-borne viral disease in Southeast Asia. Humans and horses may exhibit disease ranging from febrile illness to a meningo-encephalitis with a significant mortality when infected by the virus carried by a mosquito vector. We developed a reverse-transcriptase-polymerase chain reaction (RT-PCR) method to detect JE virus from experimentally infected equine tissue or serum samples. JEV and WNV were grown and titrated in porcine stable (PS) cell lines. RNA was extracted from serial ten-fold diluted cell culture lysate, virus-infected suckling mice brain tissue and virus-spiked horse sera. RT-PCR using primers targeted against 3' non-translated region (NTR) of flaviviruses could amplify 146 bp 3' NTR segment from both JE and WNV infected tissue and sera. These primers did not amplify any fragment from genome of equine rotavirus, equine herpes virus-1 and equine viral arteritis virus. Since both JE and WNV co-circulate in the same epidemiological setting in India, to specifically detect JE, another RT-PCR was designed that specifically amplified 291 bp fragments of envelope protein gene (E-gene) of JE virus. This RT-PCR assay had a viral detection limit of 0.7 $TCID_{50}$ from infected equine sera and was found to be 10 times more sensitive for JEV detection as compared to that of suckling mice brain inoculation (detection limit 1.7 $TCID_{50}$). Using these dual RT-PCR primers, JE and WNV infection can be specifically diagnosed from infected equine tissues or serum samples.

ISOLATION OF BUFFALO POX VIRUS AND ITS MOLECULAR AND EM CHARACTERIZATION

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Present communication describes investigations of buffalo pox outbreaks and isolation of virus from these outbreaks. During our study, Pustular pox lesions were observed on teats and mammary parenchyma of cattle and buffaloes apart from taking toll of several calves further, in the areas of outbreaks, infection was of significant zoonotic importance involving hands, legs and face of people in close contact with the affected animals. Scab materials in viral transport media (VTM), pustular fluid in VTM and blood were collected from affected animals and human beings. The materials collected were processed in the laboratory for virus isolation as per the procedure outlined in OIE manual and the for pox virus isolation. The processed, filter sterilized materials were inoculated to 9-11 day old chicken embryos by chorio allontoic membrane (CAM) route and Vero cell line . The characteristic pock lesions were noticed after seven blind passages on the CAM and the virus produced cytopathic effect (CPE) after five passages in cell culture system. The virus was confirmed by inclusion body staining which revealed characteristic eosinophilic, intracytoplasmic; cow dry Type-A inclusions. Further the virus was confirmed by a sensitive and rapid PCR using the primers that amplify "A type

inclusion" gene. The pock lesions on the CAM were subjected for electron microscopy for further confirmation.

GAP GENE BASED MOLECULAR DETECTION OF STAPHYLOCOCCUS SPP. IN SUBCLINICALLY INFECTED MILK OF MURRAH BUFFALOES

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Sub clinical mastitis has been proved to be one of the most economically important diseases affecting dairy animals worldwide. The public health importance of staphylococcal mastitis in high yielding world's best dairy type buffalo "*Murrah*" cannot be overlooked due to emergence of antibiotic resistant bacteria and change in sensitivity pattern. Presence of staphylococci in milk may lead to high degree of risk to consumer since they produce a battery of enzymes/toxins. Therefore, early detection of Staphylococcal strains is essential for initiation of proper therapeutic, control and preventive measures. The *gap* gene encoding glyceraldehydes-3-phosphate dehydrogenase is a part of glycolytic operon in Staphylococci and its amplification can be used for detection of Staphylococci associated with mastitis. In present study, under optimized conditions, primers GF-1 and GR-2 encoding *gap* revealed an amplified product of size approximately 933 bp when DNA from milk of 228 culturally positive samples collected from functional quarters of *Murrah* buffaloes were tested. None of the streptococcal and *E.coli* isolates were found positive with PCR when amplified with primers encoding *gap* gene, although all these samples amplified 210 bp product with Universal primers showing 100 % specificity of primers. The assay is an important tool for molecular epidemiology of *Staphylococcus* spp. which allows faster establishment of effective preventive measures at organized farms.

MOLECULAR DETECTION OF STREPTOCOCCUS DYSGALACTIAE BY 16S-23S R RNA GENE BASED POLYMERASE CHAIN REACTION ASSAY IN MURRAH MILK

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Mastitis continues to be the economically most important infectious disease of dairy animals throughout the world. In India, *Streptococcus dysgalactiae* have been reported as one of the important causative agents of mastitis in *Murrah* buffaloes and so its early detection is necessary for control and epidemiological studies of mastitis. The present study was carried out to develop polymerase chain reaction (PCR) assay using primers encoding target sequence of 16S-23S rRNA gene for detection of *Streptococcus dysgalactiae* from *Murrah* milk. The milk samples which were found positive by bacteriological examination were processed for standardization of Polymerase chain reaction (PCR). DNA from milk samples was extracted and PCR reactions were standardized for optimum concentrations of $MgCl_2$, primer and *Taq* DNA polymerase, and annealing temperature. Finally, PCR products were analyzed and amplified products of 281 bp approximately were yielded under standardized conditions. The specificity of assay was checked using DNA from milk samples positive for mastitis causing organisms other than *Streptococcus* dysgalactiae. This assay was completed within four hours and was found to be sensitive and specific for detection of *Streptococcus dysgalactiae*. The assay can be used as an innovative diagnostic tool for screening of large buffalo herd for early detection of streptococci directly from milk in both clinical and sub-clinical mastitis cases.

CURRENT APPROACHES TO UNDERSTAND ANTIGENIC VARIATION IN TRYPANOSOMES

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Trypanosomes evade host immune responses by constantly undergoing antigenic variation and they do so by changing the ex-pression of their variant surface glycoproteins (VSG), which forms the surface coat. VSG is expressed from specialised gene loci called bloodstream ex-pression sites. There are around 2000 silent VSG genes, which located in four subtelomeric locus types and could be potentially copied into the ex-pression site to create new variants. Most of these silent VSGs reside in subtelomeric arrays and are prone to ectopic recombination events, which provide one mechanism for hyperevolution. Array VSG genes also accumulate point mutations much faster than conventional genes, which raises the question whether arrays are replicated by a distinct, error-prone mechanism, at a different time than the cores of chromosomes. During chronic infection it has been observed that VSGs are expressed as complex gene mosaics and molecular and immunological approaches are being adopted to test whether these mosaics are functional at the protein level and how unique immune responses are. The role of gene flanks in initiating VSG switching is also being evaluated by the development of an assay that will allow measurement of VSG switch on and switch off rate.

DETECTION OF DICHELOBACTER NODOSUS IN CASES OF FOOTROT IN SHEEP BY 16SRRNA PCR

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Footrot is a specific contagious disease of the feet of sheep, goats and other ruminants caused by *Dichelobacter nodosus*. It is characterized by an exudative inflammation followed by necrosis of the epidermal tissues of the hoof with a foul odour. Footrot is distributed world wide but it has significant impact in those regions that have temperate climate including Indian state of Jammu & Kashmir in the northern Himalayas where this disease severely reduces the profitability of sheep husbandry. Scanty information is available on detection, isolation and characterization of *D. nodosus* from India particularly in J & K. Present work documents the isolation of *D. nodosus*, its detection by 16S rRNA PCR and detection of B serogroup of in *D. nodosus* in this region. Dichelobacter nodosus was confirmed by culture and PCR using species specific16S rRNA primers. When cultured, the organism appeared as flat colorless colonies having fine granulated structure with irregular margins showing characteristic Gram negative rods with swollen ends. Detection by PCR from cultured bacteria resulted in amplification of a 783bp product. Serogrouping based on detection of *fimA* gene by multiplex PCR using group (A-I) specific primers revealed the presence of serogroup B specific 283 bp bands. Extensive sheep husbandry is practiced by poor farmers of Himalayan region. Appropriate control strategies through rapid diagnosis, vaccination and management practices are urgently needed to control the disease. In order to better define the pathogenic nature of *D. nodosus*, its survival and transmission, and its specific role in sheep breeds of the state, systematic studies need to be undertaken.

PRESENCE OF PESTE DES PETITS RUMINANTS (PPR) VIRUS IN THE SERUM OF SHEEP AND GOATS IN THE ABSENCE OF VIRUS SPECIFIC ANTIBODIES

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Peste des petitis ruminants (PPR) is a highly infectious OIE notifiable viral disease of small ruminants. For Rinderpest and PPR viruses, seromonitoring and serosurveillance data has a pivotal role in ascertaining the nature of infection and also to assess the protective immune response. Serum samples are usually vital material for the aforesaid conditions. The animals with a titer of PPR virus neutralizing antibodies = 1:8, are most often considered to be protected against the disease. For an overall protection against a virus, neutralization is a feature of serum that is most often accepted as conclusive, with few exceptions viz, HIV, hepatitis, etc. In this study, we report first time, the presence of PPRV in the serum of animals in the absence of virus specific antibodies. Five groups of sheep and goats (about 25-40 animals in each group) purchased from local hill villages for experimental purpose were assessed for the presence of PPR virus/antigen and virus specific antibodies in the serum samples during quarantine period of three weeks. The PPRV in the serum samples was detected by using PPR S-ELISA kit, RT-PCR, QPCR assays and confirmed by virus isolation and sequencing of partial N and F gene. Out of 168 animals tested, 10 sheep and 23 goats serum samples were found positive for PPRV without virus specific antibodies. From these positive animals, RNA was extracted from serum and subjected to F and N gene based PCR and M gene based QPCR and, further virus was confirmed by sequencing. PPRV serum isolates from these ten animals were adapted successfully in Vero cells and characterized by partial sequencing. The PPRV specific antibodies status was assessed by employing the PPR c-ELISA kit, anti N MAb based ELISA, polyclonal indirect ELISA and SNT, as these assays are standard methods for screening of sero-negative animals. The serum samples from these 23 animals were found negative for the presence of antibodies in respect to PPR virus. Further protein profile and molecular characterization of theses serum isolates have to be carried out to understand the pathogenesis of the disease as a whole in sheep and goats. The results of the study will be discussed during presentation.

FIRST DETECTION OF BOVINE GROUP B ROTAVIRUS AMONG BUFFALO CALVES IN INDIA BY RNA-PAGE AND RT-PCR

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Rotaviruses are major causes of acute diarrheal diseases in newborn animals and humans. Rotaviral diarrhea is very common in calves. Affected young calves may die as the result of severe dehydration or secondary bacterial infection. To determine the prevalence of bovine rotavirus, 156 diarrheic feacal samples were collected from buffalo calves in the age group 1 day- 6 month from organized dairy farms located in Hisar. Genomic RNA was isolated from feacal samples by Phenol-chloroform-isoamylalcohol (PCI) method. The extracted viral RNA was screened by RNA-Polyacrylamide gel electrophoresis (RNA-PAGE) followed by silver staining. PAGE Positive samples were further extracted by Guanidium isothiocyanate (GIT) lysis method and the RNA was subjected to cDNA synthesis by reverse transcriptase (RT). The cDNA was amplified using gene specific primers. Out of 156 samples, 23 (14.74%) samples were positive for group A rotavirus (GAR) in RNA-PAGE. The viral RNA of group A rotaviruses migrated in a characteristic 4:2:3:2 banding pattern with migration of segments 7, 8 and 9 as a triplet confirmative of their presence. Of 156 samples, 7 (4.49%) samples were found positive for group B rotavirus (GBR) which presented a characteristic banding pattern of 4:2:2:2:1 on PAGE analysis. The GAR and GBR were further confirmed and characterized by RT-PCR assay. The results will be discussed in the presentation. Group A bovine

rotaviruses are recognized as a significant cause of calf diarrhea, whereas group B rotaviral diarrheas are viewed as an emerging and increasingly important disease. The GBR cause diarrhea in humans, pigs, cattle, lambs, and rats. However, Detection of GBR in diarrheic buffalo calves has been reported for the first time in India. In literature, in contrast to GAR, which are a major cause of acute gastroenteritis in young animals and children, the prevalence and characterization of GBR remains unclear. To our knowledge, this is the first report of detection of bovine group B rotavirus in India as causative agent of diarrhea in buffalo calves.

ISOLATION AND TYPING OF CANINE PARVOVIRUS IN CRFK CELL LINE IN PUDUCHERRY, SOUTH INDIA

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Canine parvovirus infection, a highly contagious disease of canines is prevalent all over the world, mainly because the virus can survive in adverse environmental conditions for a long time. The disease is clinically characterized by severe vomiting, pyrexia, anorexia and diarrhoea leading to fatal dehydration as well as myocarditis particularly in young pups. A total of 128 faecal samples/rectal swabs were collected from dogs showing signs of diarrhea/enteritis in and around Puducherry, south India. Eighteen (18) clinical samples, showing high HA titre of 1:512 and above and positivity by PCR with CPV-2ab primers, were subjected to virus isolation in CRFK cell line. Of the 18 samples processed, 3 samples (16.6%) were positive for CPV and were confirmed by haemagglutination, dot-ELISA, and IFAT. The three cell culture isolates were characterized as CPV-2b types by multiplex PCR as well as by monoclonal antibody typing.

SESSION – II
IMMUNOLOGY & IMMUNOTECHNOLOGY

Chairman, Dr. V.D.P. Rao
Co-chairman, Dr. V. Purushothaman
Rapporteur, Dr.. N.K. Rakha

Date
26 February, 2009, 5.15 - 6.30 PM

Venue
SEMINAR ROOM
Department of Veterinary Animal Husbandry Extention

TOLL LIKE RECEPTORS IN IMMUNITY AND DISEASE

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Animals have developed an immune system which they protect themselves against invasion from harmful exterior agents. There are five classes of disease causing agents that trigger an immune response: viruses, bacteria, fungi, protozoa, and helminthes. When any one of these agents penetrates the physical ,epithelial barrier between the external environment and the mammal's interior environment it elicits a two-pronged immune response. The first response is the innate that initially recognizes and controls the spread of the pathogen. After, a secondary adaptive immune response is triggered which eliminates the pathogen and has a memory function that allows the immune system to more easily recognize and eliminate subsequent infection by the same pathogen. The former involves the direct recognition of the pathogen, which is immediate, unspecific and the adaptive response is of a diverse receptor repertoire, in order to develop an appropriate immune response against invading agents. This difference in reaction suggest that all innate immunity responses must be based on the recognition of molecular patterns associated to microorganisms. In contrast, the adaptive immune response depends mainly on two types of specialized lymphocytes, T lymphocytes and B lymphocytes.. However, acquired immunity does not occur immediately in response to a new antigen or pathogen and the delay in the response could have a devastating effect on host survival. Therefore, the innate and the acquired immune responses are coordinated in such a way that the innate immune response represents the initial process which directs the defense of the mammal host.

The immune system has developed different methods to discriminate foreign antigens from its own.The strategy of microbial recognition is based on the detection of conserved molecular patterns which are essential byproducts of microbial physiology. The major molecular targets of the innate immune response are pathogen associated molecular patterns (PAMPs) . PAMPs have many features in common ; perhaps the most important is that they represent molecular structures that are produced only by the microbial pathogens and not the host. This prevents the trigger of the innate immune response against the host's own molecular signals. For example, peptidoglycan is a PAMP that is only present in bacteria not in host eukaryotic cells. This is the basis for the immune system's discrimination against self and non-self. Another feature of PAMPs is that they are essential for the survival of the microorganism.. PAMPs are often shared by a large group of microorganisms. Lipopolysac charide (LPS) is a feature of all gram-negative bacteria, while lipoteichoic acids (LTA) are only present in gram-positive bacteria. Not only do large groups of microorganisms share PAMPs but they are also the signatures of these microorganisms. Recognition of LPS, for example, not only tells the host that there is an infection present, it also informs the host that it is a gram-negative bacteria allowing the appropriate defence mechanism to be triggered.

It is evident that in the case of viruses that are synthesized and assembled in the host this detection method would fail, therefore, the innate immune response must include a mechanism not only for non-self recognition but for altered-self recognition and absence of self recognition. The properties of PAMPs previously explained make them excellent targets for the innate immune response. There exists a set of receptors designed to recognize PAMPs, called pattern recognition receptors. These receptors can be secreted proteins, cytoplasmic proteins, or cell surface expression proteins. Several different protein domains have been identified in the recognition of PAMPs, C-type lectin domain, scavenger receptor cysteine-rich domain, and leucine-rich repeat domain Unlike the adaptive immune system, the innate immune system is conserved and present in almost all multicellular organisms. Pattern recognition receptors are the first line of innate immune response elements, therefore, it would be expected that these molecules might be evolutionarily conserved.

FEATURES OF IMMUNOGLOBULIN GENES AND DEVELOPMENT OF PRE-IMMUNE REPERTOIRE AMONGST IMPORTANT SPECIES OF LIVESTOCK

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Introduction

One of the most remarkable features of the vertebrate immune system is its ability to respond to apparently limitless foreign antigens by defensive measures that are both specific and non-specific in nature. The non-specific component, innate immunity, is a set of disease resistance mechanisms that are not specific to a particular pathogen and can be triggered by molecules such as lipopolysaccharides, lipoteichoic acids, peptidoglycan and hypomethylated CpG DNA. These products are perceived as foreign and their recognition by host innate immune system can signal the presence of infection (Janeway, 1989; Janeway, 1992). On the other hand, the specific component, adaptive immunity, displays a high degree of antigenic discrimination, diversity, the ability to distinguish between closely-related molecules that are of foreign rather than self origin, and the unique property of memory. Adaptive and innate immune responses do not operate independently of each other but work in a highly interactive and co-operative manner to mount a response which is much more effective than either could do alone.

Adaptive immunity can be divided into humoral and cell-mediated responses. Antibody functions as the effector of humoral responses by binding to antigen and neutralising it or facilitating its elimination whereas effector T-lymphocytes generated in response to antigen are responsible for cell mediated immunity. Although there are intrinsic differences in the specific structure of immunoglobulins (Igs), generally speaking, most immunoglobulins have a four chain structure composed of two identical light chains and two identical heavy chains. Both the heavy and light chains are divided into two regions (variable and constant) based on variability in the amino acid sequences. As immunoglobulin (Ig) sequence data became available, it was found that virtually each individual antibody molecule carried a unique amino acid sequence in its variable region but only one of a limited number of invariant sequences in its constant region. The genetic basis of such tremendous variation coupled with consistency in a single protein molecule was found to lie in the organisation of the Ig genes.

Immunoglobulin genes and mechanisms of antibody diversification

Sheep

Studies in sheep suggested that a single V_H family consisting of nine V_H segments was utilised in the formation of the IgH repertoire (Jenne *et al.*, 2006). More recent studies have identified a number of V_H segments that did not match any of the published germline segments (Charlton *et al.*, 2000; White *et al.*, 1998) and were so divergent from V_H segments reported initially that they were classified into an additional eight families. Whatever the final position, the sheep V_H locus contains a limited number of V_H segments compared to those present at the V_H loci of mice and humans. None of the D_H segments have been sequenced in sheep, however from the analysis of rearranged CDR3 in sheep it appears that these segments consist of short and heterogeneous sequences. The J_H locus comprises six J_H segments, two of which are functional (Dufour and Nau, 1997). Both are utilised in sheep during IgH rearrangement.

The information on the Vê locus is limited; only six germline Vê genes have been reported. Kappa chains appear in 20 to 25 % of mature Ig molecules only (Griebel *et al.*, 1992). In contrast, the $V_ë$ locus contains more than 100 germline segments divided into six $V_ë$ families. Analysis has revealed that they possess little diversity and thus are unable to generate significant diversity to the combinatorial repertoire (Jenne *et al.*,

2006). The Jê locus consists of three segments, two of which are functional, whereas $J_ê$ possess two segments and both rearrange functionally. In summary, although a large number of Ig germline segments have been identified recently in sheep, they have very similar nucleotide sequences and thus overall capacity for Ig diversity through combinatorial rearrangement is limited (Jenne *et al.*, 2006).

The Ileal's peyer's patches (IPPs) were first described as aggregates of mucous secreting tissue by Johann Conrad Peyer in 1677 as cited by Griebel and Hein (Griebel and Hein, 1996). With the passage of time, the work of numerous researchers including that of Reynolds and associates identified these as a site of B cell proliferation and differentiation and the site where B cells undergo primary antibody repertoire expansion (Griebel and Hein, 1996).The exact nature of the B cell precursors which populate the organ in the sheep is not known. The IPPs follicles are oligocolonal and do not support ongoing Ig gene rearrangement (Reynaud *et al.*, 1991a). As indicated, combinatorial mechanisms do not contribute greatly to the overall diversity of the Ig gene repertoire in this species (Jenne *et al.*, 2006; Reynaud *et al.*, 1991a). The limited repertoire established through rearrangement is diversified through a process of somatic hypermutation (Reynaud *et al.*, 1997). In sheep, the neonatal repertoire essentially represents what can be achieved through rearrangement (Reynaud *et al.*, 1995). The process of pre-immune repertoire expansion takes place in the IPPs and substitutions build up over a period of time often targeting hotspots in the CDRs resulting in the pattern of transitions over transversions consistent with the features of SHM. The analysis of rearranged Ig genes recovered from foetal sheep has indicated that SHM occurs at a low level before birth (Jenne *et al.*, 2003). Following birth and exposure to exogenous antigens, a rapid build up of mutations in the Ig genes is seen (unpublished work cited in (Jenne *et al.*, 2006)). However, experiments have revealed that the exclusion of external antigens from the system (for example, when a section of lamb intestine was removed and ligated to peritoneal cavity) does not reduce the extent of mutations (Reynaud *et al.*, 1995), suggesting that somatic hypermutation may not always be linked to antigen-driven selection. A review from Reynold's group (Jenne *et al.*, 2006) reported that B cells in the IPP undergo very high mutational frequencies and that in apoptotic B cells this frequency is 5 times less in the V gene segment as compared to positively selected cells. These authors hypothesised that for a B cell to survive and emigrate from the sheep IPP it must mutate its genes, but if it fails to do so adequately, it undergoes apoptosis and dies *in situ* (Jenne *et al.*, 2006). Whilst diversification in sheep shows many features of a SHM-mediated process, there are large number of Ig pseudogenes and a possible role for gene conversion in the diversification of the Ig gene repertoire has been proposed (Reynaud *et al.*, 1997; Reynaud *et al.*, 1995), but convincing evidence has yet to be gathered.

Rabbits

Rabbit B cells are formed in the liver and omentum during foetal life. Shortly before birth, the site of production switches to bone marrow (Mage *et al.*, 2006). The evidence for Ig gene rearrangement in the foetus comes from detection of B cell recombination excision circles (BRECs) in bone marrow at 12 days gestation (Tunyaplin and Knight, 1995). A few months after birth, B lymphopoiesis appears to decrease rapidly as pro B or pre B cells, and BRECs were not found in any haematopoietic tissues tested (Jasper *et al.*, 2003). However, the demonstration of germinal centres containing B cells with undiversified Ig genes in the spleen of adult rabbits may be an indication that B lymphopoiesis can occur in older animals (Sehgal *et al.*, 1998).

Although there are more than 100 V_H gene segments available within the rabbit Ig heavy chain locus, many of which appear to be potentially functional (Currier *et al.*, 1988), the 3'-most V_H gene segment, V_H1, is utilised in 80–90% of rearrangements (Knight, 1992). Most of the remaining 10–20% of rearrangements utilise only two other V_H gene segments, V_Hx and V_Hy (Friedman *et al.*, 1994). The information on the V_L gene usage is not available, but it is predicted that variety of Vê and $V_ê$ gene segments appear to occur in rabbit based on the presence of several V_L sequences found in expressed cDNA sequences (Mage *et al.*, 2006). It is thus apparent that the capacity of the system to generate diversity (at least at the IgH locus) through rearrangement in severely limited.

As in several other species, it appears that events in or around the intestine are crucial to Ig diversification. B

cells leave the bone marrow to seed the gut-associated lymphoid tissue (GALT) sites including the appendix and sacculus rotundus, which then under the challenge of exoantigens and intestinal microbiota expand to form B cell follicles (Butler, 1997). Novel experiments in rabbits raised under germfree conditions or rabbits manipulated surgically to prevent the interaction between intestinal microbiota and GALT have shown the importance of intestinal bacteria in the development of GALT and VDJ gene diversification (Lanning *et al.*, 2000; Perey and Good, 1968). Diversification of rabbit Ig occurs in the GALT at 1–2 months of age through two, targeted mutational processes. Somatic hypermutation has been implicated in the diversification of the rabbit repertoire on the basis of appearance of point mutations in the V and D regions of the diversified V(D)J genes (Short *et al.*, 1991; Weinstein *et al.*, 1994). In addition to SHM, gene conversion-like mechanisms also generate antibody diversity in rabbits in the appendix. A somatic gene conversion-like mechanism inserts nucleotide sequence from V_H donors into the rearranged V_H gene segment (Becker and Knight, 1990; Lanning and Knight, 1997). The changes are referred to as gene conversion-like because the non-reciprocal nature of the alterations introduced has not yet been demonstrated; the large number of V_H segments that lie upstream of the functional V gene hindering this sort of analysis. In chickens (Carlson *et al.*, 1990) it has been shown that donor sequences were unaltered during diversification, providing formal confirmation of GC (Carlson *et al.*, 1990).

Horses

The IgH locus in horse is located on horse chromosome 24 (Wagner *et al.* 2004) and the work on characterization of the germline IgH V_H genes including their diversity (D) and joining (J) segments is continuously under investigation by a group of researchers such as B. Wagner, D.F. Antczak and Juan C. Almagro amongst many others. One such study analysed the cDNA library prepared from a mesenteric lymph node of the horse and reported that it contained 7 V_H genes belonging to two distinct families, corresponding to either the human $IgHV_H1$ or $IgHV_H4$ families, at least 7 D_H and 5 J_H segments (Schrenzel et al. 1997). However there is evidence that additional 3 V_H genes exist in the IgH locus of the horse (Navarro et al. 1995, Watson et al. 1997, Genbank accession number AF288214) thus making the total number of V_H gene segments not more than ten.

Horses in general have been considered within the group of species having limited diversity in the IgH V germline gene repertoire (Butler, 1998). This classification has mainly been established based on the limited information available on IgH V gene segments, D segments and J segments that has come from the sequencing data of limited cDNA clones from various studies (Navarro et al. 1995, Schrenzel et al. 1997, Watson et al. 1997). However, a horse:human chimeric antibody phage-displayed library constructed by combining an invariant human V_L domain with the repertoire of V_H domains amplified from a horse (Almagro et al. 2005) had revealed that a diverse sample of the expressed horse V_H repertoire exists. This information therefore has provided further impetus for future studies on equine IgH locus.

There are in total 11 constant heavy chain genes in equine and the organization of this region is very similar to that of mouse and rat (Shimizu et al. 1982, Brüggemann et al. 1986), containing the IgH Cμ and IgH Cδ genes as the most 5' located IgHC genes, followed by seven IgH Cγ genes (all of which are expressed and have distinct functions), and the IgH Cε and IgH Cα genes at the 3' end. The number of Cγ genes is 7 in haploid genome which is in contrast to relatively on small number (1 to 5) compared to other mammalian species.

In equines, there seem to be a relatively restricted germline repertoire of both lambda (λ) and kappa (κ) light chain, in total only 20–30 germline Vλ and less than 30 Vκ genes are present as revealed by Southern blot analyses. This species expresses predominately λ light chains which is evident from the fact that over 90% of the antibodies contain ë light chains (Gibson, D.M. 1974), corresponding to a ë:ê ratio of 13:1 both at the level of mRNA and protein (Ford et al. 1994). Out of a total four different immunoglobulin λ constant (Cλ) genes, three are functional having high nucleotide sequence homologies and the fourth is non-functional because of its association to a ψJ segment (Home et al. 1992). The germline Vλ genes are grouped together upstream of the Jλ and Cλ genes in the IgL locus. Nucleotide sequencing of the Vλ genes encoding ë light chains from splenic B-cells indicated that only a few of

these genes seemed to be used at a high frequency. Researchers still have to confront the challenges associated with questions as to where and how B-cell lymphogenesis and antibody repertoire development occur in the horse. However it has been hypothesized that horses might develop most of their antibody diversity by SHM (Sitnikova and Su, 1998) because it carries apparently a restricted germline variable gene repertoire of which only a few genes seemed to be frequently used (Ford et al. 1994, Home et al. 1992).

Pigs

Plenty of useful information had been gathered by concerted efforts of porcine immunologists and molecular biologists active in an area of porcine immunology, especially pertaining to the immunoglobulin organisation development of antibody repertoire that had resulted in greater understanding of the evolution of the antibody system of swine. Current knowledge is that the pig expresses IgM, IgD, IgG, IgE and IgA antibodies as is the case in mice and humans, albeit with a larger number of IgG subclasses, seven in total. Sequence comparisons of genes encoding IgM, IgA, IgG and IgE to those from other species have revealed highest sequence similarity with their apparent sequence homologs from humans (>70%) and ruminants (Kacskovics et al. 1994; Sun and Butler, 1997). The swine Cá locus contains an allelic variant that encodes an IgA lacking four amino acids in the hinge region (Brown et al. 1995), the significance of which is unknown. Despite this information, the characterization of the V_H and V_L repertoire at the genomic level is still incomplete and only partial map data for the κ locus (Butler et al. 2004), and the J_H–Cμ–Cδ region (Sun and Butler, 1997; Zhao et al. 2003) are available. The majority of information available on the germline V_H repertoire of this species has come from sequence analysis of rearranged or transcribed V_H and D_H segments amplified from foetal and newborn piglets (Sun and Butler, 1996, Butler et al, 2000; Sun et al. 1998 and McAleer et al. 2004). Southern blot studies carried out in the past have indicated that there are more than 20 porcine V_H genes in the genome (Sun et al. 1994), although many of them may include allelic variants. It will be difficult to assess the exact number of correct sequences for each gene or its allele, until the porcine V_H locus has been completely mapped. Another interesting finding of the swine Ig repertoire is that V_H genes that have so far been recovered all belong to the V_H3 family, whereas most expressed V_H genes in sheep and cattle are of the V_H4 family even though these mammals belong to the same order as swine (Butler et al. 2006) emphasizing that caution must be exercised while extrapolating results from one species to another. Pigs like humans make equal usage of λ and κ light chains (Hood et al., 1967). This is in contrast to mice where 95% of the light chains used are κ; and to cattle, sheep and horses where >90% light chains expressed are λ light chains. In swine, the $V_ė$ pre-immune repertoire (Ig repertoire that exists prior to antigenic exposure) is restricted since >95% of $V_ėJ_ė$ rearrangements use only a few members of the IgκV2 family and only $J_ė2$. The lambda locus has not yet been mapped or the genomic $V_ė$ repertoire determined.

It has been demonstrated that B cell lymphogenesis first occurs in the yolk sac. Class switch recombination without SHM has been shown to occur in utero, so pre-immune antibodies may be particularly important in swine because this could happen as early as 50th day of gestation (Butler et al. 2009). Pig possesses Ileal Peyers Patches like sheep which may be important for antigen-independent B cell repertoire diversification. The presence of pro B-like cells in interlobular areas of thymus and mature B cells in the thymic medulla that has switched to especially IgA in early gestation is so far unique among mammals (Butler et al. 2006). The pig uses very few V_H genes to create their pre-immune repertoire. Also there is near exclusive use of only two D_H segments (Sun and Butler, 1996 and Butler et al., 2000 and a single J_H (Butler et al. 1996) segment resulting in substantially diminished combinatorial diversity. This implies that junctional diversity plays relatively a greater role in Ig repertoire development as compared to humans and mice. In swine, the mechanism of gene conversion has been suggested but not been demonstrated convincingly (Sinkora et al. 2000) and the role of SHM still needs to be investigated.

Two $V_ė$ families (IgλV3 and IgλV8) are used in forming the pre-immune repertoire (Butler et al. 2005 and Butler et al, 2004). The analysis of early rearranged VDJ sequences from yolk sac/foetal tissues have revealed that these rearrangements are nearly 100 % in-frame and N-region additions are already present as opposed to what is seen in mice and humans. It has been

observed that the length of heavy chain CDR3 remains more or less same throughout the period of foetal life and it is because that constant level of TdT activity is maintained through this period (Butler et al. 2000; Sinkora et al. 2003). As is the case in rabbits and chickens, expressed V_H genes belong to the ancestral V_H3 family and that four V_H genes, designated V_HA, V_HB, V_HC and V_HE account for approximately 80% of total V_H gene usage in the pre-immune repertoire (Sun and Butler, 1996, Butler et al, 2000; Sun et al. 1998 and McAleer et al. 2004).

Camelids

Antibodies throughout mammalian species are composed of two identical H chains and two identical L chains. However, the sera of camelids (i.e. *Camelus dromedarius*, *Camelus bactrianus*, *Lama alpaca, Lama glama*, *Lama guanoco*, and *Lama vicugna*) (Hamers-Casterman et al., 1993) and probably the ratfish and some species of sharks contain antibodies that lack L-chains (Greenberg et al., 1995; Rast et al., 1998). These antibodies are called Heavy-chain antibodies (HCAbs) which are always of ã-isotypes and are functional in antigen binding. The differential adsorption of the dromedary serum on protein A and protein G results in recovery of double the amounts of HCAbs as compared to conventional antibodies (with L chains) suggesting their high concentration in blood. The IgH chain of HCAbs is composed of three instead of four domains because the domain corresponding to the CH1 of classical antibodies is not present. Hence, the variable domain is joined directly to the hinge region in HCAbs and as a result the Fab is reduced to a single variable domain in the HCAb (VHH) which is functional in antigen binding in the absence of a variable light chain domain (Muyldermans et al., 1994, Harmsen et al., 2001 and Maass et al., 2007). The other two constant domains are homologous to the CH2–CH3 domains of classical antibodies (Nguyen et al., 1999 and Woolven et al., 1999). The VHH amino acid sequences resemble closely that of a human V_H3 family, with important differences in their FR 2 and CDRs (Vu et al., 1997 Muyldermans et al., 1994, Harmsen et al., 2001). The genomic organization is also remarkably similar between the variable domain of the H chain of a classical antibody (VH) and that of an HCAb (VHH) with minor but very important differences that explain the antigen-binding capacity of the VHH's in a single-domain format (Conrath et al., 2003). The one such difference which is evident is the extended CDR1 and CDR3 in VHH (Conrath et al., 2003). Secondly, the FR2 in a VHH contains more hydrophilic amino acids as compared to VH domains which have higher concentration of hydrophobic amino acids. The H-chain of a classical antibody have few highly conserved residues of the VH domain that interact constitutively with the V_L in classical antibodies, namely Val42, Gly49, Leu50, Trp52 are substituted in the VHH domains of HCAbs by Phe/Tyr42, Glx49, Arg/Cys50, Leu/Gly52 (Muyldermans et al., 1994). This is probably the reason why V_L chains do not associate with VHH and that VHH can exist as soluble single-domain entity (Nguyen et al., 2001). The paratope of a classical antibody is expected to be much larger than that of a VHH due to the presence of the three CDR's in the V_L domain. However, longer loop lengths as a result of longer CDR1 and CDR3 in VHHs increase the paratope size of VHHs (Nguyen et al., 2000, Vu et al., 1997 Muyldermans et al., 1994, Wu et al., 1993). In Camels, five functional γ genes on the basis of their recovery from cDNA clones have been identified from a total of nine γ genes (De Genst et al,, 2006). Three of the five functional γ genes participate in forming HCAbs that lacks CH1 as mentioned before which is removed during splicing due to a point mutation at the 5' end of the CH1-hinge intron (Nguyen et al. 1999). In the genome of the dromedary, very similar but separate 50 VH and 42 VHH germline genes have been identified; however, the allelic relationship of these genes is unknown (Nguyen et al, 2000). VHH are distinguishable from VH by the presence of larger number of hotspots for mutation upstream of the CDR1 (Nguyen et al, 2003) in addition to the hallmark VH–VHH differences as mentioned before (Nguyen et al, 2000). Classical antibodies containing either ë or ê types are also present in the serum of camelids (Legssyer et al, 1995). Southern blot analysis on camel genomic DNA using specific light chain probes estimates the total number of Vë segments to be no more than 20 in the dromedary genome (De Genst et al., 2006). The sequence analysis of Vκ segments have shown that there is a limited diversity amongst them and all the Vê cDNA clones could be grouped into a single family. There is no information yet available about the detailed genomic organisation of the

genes residing in the H-chain locus or the L-chain locus. The mechanism how the B cells expressing only HCAb class switch remains speculative, but it has been suggested that it might be that the lymphocytes with a properly recombined VHH-D-J gene associated with a ì chain will fail to initiate V–JL recombination, but can class switch to one of the dedicated HCAb ã genes, possibly by an antigen-independent mechanisms (De Genst et al., 2006).

The detailed analysis of cDNA H-chain sequences had shown that a VHH germline-derived sequence never participate in formation of classical four-chain antibody (i.e. combined with a IgG1a or IgG1b gene), on the contrary VH germline-derived sequence can associate themselves with approximately 10% of the HCAb which are functional in antigen binding (i.e. combined with a IgG2 or IgG3 gene) (De Genst et al, 2006). The comparison of the sequences of the VH, VHH germline genes with rearranged and cDNA sequences had revealed that SHM plays a major role in antibody repertoire development in camels. The involvement of GC in immunoglobulin diversification has not been ruled out for conventional antibody generation, but possibly to a lesser extent which correlates with the lower frequency of palindromic sequences and Ig heptamer RSS in the VH relative to the VHH (Nguyen et al., 2000).

Chicken

The bursa of Fabricius is required in birds for B cell development (Cooper *et al.*, 2006). The B cells enter the bursa within a short time window and depletion of B cells from the organ a few weeks after hatching resulted in complete failure to restore the B cells population of chicks (Weill *et al.*, 1986). Thus, B cells move out from the bursa to seed the secondary lymphoid tissue early in life, after which the organ degenerates and further B lymphopoiesis in not possible. This is quite different to humans and mice where B cells are generated in the bone marrow throughout the life of the individual.

In chickens and possibly many other avian species, there are single functional V and J genes, thus limiting the capacity of combinatorial rearrangement to generate diversity. The chicken heavy chain locus contains a single J segment and a unique functional V gene (V_H1) 15 Kb upstream, with approximately 15 D elements in between. About 80 pseudo V genes similar to V-D joints exist upstream of V_H1 (Reynaud *et al.*, 1989). The rearrangement takes place outside the bursa early in development and the expression of surface IgM is a pre-requisite for entry into the bursa (Reynaud *et al.*, 1992). Diversification of the unique rearranged V_H1 gene takes place during bursal ontogeny by a hyperconversion mechanism, with V_H pseudogene segments acting as donors (Reynaud *et al.*, 1987a). All the 15 D elements in chickens are very similar and there is lack of N terminal additions limiting the diversity of CDR3.

The features of the chicken ë locus are equally unsuited to the generation of combinatorial diversity. Upstream of the Vλ1 gene segment, 25 Vλ pseudogenes are found, organised in either orientation (Reynaud *et al.*, 1987a). All pseudo V genes lack promoter, leader exons and recombination signal sequences. Only a few of the pseudogenes contain stop codons or frameshift mutations, but rather more are truncated at their 5' or 3' termini. Diversification is characterised by clusters of nucleotide changes, generated mainly by gene conversion by templated replacement from the many V pseudogenes that exist to the 5' side flank.

One feature of the process confirming that gene conversion takes place is the observation that sequence identical to the diversifying substitutions is always to be found in one of the pseudogenes which themselves remain unchanged (Carlson *et al.*, 1990; Reynaud *et al.*, 1987a). Only pseudogenes on the same chromosomes are used as donors (Carlson *et al.*, 1990) and the pseudogenes that are commonly utilised are those with the best match to the recipient sequence, lie close by at the Ig locus or are in the opposite orientation to the rearranged V segment (McCormack and Thompson, 1990). Conversion tracts range from 8 bp to around 200 bp (McCormack and Thompson, 1990) with a 5' to 3' polarity in the gene conversion mechanism. Of the two sets of parental Ig alleles, only one is rearranged as a result of a strong silencer that makes rearrangement inefficient (Ferradini *et al.*, 1994; Lauster *et al.*, 1993). Strong silencing activity is important because gene conversion events otherwise may correct the out of frame rearrangements thus failing to preserve allelic exclusion (Sayegh *et al.*, 1999). Overall, the mechanism for generation of immunoglobulin diversity in chickens is radically different from that of non-avian vertebrate species.

Immunoglobulin genes and mechanisms to generate immunoglobulin diversity in cattle

Antibody diversity in vertebrates is generated by the recombination of separate germline gene segments both for the heavy (V_H, D_H and J_H) and light (V_L and J_L) chains and association of two identical heavy and light chains that are independently formed (Tonegawa, 1983). This diversity is further increased as a result of junctional flexibility from nucleotide deletions or additions during the recombination process. More antibody diversification is brought about by SHM and insertions or deletions. While the recombination process is common across vertebrate immune systems, germline immunoglobulin gene sequence diversity differs considerably.

Most studies in the past have shown that in cattle both the light chain and heavy chain repertoires are founded upon the frequent expression of single gene families and sub-groups of segments, which are of conserved sequence. Bovine IgH chains possess CDR3 sequences that are frequently long (Berens *et al.*, 1997; Saini *et al.*, 1997; Sinclair *et al.*, 1997) and sometimes in excess of 50 amino acids in length (Saini *et al.*, 1999). It is now known that all the antibody classes (IgM, IgD, IgG, IgE and IgA) that are present in human and mice are also found in cattle (Zhao *et al.*, 2002). Present understanding of bovine humoral immunity and the mechanisms generating the bovine immunoglobulin repertoire is far from complete but its structure and the levels of diversity which are generated through rearrangement reveal that this is another species which departs significantly from the murine/human paradigm (Aitken *et al.*, 1999). The following sections highlight the extent of these differences.

Cattle heavy chain locus

Limited VH genes

The bovine heavy chain locus is located on bovine chromosome 21q23-24 (Gu *et al.*, 1992; Tobin-Janzen and Womack, 1992). The exact size of the locus and the number of segments present is not known. However, studies have shown that a family of V_H segments homologous to mouse V_H Q-52 and the related human family are used in rearrangement. The gene family has been designated as BoV_H1 (Berens *et al.*, 1997; Lopez *et al.*, 1998; Saini *et al.*, 1997; Sinclair *et al.*, 1997). Data from Southern blotting, ssCP analysis and sequencing indicate that the size of the dominant gene family is small, comprising no more than 20 members. Further, according to Sinclair *et al.*(Sinclair *et al.*, 1997) it carries few distinct CDR sequences and diversity apparent in these sequences is extremely modest. Southern blot analysis of bovine genomic DNA have also demonstrated that homologues of at least three other murine families are present in the genome (Berens *et al.*, 1997; Lopez *et al.*, 1998). Transcripts from these families have not been detected and the reason why they do not undergo rearrangement is unknown. Gene rearrangement through combinatorial joining of limited V_H segments with D_H, and J_H gene segments thus seems unable to impart significant diversity to the primary heavy chain repertoire in cattle.

Unique Complementarity Determining Region (CDR) 3 (heavy) H region

The CDR3H region of an antibody is typically the most diversified of the CDRs and determines largely the antibody specificity. Various investigators have observed that cattle IgH chains carry CDR3s of exceptional length with an average size in bovine IgM and IgG antibodies being 22.18±10.86 and 19.56±6.64 amino acids, respectively (Berens *et al.*, 1997; Saini *et al.*, 1997; Sinclair and Aitken, 1995; Sinclair *et al.*, 1995). The unusual long CDR3H region can extend up to 61 residues in size and often contain multiple even numbered Cys residues. Such multiple Cys residues have been noted CDR3H of other vertebrates like sharks and camels but they do not attain the massive size seen in cattle antibodies (Ramsland et al., 2001).This sets apart the cattle immunoglobulin system from sheep which otherwise are very similar at the molecular level. Three different potential mechanisms have been proposed to explain the unusual length of CDR3s in cattle (Sinclair *et al.*, 1997). These include long bovine D segments, terminal transferase activity to expand this part of the reading frame to an exceptional degree, and rearrangement of D segments in pairs. Camels use long CDR3s in antibodies that lack light chains and it has been postulated that a long CDR3 produces a loop that compensates for this light chain deficiency (Desmyter *et al.*, 1996; Hamers-Casterman *et al.*, 1993). D-D gene fusions in humans have been shown to generate long CDRs (Sanz, 1991) as is the case in

chickens (Reynaud *et al.*, 1989).

More recently, bovine germline D_H segments have been isolated from a bovine genomic DNA library using a radiolabelled probe. The partial characterisation of a 2.3 kb fragment led to the identification of three bovine germline D_H segments of 42 bp, 58 bp and 148 bp. Phylogenetic analysis suggested that these are closest to rabbit and chicken D_H gene (Shojaei *et al.*, 2003). All the segments are flanked by classical RSSs with typical heptamer and nonamer sequences on either side though these differ significantly in one of the D_H segments. Rearrangement of a D segment of 148 bp could potentially generate a long CDR3H region. However, the isolation of 148bp bovine D_H gene does not fully explain the generation of CDR3H sequences as long as 61 codons as has been observed in some bovine antibodies (Shojaei *et al.*, 2003). There is a possibility that even larger germline segments exit or other mechanisms such as D-D fusion might contribute to the generation of long CDR3H (Meek *et al.*, 1989; Reynaud *et al.*, 1991b).

Mechanisms to diversify cattle antibody repertoire

Several investigations have studied the development of the bovine Ab repertoire. Berens and colleagues showed that sequences from 150-day-old foetuses showed little variability indicating that rearrangement in cattle generates minimal Ab diversity (Berens *et al.*, 1997). This is in agreement with the predictions based upon the sequencing of germline VH segments. The sequences from adult IgM transcripts were mutated as much as those from antigen-driven heterohybridomas indicating a post-rearrangement diversification process takes place before antigen encounter. In common with other reports, these authors showed the rearrangement of single family of V_H segments ($BovV_H1$), although the germline carries other V_H gene families. The reasons for the lack of expression of these families are not known. It has been suggested that chromosomal translocation of V_H genes from other families to different chromosomes (Matsuda *et al.*, 1990), closer proximity of $BovV_H1$ genes to the D gene cluster (Yancopoulos *et al.*, 1984) and/ or differences in the regulatory signals could explain the predominant expression of the $BovV_H1$ family. Little or no mutation was detected in FR4 from foetal and adult cattle, which resembles findings in other species.

Studies have indicated that the spleen is a likely site for Ig rearrangement in cattle (Meyer *et al.*, 1997). The expression of RAG-1 gene has been shown concurrently in thymus and spleen in 14 days old calves. No expression of RAG-1 was observed in the spleen of 32 week old cattle but it continued to be expressed in the thymus suggesting that Ig gene rearrangement occurs early in the life of the animal only (Meyer *et al.*, 1997). By analogy with the sheep, it is likely that rearrangement in B cells begins during foetal development and that the lymphocytes then go on to populate the IPP before birth and for weeks thereafter (Reynolds and Morris, 1983). In chicken, and sheep, the post rearrangement diversification of the Ig occurs in the GALT. Both the bursa (chicken) and the IPP (ruminants) begin involuting 3 to 6 months after birth following the completion of diversification. The released B cells then populate the periphery where they form a self-renewing pool (Griebel and Ferrari, 1994; Reynolds *et al.*, 1991).

In species that are unable to generate significant Ig diversity by rearrangement, gene conversion and somatic hypermutation have been shown to drive diversification, post-rearrangement. Chickens form the best characterised example of a species that undergoes frequent gene conversion during antibody diversification (Reynaud *et al.*, 1987b; Thompson, 1992). Gene conversion as a process is thought to have been responsible for the duplication of Ig genes during the evolution of the Ig locus in mammals (Baltimore, 1981). The first demonstration of gene conversion as a mechanism for generation of diversity in mammals came from studies in rabbit where it was shown to diversify the preferential V_H1 (D) J rearrangement (Becker and Knight, 1990).

Gene conversion has been suggested as one mechanism for diversification of Vλ genes of cattle (Lucier *et al.*, 1998; Parng *et al.*, 1996). This conclusion was based upon the identification in light chain cDNA of substitutions which matched the sequence of putative pseudogene donors. However, these experiments were unable to discriminate between differences that might have been the result of allelic variation and sequences donated from pseudogenes. The investigators (Parng *et al.*, 1996) compared cDNA sequences to a functional germline segment which was not typical of the majority and therefore might constitute an unlikely candidate for the rearranged segment. Moreover, many

of the substitutions to which they drew attention were common to several cDNAs and the donors possessed near-identical sequences in the CDRs suggesting that little meaningful diversity could be generated in this way. In sheep, somatic hypermutation is thought to generate diversity post-rearrangement in the IPP (Reynaud *et al.*, 1995; Reynaud *et al.*, 1991c). These studies were centered on analysis of the lambda light chain repertoire and for many years, it was assumed that hypermutation was the dominant factor in diversification. More recently, it has been shown that rearrangement is capable of generating higher levels of light chain diversity than previously supposed (Jenne *et al.*, 2003). This study suggests that the sheep is not as heavily reliant upon mutation as might have been inferred from initial reports. However this work by Jenne and colleagues does not question the basic view that somatic hypermutation acts post-rearrangement to further diversify the ovine primary Ig repertoire. Studies of the bovine Ig heavy chain repertoire have generally favoured somatic hypermutation as a diversification process (Aitken *et al.*, 1999; Berens *et al.*, 1997; Kaushik *et al.*, 2002) but until recently, definitive evidence has been unavailable.

The recent experiments by Kaushik et al , 2009 examined the degree and pattern of diversification in the variable-region of IgM and IgG antibody isotypes from 18-month-old cattle afflicted with bovine leukocyte adhesion deficiency. These authors reported that both somatic mutations and exceptional CDR3H size generation contribute to IgM and IgG antibody diversification in cattle during the development of immune response to naturally occurring chronic and multiple microbial infections. The laboratory of Aitken (Verma and Aitken, unpublished) at the University of Glasgow recently have gathered evidence about the diversification process by looking at IgH variable and downstream $J_H1/\psi J_H4/J_H2$ regions from cattle of a different age groups. This data has revealed that features of diversification within the rearranged Ig V segments showed many of the characteristics of SHM as documented in other systems (Gonzalez-Fernandez *et al.*, 1994; Sinclair *et al.*, 1997; Wagner and Neuberger, 1996). The mutations were random in nature with some exception for certain regions which were highly mutated. Such hotspots were encountered not only in the hypermutating domains of CDR1 and CDR2 but also in the intronic sequences downstream of FR4, and the target region for the hypermutation extended well into the $J_H1/\psi J_H4/J_H2$ introns. In this study of cattle, the frequency of mutations for this non-coding region was at least as high as seen for the variable coding region of IgH. The analysis of uniqueness of each mutation argues against the gene conversion as a mechanism for generating somatic diversity. Random point mutations apparently linked to the transcriptional state of the gene is more consistent with SHM as the diversifying process. The existence of multiple V_H segments is unquestioned in the genome of cattle but there are no data to suggest that this is true of the J_H locus aside from the known duplication at BTA11. Gene conversion is therefore unlikely as a mechanism for introduction of mutations to the $J_H1/\psi J_H4/J_H2$ region. This study did not rule out the possibility of the involvement of gene conversion in antibody diversification in cattle but it seems unlikely that it plays a significant role to diversify the heavy chain repertoire.

The current uncertainties associated with diversification mechanisms will clear away in future as the data become available from the cow genome project. The genome project will provide not only the better definition of number of total V_H and V_L segments available in the genome but also the understanding of their organisation in their respective loci. It will also allow investigation of the reasons why only certain gene segments are used in rearrangement and expression preferentially when others appear to be present. It will be both very interesting and challenging if procedures could be optimized in bovine to understand particularly the primary and antigen driven Ig diversification which otherwise is difficult to separate.

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SIMPLE METHODS TO ENHANCE THE SENSITIVITY OF THE ROSE BENGAL PLATE TEST FOR THE SERODIAGNOSIS OF BRUCELLOSIS

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The most common serological test used for the field diagnosis of Brucellosis is the Rose Bengal Plate Test (RBPT) based on the agglutination of colored particulate antigen (killed Brucella organisms) by the antibodies present in the patient's serum. RBPT is one of the tests prescribed by the OIE for screening the animals for international trade purposes. Although it is a simple, cheap and effective test, the RBPT is generally considered to be less sensitive than other tests like Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT) and ELISA and false negatives are not uncommon with RBPT. Although, ELISA has been claimed to be a good screening test, its cost and requirement of skilled personnel prohibits its widespread use in the field. STAT and CFT are also too cumbersome and tedious to be used as screening tests in the field.

Simple innovative modifications in the conventional Rose Bengal Plate Test can significantly enhance the sensitivity of the technique and minimize the false negative results aiding in accurate, quick and cheapest diagnosis of Brucellosis. False negative or weak positive samples that are hard to assess as positive or negative show higher agglutination, when antiglobulin is added to the antigen – antibody mixture. Anti bovine immunoglobulin binds to the antibody in the antigen - antibody complex and thus larger aggregates are observed due to cross – linking of smaller clumps. However, such aggregates are loose and give a diffused pattern. The use of a modified antiglobulin can help by consolidating the loose and diffused network of smaller clumps which will be easier to detect. Prior treatment of the antibodies in the test serum can help in differentiating non – specific aggregates of antigen particles alone from specific antigen – antibody agglutinates. With the new method, antigen is visually distinguishable from the antibody in the agglutinate. The results of such a modified test would

be easier to read and interpret than the conventional RBPT results making it highly sensitive.

CYTOKINES AND IMMUNE RESPONSE

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Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems. They are produced by virtually all the cells involved in innate and adaptive immunity, but especially by T helper (Th) lymphocytes. The binding of a cytokine to a corresponding receptor in the immune cells initiates intracellular signal cascades, ultimately leading to changes in gene expression.

Cytokines can be classified according to structure as well as function: (i) Type 1 cytokines signal via type I cytokine receptors eg. IL-2, IFN-ã, TNF-â and/or IL-12. (ii) Type 2 cytokines includes IL-4, IL-5, IL-6, IL-10 and/or IL-13. Besides these, there are two other cytokine families have been described based on similarities in their receptor structure. These are the tumor necrosis (TNF) family, which includes TNF- á, TNF- â, CD40 ligand and Fas ligand, and immunoglobuline superfamily, which includes B7.1 and B7.2 that are responsible for costimulation of T cells. Cytokines may also be classified in terms of their production by T-helper (TH) lymphocytes (a) TH1 cytokines: they are primarily involved in promoting a cell-mediated immune response. These cytokines are generally pro-inflammatory and includes primarily IFN- ã and IL-2. (b) TH2 cytokines: are those primarily involved in humoral immune response and resposes to disseminated infection. These cytokines are generally anti-inflammatory and include IL-4, IL -5, IL - 6, IL-10 and IL-13. Both TH1 and type 1 cytokines elicit predominantly cell-mediated immunity (CMI), where as Th2 and type 2 cytokine elicit predominantly humoral immunity. Some TH1 (IFN- ã) and Type-I (IFN- ã and IL-12) cytokines down regulate humoral immunity by decreasing the level of TH2 and type 2 cytokine. Some TH2 (IL-4) and Type 2 (IL4 and IL-10) cytokine down regulates CMI by decreasing the level of TH1 and Type 1 cytokines.

ANTIMICROBIAL ACTIVITY OF RECOMBINANT BUFFALO NEUTROPHIL BETA DEFENSIN-4 (BNBD4) AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* ISOLATED FROM MASTITIC MILK

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Bovine neutrophil â defensin-4 (BNBD4), a small cationic peptide, exhibits a broad range of antimicrobial activity. It plays an important role as effector molecules of innate immunity that provide a first line of defense against microbial invasion. For the purpose of this study, buffalo BNBD4 cDNA was amplified from bone marrow cells by RT-PCR. The amplified product (192 bp) was cloned, sequenced and nucleotide sequence submitted to GenBank (AY392452). The amplified product was sub-cloned into expression vector pET32a. The expression construct (pET32-BNBD4) was transformed into *E. coliBL21* (DE3) and recombinant protein was expressed as fusion protein with thioredoxin at N-terminus along with a histidine hexamer tag (His-tag) at C- terminus. Recombinant protein was analysed by SDS-PAGE and the expected bands of approximately 21kD and 28kD in size of fusion tag (thioredoxin) and thioredoxin-BNBd4 were observed. The recombinant buffalo BNBD-4 protein was purified using a nickel affinity column. Antimicrobial activity of the rBNBD4 against *Staphylococcus aureus* and *Escherichia coli* isolated from mastitic milk, was studied by colony counting method. Tested microorganisms were incubated with different concentrations (5, 10, 15, 20, & 25 ug/ml) of recombinant BNBD4 in 100ul of 10mM sodium

phosphate buffer (pH7.4) containing 1% v/v tripticase soy broth for three hours at 37°C. Concentration of the tested organisms was 1x 10^5 CFU/ml. Following incubation, the serial dilutions of the mixture were plated and colony counts were performed the following day. Recombinant fusion BNBD4 showed significant bactericidal activity against tested microorganisms. Hence, recombinant neutrophil beta defensin-4 protein could be use as an antimicrobial molecule effective against mastitis causing *S. aureus* and *E. coli* microorganisms.

ANTIMICROBIAL ACTIVITY OF MAMMARYGLAND MONONUCLEAR CELLS STIMULATED WITH RECOMBINANT BUFFALO IL-2 PROTEIN AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* ISOLATED FROM MASTITIC MILK

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Interleukin-2 is predominantly produced by the helper T-lymphocytes and large granular lymphocytes. It produces a marked effect by enhancing the cytotoxicity of CD8+ T cells and natural killer cells. Interleukin-2 enhances the bactericidal activity of the mononuclear cells. Here, we report the expression, purification and functional activity of recombinant buffalo interleukin-2 (rbuffIL-2) to stimulate bactericidal activity of the mammary gland mononuclear cells. Buffalo IL-2 cDNA was amplified and cloned into expression vector pET32a. Recombinant plasmid (pET32/buffIL-2) was transformed into *E.coliBL21(DE3)* and recombinant protein was expressed as fusion protein with N-terminus thioredoxin tag and C-terminus histidine hexamer tag. The recombinant protein was purified using nickel column and confirmed by Western blotting with specific rabbit anti- bovine IL-2 serum. The bioactivity of rbuffIL-2 was detected through MTT colorimetry by stimulating the proliferation of buffalo lymphocytes cultured with rbuffIL-2 protein *in vitro*. The rbuffIL-2 protein significantly promoted the proliferation of lymphocytes. The bactericidal activity of mammary gland mononuclear cells cultured with rbuffIL-2 was evaluated using *Staphylococcus aureus and Escherichia coli* strains isolated from clinical cases of bovine mastitis. Bactericidal activity was more evident in cell population cultured with rbuffIL-2 than those cultured in absence of this cytokine. The enhanced capacity of rbuffIL-2 activated lymphocytes to kill *S.aureus* was elevated during the 30 and 60 min incubation period. The results indicate that rbuffIL-2 protein could be use as immunomodulator to combat bovine mastitis.

TRANSCRIPTIONAL EXPRESSION OF TLR9 IN HORSES ANDPOITU DONKEYS

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Toll like receptor 9 (TLR9) is critical component in the signaling pathway for Cytosine-phosphate-guanine (CpG) mediated activation of the mammalian immune system. Significant structural differences in the extracellular domain of TLR9 account for species-specific recognition of CpG ODN sequences. TLR9 has been extensively studied in humans and mice. Although TLR9 sequences are reported in some animal species including horse but there is no report of TLR9 sequences of Indian breeds of horses and donkeys. To address these concerns, Poly A^+ mRNA transcripts from peripheral blood mononuclear cells (PBMCs) of Marwari breed of horse and poitu donkeys were purified using the oligotex particles®, reverse transcribed and amplified to assess the transcriptional expression of TLR9 in these species. Partial TLR9 gene amplicons were obtained using conserved as well as species specific primers. Cloning of the amplified products of TLR9 is in progress.

MULTICISTRONIC EXPRESSION OF BUFFALO INTERLEUKIN-2 (BUFFIL-2) AND BUBALINE NEUTROPHIL BETA DEFENSIN-4(BNBD-4) IN VERO CELLS

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The buffalo neutrophil beta defensin-4 (BNBD-4) and interleukin-2 (buffIL-2) act as effector molecules of the innate immune system. The present study was undertaken to co-express buffalo interleukin-2 (buffIL-2) and bubaline neutrophil beta defensin-4 (BNBD4) genes in eukaryotic cells. cDNAs of both the genes were successfully amplified by RT-PCR from the total cellular RNA isolated from bone marrow cells and peripheral blood mononuclear cells, respectively. The expected amplified products were 192 bp and 430 bp in length. Both the amplified products were cloned into pTZ57R/T vector and sequenced. The nucleotide sequence of buffalo BNBD-4 gene has been submitted to GenBank (accession no.AY392452). The buffIL-2 and BNBD-4 genes were subcloned sequentially into eukaryotic expression vector (pVITRO) and recombinant expressions construct (pVITRO/buffIL-2/BNBD4) was prepared. Endotoxin free recombinant plasmid was introduced into Vero cells using lipofectamine reagent. Samples were collected 48 hours after transfection for detection of expressed proteins. Western blot analysis was carried out with total protein extracts from transfected and control cells using antibodies raised against rbuffIL-2 and rBNBD4 in rabbit. Purified proteins were used as a positive control. Proteins from transfected cells showed the immunoreactive protein of ~7KD (rBNBD4) and ~ 15Kda (rbIL-2) in size. The result indicated that the transfected cells produced the primary translation product (mRNA) of rbuffIL-2 & BNBD4 genes.

DEVELOPMENT OF DOT- ELISA FOR DETECTION OF ANTIBODIES TO INFECTIOUS BURSAL DISEASE, HYDROPERICARDIUM SYNDROME AND CHICKEN ANAEMIA VIRUSES**

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The antibody status of breeder flock is important in assessing the level of protection conferred on hatchlings through maternal antibodies. An assay does not require much technical competence and sophisticated equipments is required to assess the antibody level at farm level. This helps in fine tuning of vaccination schedule of both breeders and hatchlings. The dot-ELISA test detecting antibodies to three viral diseases could reduce cost and save reagents. The dot-ELISA was developed and compared with that of VNT. The IBDV and HPSV antigens after purification were used as coating antigens for dot-ELISA. In case of CAV, the VP1 recombinant protein expressed in DH5á *E.coli* was used as coating antigen. The recombinant protein was purified by Ni CL agarose column as the protein is having *His* tag. The $TCID_{50}$ of IBDV and HPSV were found to be $10^{5.0}$ and $10^{3.2}$ per ml respectively after 4th passage. The VNT and dot – ELISA was performed for screening of antibodies to IBDV and HPSV with 302 breeder serum samples for the presence of antibodies to three viruses. All the three virus proteins at 150 ng/µl concentration gave optimum dot intensity. A special trough was fabricated to enable to dip all the legs of dot-ELISA comb with three dots and also the comb with three legs was fabricated for present study. A total of 195, 205 and 104 serum samples gave

positive results with dot-ELISA for IBDV, HPSV and CAV respectively. The results by comparing with VNT for IBDV showed 90.1% specificity, 74.1% sensitivity, 83.7% accuracy and a K value of 0.99 indicating perfect agreement. Likewise, for HPSV 96.9% specificity, 67.1% sensitivity, 83.4% accuracy and a K value of 0.99. The VNT though standard and gold standard is time consuming and cumbersome. The dot-ELISA results are comparable to that of VNT and easily performed even at pen side. So the standardized dot-ELISA can be used for screening of serum samples with nearing accuracy.

PROTEIN G BASED ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DIAGNOSIS OF BRUCELLOSIS IN BUFFALOES

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Brucellosis is a major bacterial zoonosis of global importance. Timely and correct diagnosis of this disease is important to prevent its spread among human and animal population and for international trade. Buffaloes are an important part of domestic animal population in India and *Murrah* breed of Haryana is considered as 'black gold' due its economic importance for farmers. The conventional serological tests viz. Rose Bengal Plate Agglutination Test (RBPT), Serum Tube Agglutination Test (STAT) and Complement Fixation Test (CFT) commonly used for cattle, are also used for buffaloes. However, these tests have certain inherent problems which are circumvented with the development of enzyme immunoassays such as Enzyme Linked Immunosorbent Assay (ELISA). But this assay has its applicability in those animals whose species specific conjugates are available. Till date species specific conjugate for buffaloes is not available. This leads to make use of an alternate conjugate comprised of protein-G tagged with an enzyme. The protein-G is a cell wall component of *Sterptococcus* which has the ability to bind strongly with many mammalian IgG including bovines. Since bovine includes both cattle and buffaloes the ability of protein-G to bind with buffalo IgG is exploited to develop a protein-G based ELISA which can be used to assess *Brucella* antibodies in buffaloes. The assay performance in terms of specificity and sensitivity was determined employing the serum samples from brucellosis free buffalo herd and culture positive animals. Results of potein-G ELISA will be discussed and compared with STAT, RBPT and anti-bovine ELISA.

EVALUATION OF IMMUNE RESPONSIVENESS IN PURE- AND CROSS-BRED CHICKENS USING S-RBC ANTIGEN

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The immune responsiveness of two breeds of poultry viz; one stock of Dahlem Red, one strain of Rhode Island Red (RIR-B) and the cross resulted from Dahlem Red ? x RIR-B ? maintained at the Poultry Research Farm of the University was studied using Sheep Red Blood cells (S-RBC) as an antigen at 0, 5, 10 and 15 days post primary inoculation (ppi). The pure bred groups did not differ from each other for level of natural antibodies where as the crossbred group had significantly higher levels of antibodies as compared to pure average. (1.12 vs. 1.48). The presence of natural antibody was evident in all the genetic groups. . The mean titre on day 0 averaged to be 1.24±0.08 pooled over all the groups. There was no significant difference between the pure groups viz; Dahlem and RIR-B from day 0 to day 10 titre post primary inoculation. On genetic group pooled basis the HA titre increased sharply from day 0 (1.24) to day 5 (6.47) ppi, and then declined slowly to 2.67 at day 15. The differences between pure groups are statistically significant at day 15 only with RIR-B having higher titre. The average of two pure was significantly higher than of the cross at day 10 and 15 ppi.

PREPARATION AND STANDARDIZATION OF *BRUCELLA ABORTUS* O-POLY-SACCHARIDE-FITC ANTIGEN FOR FLUORESCENCE POLARIZATION ASSAY FOR THE DIAGNOSIS OF BRUCELLOSIS IN BUFFALOES

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Brucellosis is an important disease of man and animals but due consideration has never been given to this bacterial zoonosis in India. The disease is prevalent in all livestock systems and increased demand for dairy products accompanied with changing and intensified farm practices has raised concern of its spread to the animals and human population. Isolation and identification of *Brucella* from infected animals is the definitive diagnostic method but not commonly used because of its many limitations and possibility of hazard unless the laboratory is equipped with special facilities to handle live organisms. Hence, many serological tests viz. Rose Bengal Plate Agglutination Test (RBPT), Standard Tube Agglutination Test (STAT), Complement Fixation Test (CFT) and Enzyme Linked Immunosorbent Assay (ELISA) are in use for the diagnosis of this disease. Recently, O-polysaccharide antigen (OPS) purified from *Brucella abortus* cells and tagged with a fluorescein isothiocyanate dye (FITC) has been used in Fluorescence Polarization Assay (FPA) for detecting brucellosis in animals. The FPA is a homogenous primary binding immunoassay which measures antigen-antibody binding directly, without the need of a separation procedure. In the present studies, OPS-FITC antigen was prepared in the laboratory using *B. abortus* S99. This antigen was standardized using a panel of serum samples from a brucellosis free buffalo herd where the disease has never been reported/ detected. The sensitivity of the assay was determined using serum samples from culture positive buffaloes from whom *B. abortus* was isolated. The results of FPA will be discussed in comparison to conventionally used STAT and RBPT methods for diagnosis of disease.

IMMUNOCHROMATOGRAPHIC DIAGNOSIS OF CANINEPARVOVIRUS INFECTION

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Ten suspected cases of Parvodog gastroenteritis were tested by immunochromatography. Monoclonal antibodies test .Out of ten dogs three dogs found positive by said test. The main clinical symptoms observed were rise temperatureup to105^{0}F, vomiting, blakish or blood mixed faeces, complete anorexia, dehydration followed hypothermia and collapse if dog remain untreated. The ailing dogs were treated successfully with parentral ceftriaxone, metaclopromide, ranitidine, isolyte M, ascorbic acid, botropause and ciprofloxacin-tindazole combination per os.

SESSION – IIII
GENOMICS & BIOTECHNOLOGY

Chairman, Dr. R.K. Singh
Co-chairman, Dr. Anil Taku
Rapporteur, Dr. B.R. Gulati

Date
27 February, 2009, 9.00 AM - 1.00 PM

Venue
SEMINAR ROOM
Department of Veterinary Animal Husbandry Extention

DOMESTIC BUFFALO GENOMICS: HYPE AND HOPE

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Domestic buffalo is essentially an Asian animal and is the mainstay of dairy industry in India. Applications of principles of population genetics for genetic enhancement of this species have been hampered by various socio-economic factors. Most of the improvement thus far has been possible through conventional wisdom of the farmers. Impact of progeny testing of males followed by their extensive use through artificial insemination has had limited impact on actual genetic enhancement of domestic buffalo. On the other hand domestic animal breeding in the developed word has been based upon the modern scientific methods for more than seven decades. During the last ten years DNA sequencing methods have been extended to livestock species. Using genomics methods issues related with livestock biodiversity and domestication history have been successfully addressed. Our group at CCMB has been involved with understanding genetic history of buffalo. In addition we have generated several DNA markers of various types to create genomic maps of this species. In other livestock species DNA diagnostic tools are being developed for identification of stocks with better potential. This has been possible due to several factors. Some of these include- availability of large-scale phenotyping data in cattle, chicken, pig sheep etc., sequencing of genomes of these species and thus development of DNA diagnostic tests, and finally the organised structure of the animal industry in the developed world. In domestic buffalo, we are yet to have any of these elements in place. At CCMB along with a few other laboratories in the world we have been developing genomic resources for buffaloes. Conversion of these resources into tools for genetic improvement would require large-scale genetic experiments with quality production and reproduction data. Experiences of the last several decades have vividly demonstrated that such a gigantic task is beyond the realms of public sector institutions. Therefore, there is an urgent need for strengthening public-private partnership. To begin with, the revenue model may be based upon the immediate application of the extant scientific knowledge base and production of quality buffalo bulls and or semen along with packaged breeding services from nucleus herds operating through public-private partnership mode. Such herds would help accelerate knowledge generation by combining genomic tools and phenotyping data. Given their scale of operations one can expect that these herds would act as immediate incubators for absorption of this knowledge in their breeding schemes and covert this knowledge into wealth through dissemination of domestic buffalo of high genetic merit.

MOUSE EMBRYONIC STEM CELLS- A POWERFUL TOOL FOR UNDERSTANDING GENE FUNCTIONS

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Mouse embryonic stem cells are pluripotent cells from inner cell mass of pre-implantation mouse embryos. Introduction of targeted mutations in endogenous genes into ES cells followed by generation of genetically mutant animals and their phenotyping is a powerful tool to understand gene functions. A complimentary approach is *in vitro* differentiation of mutant ES cells into embryoid bodies and into various cell lineages and then analyse the resultant cellular phenotype without derivation of animal. The organization of embryoid bodies from ES cells mimics to a large extent differential gene expression and cell differentiation pattern observed during normal development of embryos. Therefore, *in vitro* phenotyping of mutant ES cells is a very attractive option particularly when a given mutation is lethal or for analysis of critical steps in development, which are otherwise difficult to dissect in whole animal system. Further, such a strategy provides a far quicker option to study development related molecular processes. Our laboratory is extensively using ES cell based functional genomics in ascribing functions to mouse genes. In this lecture, I shall present results from our on-going work Wdr13 and Argonaute genes.

GENETIC CHARACTERIZATION OF INDIGENOUS BREEDS OF EQUIDS – MOLECULAR TECHNIQUES

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India possesses a vast genetic resource of equines including horses, donkeys and wild ass. Six different breeds of horses (*Equus caballus*) namely Kathiawari, Marwari, Manipuri, Zanskari, Bhutia and Spiti have been identified on the basis of their geographically localization. These existing indigenous equine breeds possess certain favorable genetic traits namely sturdiness, endurance, swiftness, relatively disease resistance and their adaptability to different agro-climatic conditions prevailing in the country which could have been possible through the accumulation of a special combination of certain genes or gene groups required for these traits. However, owing to lack of sound breeding policies and planned development activities, the quality of indigenous breeds of horses is undergoing rapid deterioration and these breeds are under the threat of extinction. The unique features of these breeds are required to be maintained otherwise, possibly an irreparable loss may be caused if any of these traits is allowed or wiped off from the scene. At present, it is difficult to assess future requirement of this bio-diversified material, therefore it is essential to preserve enough genetic diversified with in each breed for exploitation at hand as well as for future. Beside this, there is also an urgent need to evaluate the genetic make up of these indigenous breeds at molecular levels (blood protein and nucleic acid) and proper documentation of the scientific information in animal genetic resources data bank under the international pressure on property right and animal genetic resources of any country.

Different horse breeds are generally identified and characterized on the basis of their phenotypic traits, their pedigree and performance. However in the recent past, genetic testing has also been made essential for validation of horse pedigrees for breed registry authorities, sales companies and racetracks (Bowling, 1996). In USA alone over more than 40 horse registries have some form of genetic testing requirement. Of utmost importance to breed registries and owners are the accuracy of results, the effectiveness of the tests to detect the incorrect parentage and cost of the test etc. In genetic analysis, various types of genetic markers such as morphological, chromosomal, biochemical and molecular markers are used. Morphological (pigmentation or other related features) and chromosomal markers (numerical or structural differences) usually show low degree of polymorphism and hence are not useful. At molecular level, blood group testing, protein polymorphism and lymphocyte testing are in use as biochemical markers for the above characterization in horses as well as for other animals species (Aaltonen and Antila, 1987; Bowling and Williams, 1991; Sandberg, 1973; Scott, 1978; Stormont and Suzuki, 1964; Bowling and Clark, 1985; Bernoco *et al.*, 1987a,b; Ansari *et al.*, 1988). The efficacy of the standard test to detect an incorrectly identified sire or dam when the other parent is recorded accurately is about 97-99% depending upon breed and system used (Bowling, 1985). A standard test battery for horses typically consists of 15 systems of blood groups and proteins markers. Little work has so far been done on Indian breeds of horses.

However, biochemical markers (blood groups and allo-enzymes etc) have also been observed to be sex limited, age dependent and are significantly influenced by the environment. Beside this, these markers reflect variability in their coding sequences that constitute less than 10 % of the total genome (Hochgeschwender and Brennan, 1991). Further, because a great deal of genetic variations remained undetectable using protein markers (Ferris and Berg, 1987; Burke, 1989; Wayne and Jenks, 1991), DNA technologies seems more efficient for study of evolutionary, population and ecological genetics, genetic characterization, genetic distancing among different breeds of horses etc.

DNA based molecular markers, capable of detecting

the genetic variation at nucleic acid sequence levels, have not only solved these problems but also possess unique genetic properties that make them more useful than other genetic tool. More over these molecular markers are numerous and distributed ubiquitously throughout the genome. These follow a typical Mendelian inheritance which usually expresses in a co-dominant fashion and are often multi-allelic giving mean hetero-zygosity of more than 70%. They remain unaffected by the environmental factors and generally do not have phenotypic effect on quantitative traits loci. Such specific molecular marker based genetical analysis studies are not so simple as it is well known that in eukaryote the coding sequences – Gene constitute a minor part of the hereditary materials (~ 5-10% of the genome in mammals. The remaining DNA presents non-coding sequence for which a role has not been assigned as yet (Hochgeschwender and Brennan, 1991).

DNA techniques and markers that can be used for studying genetic distancing, breed characteristics, molecular variability among different breeds, parentage testing, linkages etc include multi-locus fingerprints (Minisatellite–Genetic profiling), restriction fragment length polymophism (RFLPs), biallelic systems (Single nucleotide polymorphism, SNPs), mitochondrial sequence polymorphism (mt-DNA), random amplification of polymorphic DNA (RAPD) and microsatellite, also known as short tandem repeats (STRs) or simple length repeats (SLRs), sequence analysis, mitochodrial DNA, Y chromosomes, autosomal DNA etc. Selection of the best system is very important for animal breeding programme. Till date, limited work has so far been carried out on genetic characterization of Indian breeds (Mamta *et al.*, 2004; Behl *et al.*, 2005; 2006, 2007a,b Bhupendrabh, 2005; Gupta *et al.*, 2005; 2007).

Some of the commonly used techniques in genetic characterization of horses in different parts of the world are as follows.

a) DNA Fingerprinting

Earlier this technique was used to assess the differences at DNA level among different breeds of horses for their characterization purpose. However at present, this technique is not considered good for breed characterization and parentage testing programs. It is good only as a solution to individual cases and can be used for gene mapping (Bernoco and Byrns, 1991; Georges *et al.*, 1988). For animal industry program, 1) this technique requires too much of DNA, 2) takes too much time to produce and analyze results, 3) too difficult to apply recorded results to other case etc.

b) Restriction fragment length polymorphism (RFLPs)

This technique was earlier observed to be quite useful as it covers whole genome and reflected natural variations present in DNA and results were reproducible. RFLP markers are codominant, so three possible genotypes for marker can be distinguished. However, this techniques also shares the same problems as finger printing for an animal industry application (i.e., requires too much DNA and processing time), although they too could be useful for individual cases and may have application for genetic mapping. (Harbitz *et al.*, 1990; Kay *et al.*, 1987; Rando *et al.*, 1986).

c) Mitochondrial polymorphism

This technique has the potential to be powerful tools for sorting out maternity but can not cover answer question about paternity. However, identifying mitochondrial sequence polymorphism is a complicated process, and may be too expensive to apply routinely to verify maternity in horses. Polymorphism in horse mitochondrial sequences exist and could be applied to the solution of special cases only. Certain bi-allelic systems are attractive, particularly in not requiring electrophoresis to detect the variants (Nikiforov *et al.*, 1994).

d) Randomly amplified polymorphic DNA (RAPD)

RAPD technique is a simple PCR based technique allowing geometric amplification of DNA templates in presence of single short primer of arbitrary sequence and lower annealing temperature than the average PCR. This technique has been observed to be more rapid and convenient than Finger printing and RFLP. In this knowledge of primer design is not necessary and can be used immediately to generate genetic markers from unchanged genomes with out going thorough rounds. RAPD markers have already be used for comparing different breeds (Bailey and Lear, 1994). Sometime it

is difficult to reproduce the results because short primers used in this amplification do not require full homology with the binding sites etc.

e) Amplified Fragment length polymorphism (AFLP)

Amplified fragment length polymorphism which is an intermediate between RFLP and RAPD, is attractive since a large number of variable genetic markers can be generated quickly from previously uncharacterized genomes, only small amount of template DNA is required, technically not demanding and the method is stringent and repeatable. In AFLP, template DNA is digested with two restriction enzymes and the resulting restriction fragments are then ligated with adapters and, subsequently, PCR amplification is carried out using specially designed primers which comprise a unique part corresponding to selective based and common part corresponding to the adapters and the RE site.

As AFLP is based upon oligonucleotide adopter – PCR primer combination which are designed by the researcher, the data derived from it is as reliable as RFLP (Karp, 1998). An important merit of AFLP is that it generates a large amount of information (e.g. amplified bands and loci) per individual per unit time from complete genome. Also different AFLP parameters can be altered such that the method can be adopted to measure various levels of genetic variability trait which readily lends this approach to DNA finger printing, genome mapping and phenotypic correlation.

f) Minisatellites

Large complex repeats, commonly called minisatellites are used mainly in finger printing procedures. The hypervariable nature of these minisatellites has been extensively exploited as a means of developing a powerful DNA fingerprinting technique to obtain individual specific restriction patterns. Many multi locus probes derived from various sources e.g. a sequence of M13 phage (Vassart *et al.*, 1987), R18.1 bovine genomic clones (Haberfeld and Hillel , 1991), pig repetitive clone pS3 (Coppieters *et al.*, 1990) have been used to detect hypervariable minisatellites in human and farm animal DNA.

g) Microsatellites

Microsatellites are a relatively new class of genetic markers. Run of simple nucleotide repeats called microsatellites or STR (short tandem repeats e.g., CACACACACA) are distinctly different from protein coding regions. Microsatellites are also examples of STS (short tagged sites) and VNTR (variable number tandem repeats). STR are proving to be effective tools for parentage studies and gene mapping which may lead to new selection tools to control genetic diseases and to improve performance traits. Microsatellites have also been used for assessing the genetic distancing, linkage mapping etc (Van Haeringen *et al.*, 1994; Sakagami *et al.*, 1995; Ewen and Matthews, 1995; Eggleston – Stott *et al.*, 1996; Swinburne *et al.*, 1997). First published horse microsatellite was HTG 6 (VHL 20) containing T and G as the repeat nucleotides. In horses, number of TG repeats at HTG 6 has varied from 4 to 26. DNA sequence flanking the repeat regions uniquely define HTG 6 somewhere in the horse genome.

Till date, most of the work is confined to phenotypic characterization of these indigenous breeds while limited work has so far been carried out on genetic characterization of these breeds (Mamta *et al.*, 2004; Behl *et al.*, 2005; 2006, 2007a,b Bhupendrabh, 2005; Gupta *et al.*, 2005; 2007). Gupta *et al.* (2005) has evaluated horses of Marwari breed using 26 different microsatellite pairs with 48 DNA samples from unrelated horses. The estimated mean (± s.e.) allelic diversity was 5.9 (± 2.24), with a total of 133 alleles. A high level of genetic variability within this breed was observed in terms of high values of mean (± s.e.) effective number of alleles (3.3 ± 1.27), observed heterozygosity (0.5306 ± 0.22), expected Levene's heterozygosity (0.6612 ± 0.15), expected Nei's heterozygosity (0.6535 ± 0.14), and polymorphism information content (0.6120 ± 0.03). Low values of Wright's fixation index, *F*IS (0.2433 ± 0.05) indicated low levels of inbreeding. This basic study indicated the existence of substantial genetic diversity in the Marwari horse population. No significant genotypic linkage disequilibrium was detected across the population, suggesting no evidence of linkage between loci. A normal 'L' shaped distribution of mode–shift test, non-significant heterozygote excess on the basis of different models, as revealed from Sign, Standardized differences and Wilcoxon sign rank tests as well as non-significant *M* ratio value suggested that there was no recent bottleneck in the existing Marwari breed population,

which is important information for equine breeders. This study also revealed that the Marwari breed can be differentiated from some other exotic breeds of horses on the basis of three microsatellite primers. Similarly Mamta *et al.*,(2004) has also characterized horse of Spiti breed. Beside this, different sets of microsatellite have also been used by Behl *et al.*, 2005; 2006; 2007a,b and Gupta *et al.* 2007) to characterize Marwari, Spiti, Manipuri and Zanaskari breeds of indigenous horse. These studies has indicated the existence of genetic distancing between different breeds, little bottlenecking among the animals of same breed etc.

Most of the present studies are able to reveal the genetic distancing between different breeds as the total genetic material representing variability in the coding sequence is only 3 to 5 percent of the total genome. These genetic markers are not able to clearly distinguish different breeds at genetic level as well as correlate them with their phenotypic characteristics as these are mainly based on the differences at the non-coding regions in the genome. These are revealing only the relatedness of animals of different breeds. However, work need to be continue on genetic evaluation for assessing the possibility of microsatellite markers if any, for clear-cut identification of different indigenous breeds as Marwari and Spiti breeds has already been differentiated from exotic ones.

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STEM CELLS IN LIVESTOCK- STATUS AND APPLICATIONS

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Evans and Kaufman 1981 and James Thomson 1998 developed first embryonic stem cell line in mice and human respectively. A stem cell is uncommitted cell and remains uncommitted unless it receives signal to develop into a specialized cell. Since human ES cells are constrained by legal and ethical restrictions, farm animals stem cells can be an efficient alternative to contributing in human health. This can be through nutrition, producing biomedicine, cell therapy and xenotransplantation. With decoding of the genome sequences in animals, stem cell promise to resolve many mysteries of the developmental biology. Other applications include animal model testing of pharmaceutical research, shortage of human cells, tissues, organs and as source of therapeutic proteins.

What is special in stem cells ?

The apparently unlimited replication potential of ES cells may be related to the extremely low level of DNA methylation, as well as the presence of telomerase, which prevents shortening of telomere involved in cell apoptosis. As a result, these pluripotent cells can be expanded seemingly indefinitely in culture while maintaining a normal karyotype. The expression of a transcription factor, Oct-4 shown to be required for the maintenance of stem cells in pluripotent state has been found to exhibit similar spectrum of action.

Source of stem cells

Depending on the source stem cells these can be

- Embryonic stem cells: from the inner cell mass of blastocysts
- Embryonic germ cells: from genital ridge
- Adult stem cells: from many tissues

Feeder layers The work on embryonic stem cells involves preparation of feeder cells for culture of embryos or ICM from embryos to obtain stem cells. Fibroblast treated with mitomycin is used as feeder, super ovulation or in vitro production of embryos, ICM isolation, its culture and long term proliferation and freezing of cells are involved in stem cell work.

Blastocyst: For ES cells blastocyst are required for initiation. These can be produced *in vitro* or *in vivo* from super ovulated animals.

ICM Isolation: Tophectodermal cells have been removed by enzyme digestion, laser, cutting with sharp razor blade or immunosurgery. ES cell lines available in human and mice are from ICM isolated using immuno-surgery.

Enzyme treatment: Zona free blastocyst is put in the trypsin solution for several minutes and observed under microscope till the outer trophectoderm cells became loose and shed from the ICMS and darker ICM cells remained intact. Longer trypsin treatment could disburse the ICM cells. The ICMs are removed from the trypsin and washed with PBS before putting them on the feeder cells for growth.

Immunosurgery: This technique was first described by Solter and Knowles (1975) for selective killing the outer trophectodermal cells of blastocyst. The principle of technique is first allow binding of trophectodermal cells with antibodies specific to them and than lyse these cells with complement. This technique involves multiple steps for selective lysis of outer trophectoderm from zona free blastocyst. Zona free blastocyst are washed in PBS and treated with goat anti-bovine antibodies washed with PBS and placed in guinea pig complement The ICMs isolated are further kept in PBS before culturing them on feeder layers. Complement time for different species needs to be standardized. Stage of blastocyst also effects lysis time.

Culture of ICMs:ICMs isolated by enzyme digestion or immuno-surgery are placed on feeder layer prepared one day advance. ICMs are given time to make colonies. After sufficient growth of primary colonies

these are trypsinised and passaged for propagation. Cells are cryopreserved at different passages for multiplication and characterization studies.

Characteristics of ES cell lines: Several criteria needs to be satisfied before stem cell line qualify for bonafide ES cell line

ES cell should meet the following criteria:

- Maintenance of classical colony and individual cell morphological characteristics
- Molecular marker including Oct-4, Nanog, Sox, SSEAs gene expression
- Teratoma formation (tissue from all three germ layers)
- Prolonged passaging
- Maintenance of normal karyotype
- Clonality
- Telomerase expression
- Alkaline phosphitase expression

Few characterization techniques are briefly discussed.

Morphology: Human ES grow in tightly compacted colonies of undifferentiated cells. These cells have bigger nucleus than normal cells in relation to nuclear cytoplasm ratio. Morphology of cells differs in different species. Adult stem cells also differ in morphology depending on the tissue of origin.

Alkaline Phosphatase Staining: Expression of AP activity can be analyzed in blastocyst, ICMs and embryo derived cells or adult stem cells. The culture medium is removed and cells are washed twice with PBS before fixing them in 3.7% para formaldehyde at 37°C for 30 minutes. After washing with again with PBS, AP staining solution for one hour. AP positive stained cells appeared red while and negative cells were colorless or brown.

Molecular marker

In gene transcriptional programme there are certain genes which are related to pluripotency. Same genes are not transcribed in all species yet there are some common genes related to pluripotency. There function aspects still need more studies. The comparative expression of markers in human, mouse and bovine and buffalo stem cells is given in table.

Table: Comparison of markers in human mouse and bovines and buffaloes

Marker name	Mouse ES/ Embryo	Human ES Embryo	Bovine ES/Embryo	BuffaloES/Embryo
Alkaline Phosphatase	+	+	+ Partially	
SSEA-1	+	-		
SSEA-3	-	+		
SSEA-4	-	+		
TRA-1-60	-	+		
TRA-1-81	-	+		
OCT3/4	+	+	+	+
SOX2	+	+		
REX1	+	+		
TERT	+	+		
FGF4	+	+		
LIFR	+	-		
STAT3	+	+		
Nanog	+	+	+	+

Status of Animal ES Cells

The transcription factor Oct-4 is essential for the germ cell lineage in the mouse where it is required at critical levels for embryonic stem cell renewal (Niwa et al 2000). In mouse and human, Oct 4 is restricted to the pluripotent cell population, both in vivo and in vitro. The bovine Oct-4 shares high sequence homology with its mouse ortholog, and the organization of Oct-4 is similar to its human and murine orthologs with which it shares 90.6% and 81.7 % overall identity at protein level, respectively. However, in bovine cell culture, the usefulness of Oct-4 was questioned due to an Oct-4 pseudogene (van Eijk et al 1999). In study by Yadav et al 2005, the Oct-4 expression was detected by RT-PCR with completely matching primers. Direct sequencing of the PCR product allowed to discriminate be-

tween the Oct-4 ortholog and pseudogene, as they differ by two nucleotide exchanges at positions 754 and 1245. Another report also showed that in contrast to the protein distribution, the Oct-4 mRNA pattern is restricted to ICM cells in bovine blastocysts (Kurosaka et al., 2004), and paralles the situation in the mouse. This made Oct-4 transcription a suitable marker for pluripotent cells in cattle.

AP activity is a marker of pluripotency in mouse and human (Mulnard and Huygens 1978). AP activity has also been used to identify pluripotent cells in cultures from livestock species (Talbot 1993, Li et al, 2003). Culture of murine and human stem cells usually requires the presence of feeder cells (Evans and Kaufmann, 1981; Thompson et al 1998). Feeder cells are reported to produce growth factors and other substances that support proliferation and prevent the differentiation of pluripotent cultured cells (Anderson, 1992; Dang et al, 1995). Livestock ES cells are commonly cultured on feeder cells in most laboratories because the molecular pathways and key molecules required to maintain pluripotency in these species are unknown (Wolf et al, 2004). Successful use of BFF cells to culture bovine and murine ICM cells indicates that BFF cells could potentially be used as feeder cells for homologous as well as heterologous ES cells.

In contrast to the limited proliferation of bovine ICM derived cells, cells from murine blastocysts could be grown well for more than 200 days over 60 passages on the same feeder layers and identical culture conditions. Alkaline phosphatase activity in these cells was negative but the cells expressed Oct-4 in the 41th and 46st passage. The cells formed embryoid bodies in suspension culture and differentiated when cultured without feeder cells. Human ES cells grown in suspension culture in the absence of feeder cells or basic fibroblast growth factor also form embryoid bodies (Desbaillets et al 2000) These results show that bovine embryonic feeder cells are suitable for murine embryonic stem cell isolation.

Adult Animal Stem Cells

In contrast to mouse and human embryonic stem (ES) cell lines, no true pluripotent ES cells have been established from livestock. Resistance by livestock species to ES could be due to species limitations, as uncovered by attempts to make livestock embryonic cells behave as do the embryonic cells in rodents. Murine ES cells are thought to arise from egg cylinder stage embryos, a stage which the domestic livestock embryos do not have. ES cells from domestic species may have different characteristics since epiblast morphology differ between rodent and non-rodent species (Anderson 1992). Development of the blastocyst and yolk sac is different in ruminants in comparison to mouse and human (Talbot et al. 2000).

Further, the cells grown from bovine and porcine ICMs fail to grow after certain time in culture with the loss of totipotency. In order to facilitate stem cell biotechnology, it would be advantageous to have readily available low-cost source of stem cells in large animal species, so that the ethical and other problems associated with embryonic stem cells procedures are overcome. In this regard, the possibility of utilizing adult stem cells appears promising. Adult stem cells, like all stem cells, share at least two characteristics. First, they can make identical copies of themselves for a long period of time i.e. long-term self-renewal. Second, they give rise to mature cell types with characteristic morphologies and specialized functions.

Adult stem cell sources:

Isolation of stem cells, having restricted differentiation potential, from postnatal tissues has been successfully attempted from bone marrow (Kucia 2005), umbilical cord (Carlin et al, 2006, Yang et al, 2004), placental tissue (Yen et al 2005), fetal somatic explants (Kues et al, 2005), amniotic fluid (Tsai et al, 2004), adipose tissue (Zuk et al, 2001) buffalo amnion, amniotic fluid, umbilical cord matrix and fetal explant (Yadav PS et al 2008 and Yadav RP et al 2008.) Amnio and skin (Toma et al, 2001). Some of the sources having good culture feasibility and better plasticity are discussed below.

Fetal stem cells: Primary cultures shall be prepared by somatic explants cultures of somatic tissue from buffalo fetuses of post-coitus around day 25. Cell cultures from adult buffaloes shall be isolated from subdermal tissue of ear clips. Small pieces of the tissue shall be placed into buffalo plasma droplets with 80mM CaCl2 for coagulation and than DMEM medium containing 10 percent serum. For High serum cultures 30 Percent serum shall be supplemented in standard medium.

Umblical cord matrix cells: The umbilical cord (UC) shall be collected from new born calves of the institute buffalo's immediately after birth and placed in sterile solution. Umblical cord segments 1-3 cm in length shall be cut longitudinally to expose the two umbilical arteries and umbilical vein. The remaining UC tissue including the Whartons Jelly shall be dicedinto 2-5 MM^3 explants and transferred to 6 well tissue culture plates. The culture medium shall be DMEM FBS and growth factors and antibiotics. After 24-48 h the cells shall be trypsinsed and maintined in cultures and periodically sub-cultured.

Amniotic fluid derived stem cells: Cells shall be isolated from the buffalo amniotic fluid (BAF)and shall be used for cultures with in six hours of collection. The BAF shall be centrifuged at 300g and resulting pellets shall be washed twice with DMEM to remove blood and cell debris. Cells isolated shall be plated in $25cm^2$ culture flasks having DMEM and 10 percent serum. After attaining confluence the cells shall be trypsinised and released cells shall be replaced.

Amnion cells:

Amnion is separated from chorin by peeling and further trypsinization (0.25% trypsin-EDTA solution) is performed for 20 minutes. Coarse particles were allowed to settle down for 5- minutes, supernatant was collected into fresh centrifuge tube and centrifuged to obtain cell pellet by centrifugation at 450g for 10 minutes. Yadav et al 2008 have first time reported that these cells exhibit a fibroblast-like phenotype in colony, with the cells positive for alkaline phosphatase on staining, expression of Nanog a marker for pluripotenty.

Plasticity :

Adult stem cells with high degree of plasticity were identified among hematopoitic and mesenchymal cell population from bone marrow and central nervous systtem (Weismann 2000,; Jiang et al. 2002). It has also been demonstrated (Kues et al. 2005) that murine and porcine fetal somatic explants contain a subpopulation of somatic stem cells (FSSCs), which can be induced to display features of lineage uncommited cells. In vitro, these FSSCs exhibit characterisitics of ES cells, including expression of stem cells marker genes Oct4, Stat3 and Akp2, and growth of multicellular aggregates. More recently, it has been shown (Tsai et al. 2006, Kim et al. 2007) that ameniotic fluid derived stem cells express NANOG and POU5F1 Family Oct4. Presence of stem cells in amniotic fluid has also been reported by (Trounson 2007,). Cells isolated from porcine umblical cord (PUC) express three stem cell marker transcription factors found in pluripotent stem cell both at mRNA and protein level. The presence of these transcription factors and alkaline phosphatase expression suggest that PUC cells have properties of primitive stem cells (Carlin et al. 2006).

India is also doing well in livestock stem cell research, work has been reported on isolation of ICM from in vitro produced buffalo embryos by mechanical methods (Chauhan et al. 2005, Verma et al. 2007). Among the various intracellular expression-based markers for the charaterization of ES cells, Oct-4 expression has been studied in buffalo (Verma et al. 2006,). Isolation of stem cells from early stage buffalo embryos has emanated (Mahere et al, 2005). Adult stem cells from amnion, amniotic fluid and umbilical cord matrix from buffaloes. (Yadav PS et al 2008 and Yadav RP et al 2008)

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CLONING AND EXPRESSION OF IMMUNOGENIC S1 GENE OF INFECTIOUS BRONCHITIS VIRUS ISOLATED FROM FIELD OUTBREAK

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Infectious bronchitis (IB) is an acute and contagious disease of poultry. The spike glycoprotein (S) of IBV is a dimmer and is cleaved into two glycopolypeptides, S1 and S2 post-translationally. S1 gene defines the serotype and plays a major role in induction of protective immunity. Eukaryotic expression systems are frequently employed for the production of recombinant S1 proteins as it is highly glycosylated protein. In present study the S1 gene was amplified from suspected field sample. The gene was amplified with the help of S1 gene specific primers. The PCR amplified 1.5kb (1448 bp) S1 gene was cloned into T/A cloning vector. Subsequently the clone was digested with *Sac*-I and *Hind*- III restriction enzymes to produce compatible ends to ligate into the eukaryotic expression vector pQE-TriSystem. The pQE-TriSystem-S1 gene clone was then transfected into the Vero cell line. The successful expression was confirmed at 24 and 48 hrs post transfection by Reverse Transcriptase-PCR. These promising observations emphasize the need of expression of S1 gene recombinant protein for the development of recombinant DNA vaccine against IB in near future.

GENE SILENCING AND ITS IMPACT ON LIVESTOCKS

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Gene silencing, "switching off" a gene, is the interruption or suppression of a gene at transcription [transcriptional gene silencing (TGS)] or translational levels [post-transcriptional gene silencing (PTGS)]. TGS is the result of histone modifications & PTGS is of mRNA of a particular gene being destroyed. Micro RNA (miRNA), small interfering RNA (siRNA) and dicer are the main components of gene silencing. The guide strand of miRNA integrates with RNA-induced silencing complex (RISC) and inhibits translation of mRNA. siRNAs play key roles in RNAi process and having complementary nucleotide sequences to the targeted RNA strand. Dicer, ribonuclease, catalyses the first step in the RNAi pathway and initiates formation of RISC. Mechanism of gene silencing is to protect the organism's genome from transposons and viruses. The RNAi studies in livestock species are in early stages. In bovine cells, the direct injection of dsRNA results in the transient ablation of gene expression. Novel bovine RNA polymerase III promoters continue to be developed and evaluated for use in bovine-specific RNAi research. A point mutation within the 3' UTR of myostatin creates a target site for at least 2 endogenous miRNA that are highly expressed in the skeletal muscle of Texel sheep. Vaccination against the porcine reproductive and respiratory virus replication machinery in cells can be done by gene silencing. The rapid development of antisense technology offers almost unlimited scope for new & high specific therapeutics. It also permits loss of function genetic screens & rapid test for genetic interaction in mammalian cells. More studies and research works should be done for the benefit of livestock as well as for human beings.

MARKER ASSISTED SELECTION IN FARM ANIMAL

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A marker can be defined as any stable and inherited variation(s) detectable or measurable by a suitable method and which can be used to detect the presence of a specific genotype or phenotype other than itself. When genetic marker data are included in the selection criteria, this process being referred to as marker-assisted selection (MAS). MAS can be applied to support existing conventional breeding programs. It can be used in routine genotyping, parentage testing/pedigree verification. In a breeding scheme, use of phenotypic and marker data could provide more information than phenotypes alone. Marker data can be collected early in life. Molecular marker maps, the necessary framework for any MAS

programme, have been constructed for the majority of agriculturally important species. Currently, MAS does not play a major role in genetic improvement programmes in any of the agricultural sectors. The enthusiasm and optimism concerning the potential contributions that MAS offers for genetic improvement still remains. However, they seem to be tempered by the realization that it may take longer than originally thought and that genetic improvement of quantitative traits using MAS may be more difficult than previously considered.

GENOTYPING BY BIOFILM-ASSOCIATED PROTEIN (BAP) PCR, OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM CASES OF BOVINE MASTITIS

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Staphylococcus aureus is a common cause of intramammary infections, which become chronic associated with the ability of the bacteria to produce biofilm. The implication of biofilms in chronic infections has triggered an increasing interest in the characterization of genes involved in biofilm formation. Recently, Bap (for biofilm associated protein), was implicated in bovine *S.aureus* biofilm formation. The purpose of this study was to investigate the potentials of *S.aureus* isolates from bovine mastitis cases to produce biofilm, through amplification of Bap gene. The amplification reactions were carried out using a programmable thermal cycler. A PCR programme of initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 20 seconds, annealing at 42°C for 20 seconds and extension at 72°C for 50 seconds gave good and consistent results. Findings revealed that out of 25 *S. aureus* isolates, 9 showed amplification, where as 16 did not. This study showed the presence of an important genetic loci involved in biofilm formation process, Bap, in *S.aureus* isolates. Further, the findings of this investigation emphacised the need to either find antimicrobials that are efficient against biofilm bacteria or develop biofilm based *S.aureus* vaccine using Bap harboring isolates as vaccinal candidates.

SOX-2 GENE EXPRESSION PATTERN IN STEM CELLS DERIVED FROM DIFFERENT STAGES OF BUFFALO (*BUBALUS BUBALIS*) EMBRYOS**

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** Part of the Ph.D. thesis submitted by the first author to Tamilnadu Veterinary and Animal Sciences University, Chennai.

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The present study was undertaken to compare the *Sox*-2 gene expression in stem cells derived from various stages of *in vitro* produced buffalo embryos. Primers were designed based on the *Sox*-2 sequence (NCBI Ac. No: DQ487021.1) of chinese swamp buffalo available in Pub med GenBank by web based primer3 designing programme to obtain a product of 413bp. For zonalysis and subsequent isolation of ES cells 0.5 per cent pronase was used. RT-PCR was performed by using reverse specific primer (3' primer) for the first strand synthesis, utilizing RNA equivalent of ten to fifteen oocytes (approx. 200 ng) per reaction. The DNA sequence of the RT-PCR product submitted to NCBI Pub med GenBank was given Accession number: EU661361. Strong *Sox*-2 expression was observed in the inner cells obtained from 16-cell stage embryo, morulae and inner cell mass of blastocyst. Out of six trials, in two trials the blastomeres/ inner cells of 2-cell, 4-cell and 8-cell stage embryos did not express *Sox*-2 gene even though they were believed to be totipotent. But in four trials a faint band was observed. The *Sox*-2 gene expression pattern was low and variable in stem cells derived from early embryos but gradually became more regular, with 100 per cent expressing *Sox*-2 from 16-cell stage onward. This might be related to the exhaustion of maternally generated *Sox*-2 transcripts and then its recovery via expression of zygotic

transcripts, which takes place in buffalo embryos at the 8-16 cell stage. Epigenetic mechanisms might be the cause of the low levels of *Sox*-2 gene expression after fertilization. Based on the results it was believed that *Sox*-2 was co-expressed with *Oct*-4 in the ES cells and act synergistically with *Oct*-4 to activate *Oct–Sox* enhancers, which regulate the expression of pluripotent stem cell-specific genes, including *Nanog*, *Oct*-4 and *Sox*-2 it self. RT-PCR product was purified and sequenced. The sequence result (NCBI Ac. No: EU661361) was compared with the same gene of the other species by BLAST (Basic local alignment search tool) and was found that *Sox*-2 had 93% - 99% homology across the phylogeny with rat and mouse being distant relatives. Specifically, it had 99 per cent homology with Chinese swamp buffalo and bovine *Sox*-2 gene, 98 per cent homology with sheep *Sox*-2 gene, 97 per cent homology with dog *Sox*-2 gene, 96 per cent homology with pig *Sox*-2 gene and 95 per cent homology with human *Sox*-2 gene, 93 per cent with rat and mouse *Sox*-2 gene.

ISOLATION, CHARACTERIZATION AND MOLECULAR CLONING OF OUTER MEMBRANE PROTEIN (OMP-40) OF *SALMONELLA ENTRICA* SEROVAR GALLINARUM

Aman Kumar and Mumtesh Kumar Saxena

The present study was conducted to characterize and clone the OMP 40 gene of *Salmonella* Gallinarum .On the basis of band intensity of SDS-PAGE ,Omp-40 was found to be one of the major Omp of S. Gallinarum. Presence of three precipitation lines against Omp preparation in immunodiffusion test revealed at least three immunogenic Omps in S. Gallinarum. Further, western blot was performed using crude protein, purified Omp and hyperimmune serum in which , Omp-40 found to be the most immunopotent. Primers were designed using published sequence of Omp-40 of S. Typhi and full length Omp-40 gene of S. Gallinarum was amplified. The amplified product was cloned in pJET cloning vector using DH5á as a host. Cloned product was screened by i) growth on ampicillin incorporated LB agar, ii) Omp-40 specific PCR and iii) restriction endonuclease digestion of recombinant plasmid. Positive clones were sequenced; this reveals the size of Omp-40 as 1.4 kb. Analysis of sequence using DNASTAR software showed 98% homology of Omp-40 with other serovars like S. Typhi, S. Typhimurium etc. Above analysis conclude that Omp-40 may be the conserve protein in *Salmonella* serovars and useful in development of recombinant subunit vaccine against Fowl Typhoid.

POLYMORPHISM STUDY OF BUFFALO KAPPA CASEIN EXON-4

Jaspreet Singh Arora, Sachinandan De, Shamik Polley, Vinesh.P.V, Paras Yadav, Parveen Kumar, T.K. Datta, S.L. Goswami.

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The casein fractions of milk are alpha s1, alpha s2, beta and kappa. These casein genes are linked in a 250-kb cluster on chromosome 6 in cattle and sheep, chromosome 4 in goat and chromosome 7 in Buffalo. Kappa (ê) casein in milk determines the size and specific function of milk micelles. Eleven ê-CSN variants have been reported within the genus *Bos*. The aim of the present study was to know genetic variation in ê-casein exon-4 among five buffalo breeds i.e, Murrah, Nili Ravi, Nagpuri, Surti and Mehsana. Out of these five breeds, Murrah and Nili Ravi were analyzed so far. The ê-casein complete exon-4 was amplified by PCR using designed oligonucleotide primers. PCR-SSCP (Polymerase Chain Reaction-Single Strand Conformation Polymorphism) of 500bp fragment of ê-casein exon-4 was carried out using 12% native- PAGE for screening of different SSCP band patterns. Three patterns (A, B and C) of single strand DNA bands were found and these patterns were sequenced and compared with available coding DNA sequences from GenBank. On the basis of chromatogram data A and B pattern reveals heterozygous genotype whereas C pattern is homozygous. Both A and B patterns shows change of nucleotide at codon 404(ACCATC) which results in change of amino acid from ThrIle and at codon 408(ACCACT) which is a silent mutation, hence amino acid being the same i.e., Threonine. Pattern A was found to be 29%, B 6% and C 62% amongst the animals screened.

SESSION – IV
V A C C I N O L O G Y

Chairman, Dr. Satish Kumar
Co-chairman, Dr. Prem Singh Yadav
Rapporteur, Dr. N.K. Kakker

Date
27 February, 2009, 2.00 - 3.30 PM

Venue
SEMINAR ROOM
Department of Veterinary Animal Husbandry Extention

EVALUATION OF HUMORAL IMMUNE RESPONSE TO SHEEPPOX VACCINE

Amitha R. Gomes, Raveendra Hegde, S.M.Byregowda, Vijayashree Varadarajan, Yeshwant S. L., Giridhar, P and C. Renukaprasad

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Sheep pox is a malignant pox disease of small ruminants characterised by fever and generalized pock lesions. High mortality rates occur in young animals resulting in significant economic losses. Sheep pox is caused by sheeppox virus, a member of the *Capripoxvirus* genus in the *Poxviridae* family. Immune response to sheep pox involves both humoral and cell mediated factors. In the present study, an attempt was made to evaluate the humoral immune response in sheep vaccinated with live attenuated sheep pox vaccine prepared using Rumanian Fanar strain. Twenty five animals were vaccinated with sheeppox vaccine with a recommended dose of 200 $TCID_{50}$/animal. Paired serum samples were collected from both vaccinated and control animals on twenty one day post vaccination. Serum samples were screened for neutralizing antibodies by serum neutralization test using suitable indicator system. Preimmunization serum samples collected from these animals did not reveal any serum neutralizing antibodies. Post vaccinal serum samples from control animals also did not reveal any neutralizing antibodies. Where as the vaccinated animals demonstrated the presence of neutralizing antibodies. Average neutralization index in our study was $\log_{10}1.35$ which is a significant value. More than ninety per cent of the animals demonstrated a neutralization index of more than 1. Thus it was concluded that the serum neutralizing antibodies do play a significant role in the immunity against sheeppox.

THERMOSTABILITY OF LIVE ATTENUATED PESTE DES PETITS RUMINANTS VACCINE

Raveendra Hegde, Amitha R. Gomes, S. M. Byregowda, P. Giridhar and C. Renukaprasad

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Live attenuated veterinary and human vaccines can and do suffer serious deterioration in vaccination campaigns in tropical and subtropical environments. The cause in most cases is due to difficulty in maintaining the cold chain, which inevitably results in loss of potency. In the present study an attempt was made to study the stability of the Ani-PPRh, a live attenuated homologous PPR vaccine produced at Institute of Animal Health and Veterinary Biologiclas, Hebbal, Bangalore. The residual infectivity was determined using vero cell system after exposure at four different temperatures for varying time intervals. The present study was done to evaluate the efficacy of vaccine during storage under fluctuations of temperature under field conditions as the vaccine virus was very susceptible to higher temperatures. It was found that the vaccine was highly stable at low temperatures especially at -20^0 C. The vaccine also retained its stability at 4^0 C for few months, where as rapid deterioration of the titer was observed at higher temperatures of 25 and 37^0 C. Thus higher temperature was found to be highly deleterious to the vaccine. It was recommended to store the vaccine at lower temperatures especially below 4^0 C for effective use of the vaccine.

ATION OF *CLASSICAL SWINE FEVER VIRUS*, MOLECULAR CHARACTERIZATION AND DEVELOPMENT OF A CELL CULTURE BASED INACTIVATED CLASSICAL SWINE FEVER VACCINE

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Present study describes development of a cell culture based vaccine for classical swine fever virus. To initiate, we investigated first few outbreaks of classical swine fever in southern states of India and the virus was isolated in porcine kidney cells (PK-15 cell line) from samples collected from outbreaks in Kerala state. The virus was confirmed by Reverse Transcription Polymerase chain reaction (RT-PCR) targeting the 5'UTR and N pro regions of classical swine fever virus and restriction endonulcease analysis of the amplified genome using unique *Pst 1* enzyme. An inactivated cell culture based classical swine fever vaccine was prepared using this virus. The vaccine efficacy was carried out in Rabbits with different doses. Blocking (Competitive) ELISA was employed on serum samples for relative quantification of serum antibodies. The vaccine was found effective when used with a booster dose two weeks after first vaccination. The details of vaccine preparation protocols, vaccination trials and results obtained during the study will be elaborated and discussed during presentations.

EVALUATION OF PESTE DES PETITS RUMINANTS VACCINE UNDER FIELD CONDITIONS

A. Thangavelu[1], G. Dhinakar Raj[2], K. Saravanabava[3] and K.S. Palaniswami[4]

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Live attenuated vero cell adapted peste des petits ruminants (PPR) vaccine developed using sheep origin PPR AR 87 virus at TANUVAS was subjected to large scale field trial. Around 13 lakh doses of the vaccine were tested under field conditions in Tamilnadu, Andhra Pradesh, Karnataka and West Bengal. There was no untoward post vaccine reaction confirming the safety of the vaccine. Sera samples collected before and 21 days after vaccination were tested by neutralization test in vero cells. The post vaccination titres ranged from $2^{3.3}$ to $2^{7.3}$ indicating adequate seroconversion. A portion of the samples (230) were also tested using IVRI c ELISA kit. Ninty one percent of the samples were found to be positive.

PROTECTIVE IMMUNE RESPONSES AGAINST PPR AND GOATPOX VIRUSES CHALLENGE IN HILL GOATS VACCINATED WITH COMBINED VACCINE CONTAINING THE THERMO RESISTANT PPR AND HIGHLY ATTENUATED GOATPOX VIRUSES

V. Balamurugan*, V. Bhanuprakash*, A Sen, M. Hosamani, G. Venkatesan, and R.K. Singh

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* Equal contribution

Peste des petitis ruminants (PPR) and goatpox are OIE notifiable diseases of small ruminants especially goat and sheep. Both the diseases are economically important, in enzootic countries like India and cause significant loss and are major constraints in the productivity of animals. Considering the geographical distribution of both PPR and goatpox infections and prevalence of mixed infection, in the present study, safety and potency of the experimental combined vaccine comprising attenuated strains of thermo-resistant (TR) PPR virus (PPRV Jhansi, P-50) grown at 40°C and highly attenuated goat poxvirus (GTPV Uttarakashi, P100) was evaluated in sub-Himalayan local hill goats. Goats immunized subcutaneously with 1 ml of vaccine consisting of either 10^3 $TCID_{50}$ or 10^5 $TCID_{50}$ of each of PPRV and GTPV were monitored for clinical and serological responses for a period of 3-4 weeks post-immunization (pi) and post-challenge (pc). Specific immune responses i.e., antibodies directed to both PPRV and GTPV could be demonstrated by PPR competitive ELISA kit and goat pox indirect ELISA, respectively following immunization. All the immunized animals resisted infections when challenged with virulent strains of either GTPV or PPRV or GTPV& PPRV on day 28 pi, while in contact control animals developed characteristic signs of respective disease. PPR viral antigen could be detected in the excretions (nasal, ocular and oral swab materials) of unvaccinated control animals after challenge but not from any of the immunized goats. Combined vaccine was found safe at dose as higher as 10^5 $TCID_{50}$ and induced protective immune response even at lower dose (10^2 $TCID_{50}$) in goats, which was evident from sero conversion as well as challenge studies. The study indicated that both the viruses are compatible and did not interfere with each other in eliciting immune response, paving the feasibility of use of this combined vaccine in combating both infections simultaneously.

TRIVALENT INACTIVATED BLUETONGUE VACCINE TRIAL IN SHEEP

Y.Krishna Jyothi, M.Srinivas, Y.N.Reddy, K.Dhanalakshmi, P.Panduranga Rao, B.Susmitha and B.J.R.Sarma

Department of Veterinary Microbiology, College of Veterinary Science,
Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad – 30

A trivalent Binary Ethylene Imine (BEI) inactivated Vero cell based bluetongue virus vaccine comprising three indigenous isolates of serotypes 2, 9 and 15 was developed. Virus was found to be completely inactivated when 3 mM BEI with addition of 0.04% formaldehyde at 26°C for 24 hours was used rather than BEI alone for inactivation. Vaccine was found to be sterile and safe as per OIE protocols. Immunogenecity of the trivalent vaccine was assessed in two groups of 6 sheep each (Group I & Group II) by inoculating 2ml of vaccine subcutaneously. Group III (control) consisting of 5 sheep received normal cell culture fluid and processed in similar manner. Each dose of vaccine consisted of 10^6 $TCID_{50}$ of each of the three serotypes. Group I received a booster dose at 21 days post inoculation (PI). Group specific humoral antibody response was detected as early as day 7 PI by cELISA while all were positive by day 10 PI. Neutralizing antibodies were detected in all vaccinated sheep by 10th day against BTV-2, 14th day against BTV-15 and 21st day, against BTV-9 post vaccination. Neutralizing titers ranged from 1:32 to 1:256. CMI response could be detected by Lymphocyte stimulation test first on 7th day after vaccination by MTT assay. Of the three serotypes, BTV-9 elicited a poor and late neutralizing antibody response. There was no significant effect of booster dose on immune response to BT vaccine.

SESSION – V
MOLECULAR FORENSICS & BIOSECURITY

Chairman, Dr. V. Ramaswami
Co-chairman, Dr. V. Purushottam
Rapporteur, Dr. S.R. Garg

Date
27 February, 2009, 3.45-5.00 PM

Venue
SEMINAR ROOM
Department of Veterinary Animal Husbandry Extention

RAPID AND SENSITIVE IDENTIFICATION OF ANIMAL SPECIES USING FEATHER AND HAIR FOLLICLES

Alisha, Harimohan, G. Prasad and Minakshi

Department of animal Biotechnology, CCS Haryana Agricultural University, Hisar

Species identification at many times becomes a challenge for forensic experts and is of great importance in settling disputes regarding the species of the samples presented to the investigators. It becomes more difficult especially when samples such as pieces of hide and/or few hair follicles are provided. A rapid and less cumbersome protocol for species identification utilizing the hair follicle is thus of relevance to the research workers. A quick single tube method for DNA extraction was developed to get PCR quality DNA from single feather follicle and hair follicles from horse, cattle and buffalo. The DNA extracted by using this single tube method was amplifiable by PCR using species specific primers. A 10% aliquot of the PCR reaction mixture after 30 cycles of amplification was analyzed by agarose gel electrophoresis in 1% gel containing 0.5ug/ml ethidium bromide. The amplicons of expected sizes were visualized under U.V. light in gel documentation system. The method was found rapid, inexpensive and suitable for small amount of samples. The use of single tube makes this method more convenient and suitable for handling large number of samples with reduced chances of cross contamination of samples. The method is free from use of hazardous organic chemicals such as phenol, chloroform and isopropanol that are required for conventional DNA extraction protocols. In conclusion the method is very rapid, sensitive, inexpensive environmental friendly and useful for molecular forensic studies.

SESSION – VI
ACADEMY INDUSTRY INTERACTION

Chairman, Dr. M.P. Yadav
Co-chairman, Dr. K.S. Palaniswamy
Rapporteur, Dr. J. Rawat

Date
27 February, 2009, 9.00 -11.00 AM

Venue
SEMINAR ROOM
Department of Animal Biotechnology

INSTITUTIONALISATION OF RESEARCH, DEVELOPMENT AND COMMERCIAL CAPABILITIES IN NANO-ANTIBODY TECHNOLOGIES AS TOOLS TO COMBAT BIOTERRORISM: A CASE FOR HISAR AS BIO-INDUSTRIAL HUB OF THE COUNTRY

Jagveer Rawat

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Indian media has frequent news-items of food-frauds, namely, mixing of urea in milk or making of ghee from animal fat or mixing of any toxic but mimicking substance in any of the food item. It clearly shows vulnerability of the FOOD SUPPLY CHAINS of various products, from 'FARM-to-FORK'. That is why campaign for 'SAFE FOOD or ORGANIC FOOD', caught national imagination so much so that issue of pesticides in Coca Cola led to establishment of a Joint Parliamentary Committee for its investigations. This media and public fear reaction was further compounded by Swami Ram Dev, who questioned not only its nutritious value but 'pressed fear button' that it 'rather harmed' human being. Considerable decline in its consumption was the result. Other scares were related to Bird Flu in the recent past which almost paralysed the poultry industry.

1. POLICY DEVELOPMENT ISSUE ON BIOTERRORISM ARE COMPLEX AND TRANSCEND FROM THE USUAL TECHNICAL BOUNDARIES AT IMPLEMENTATION LEVEL

A considerable upsurge in 'mall-retail format' entering into 'food supply chain' in India is an accepted reality in the recent years but the 'farm-level and factory level production' has not been stringently enforced. 'Point-of-origin and labelling requirements' were resisted in the 'Food Act' by 'Captains of Indian Food Inc.' which probably would have serious implications not only for export market but for Indian domestic market. As this loophole remains, 'investigation and enforcement agencies' mandated for protection of 'food chains', might not have total sanctity and teeth to investigate origin of many kind of 'threats' (biological, chemical, contaminants, pollutants). Point of origin of a threat is a requirement for any 'criminal investigation agency's work'. Surprisingly this is also a very important work for any epidemiological investigation. Tackling with 'pandemics' could entail almost similar 'techno-administrative-managerial' responses as a 'biothreat' might have! There are many more similar threats in a typical district in India. For example, in some cities there are severe 'occupational hazards' and same may be the case for some 'villages'. Such hazards as usage of heavy dosage of chemicals for spray in 'districts growing cotton' as well as usage of 'heavy dosage of pesticides in vegetables or using sewage water with heavy metal toxicity' etc, could be more common in some districts than others. Biological and Chemical threats and hazards therefore have lot of similarity as far as there 'tracking' and then, their 'elimination' is considered. However, 'internal security' (India) or 'homeland security' (the US) systems have wide variation from country to country. Techno-business-policy variation amongst the countries are glaring. Systems of 'internal security or homeland security or home guard' etc, are guided by the 'socio-economic-political contexts'. In India for example the policy of 'internal security' from 'biological and other kind of terror events' might involve different elements than the US. For all such events there has been a debate on 'operational efficiency' of 'prevention' of such events. Recurrence of terror-strikes at heightened frequency have led the Union and State government to the demand of more 'stringent act' on one hand, on the other, some quarters regard that an agency like 'Federal Bureau of Investigation' would be more effective.

Whether there is a more stringent act and also if there is an agency of the US 'FBI' in India, there is a very important need to 'understand and study' the 'whole

system' of 'internal/homeland security' and linking it with 'disaster management'. In India and may be elsewhere in the world, there is an urgent need to create 'simplest citizen interface agencies' which could be properly linked with 'highly professional and competent organisation' at the level of district. I see lots of commonality in dealing with matters of 'food hazards, pollution, environmental health, food security (more than 90% of the food produced in India could not be processed and preserved leading to serious problem of food security), food adulteration, occupational hazards, epidemics, terror (bio and chemical included)'. It is important to understand that these 'problems' might irregular and unpredictable pattern and usual administrative departments might have to remain busy in gearing up their own machinery of 'prevention' only for the 'expected ones'.

1.1. **Looking for inspiration?**

At best, policy of preventive mindset is in place in India, however, in the US this has been a policy shift from 'prevention' to 'preparedness' as reported by Andrew Lakoff .In the wake of new disease threats—such as SARS, avian flu, mad cow disease, and drug-resistant strains of malaria and tuberculosis—led for new approaches to "securing health" against these threats. Such proposals did come not only from public health and medicine but also from such fields as emergency management, national security, and global humanitarianism, showing that 'homeland security' has become a complex and rapidly transforming terrain. The editors of the book released in October, 2008 ['Biosecurity Interventions: Global Health and Security in Question' (Edited by Andrew Lakoff and Stephen J. Collier)] focus on how experts, public officials, and health practitioners work to define what it means to "secure health" through concrete practices such as global humanitarian logistics, pandemic preparedness measures, vaccination campaigns, and attempts to regulate potentially dangerous new biotechnologies. Additionally, cultural dimension of the human organization of shifting entire response from prevention to preparedness, has been very well studied by various authors. Lakoff describes two particularly important implications of this discursive shift from "prevention" to "preparedness." Rather than statistically calculating probable risk based on historical evidence of disease, experts use the scenario-based methods of "imaginative enactment" to construct plausible singular events and potential disasters. Meanwhile, the ongoing living conditions of large populations — poverty, limited access to health care, inadequate housing – fall increasingly farther outside the purview of a biopreparedness system in which prevention is no longer the main focus.

1.2. **Ground realities**:

'Biopreparedness', therefore, is a matter of policy-debate and this article intends to grapple with understanding the organization of 'response system' of 'biological and chemical hazards, threats, epidemics in relation to a broader question of preparedness within the usual administrative structures and machinery of a typical implementation interface', which may be a district. There is a further presupposition to understand that the nature and approach of the machinery to any 'natural calamity such as cyclones and find what are the best practices in the disaster management'. Broadly it would be interesting whether there at all is a focus currently on 'prevention' as a 'broader policy objective' of 'internal security and disaster management administrative system' or not. If it is there, question have to be raised on whether a 'shift' from 'prevention' to 'preparedness' should be a 'policy option'. I would rather find it as an opportunity for 'overhauling' system of 'internal security and disaster management' at an effective administrative unit of an Indian district, wherein 'biopreparedness' should become 'watchword'.

The key 'constituents' of such a design would be derived from existing administrative structures of the government. Such structures as the 'District Biotechnology Committee' which is supposed to oversee and act as District Level Committee for Monitoring Genetically Engineered Products Usage reflects the obligation of government of the protection of the general public health system from the possible hazards of introduction of 'genetically modified food/ fiber crops'. However, this Committee System could be restructured to a high-powered DISTRICT ECONOMICAL AND ECOLGICAL COUNCIL (DEEC) wherein the issues of food chain safety should include not only GM food but also usage of any other contaminants, including purposeful introduction of any of the biothreats in food chain to be dealt under various

PFActs as well as preventative and/or preparedness team for various kind of biological and chemical threats'. This type of Committee structure has already been recommended by Administrative Reforms Commission of Haryana headed by Karn Singh Dalal, Member of Legislative Assembly of the State. Such kind of recommendations could be the template for preparing 'New Age Agile Institutions' wherein 'total stranglehold of district magistrate or deputy commissioner' on every 'function' of the district could be 'removed'. This Commission has exposed that the Deputy Commissioner heads so many Committees in a typical Indian district that he might even not remember the name of each.

This is the time therefore for Indian Institute of Public Administration and some other management schools to commission a Study on 'TERROR-PANDEMIC-FOOD SPOILAGE-FOOD ADULTERATION-ENVIRONMENTAL HEALTH MONITORING: DESIGN OF THE PUBLIC PREPAREDNESS SYSTEM IN NORTH WEST OF NATIONAL CAPITAL REGION'. The Federation of Indian Chamber of Agriculture, Trade and Services has already been in contact with various participants of some of the food chains in this region and a firm offer of collaboration was given by Prof Christopher Brown, Dean of College of Veterinary Sciences of Michigan State University of the USA during a Conference organised at Pantnagar from November, 7-9, 2008. This region comprises of some districts of Haryana and Delhi and the Chamber has made some preliminary contacts with farmers and some corporate as well institutes and universities. This then would be created into a movement at the level of this 'Region' and the design of SAFE AND NUTRITIOUS FOOD CHAINS would be targeted through creation of 'MULTI INSTITUTIONAL ALLIANCE' wherein number of institutes, private industry players, cottage industry, farmer-producers, primary food producers, safety laboratory and HACCP Certification Agencies along with Government Department/Boards and external and internal funding agencies including some international collaborative arrangements, could be made members. This kind of 'Multi Institutional Alliance' in Haryana could be designed to act first on 9-villages of Sonipat district. The villages has been identified and they would be near Kundli at Northern Border of Delhi. There are number of food related institution nearby including 'National Institute of Food Technology, Entrepreneurship and Management at Kundli' and 'Food Park at Rai' and there are international vegetable market system are also being established. For animal food chain, though there are number of processing plants which have come up but the 'food production chains of animal origin (broiler)' are located in nearby districts of Panipat and Jind in Central Haryana and Panchkula in upward North.

2. TECHNO-BUSINESS CHALLENGES AND OPPORTUNITIES: INDIA

2.1. General Scenario:

It is quite instructive to know that antibody products produced using 'technology platforms' could at best be said to be at a stage of infancy in India in human drug category whereas veterinary products are languishing in the technologies of 20th Century. Though antibody products could have niche market even in a typical domestic animal medicine in India. For example, characterised by prized milch animals such as Murrah buffaloes; prized race horses and 'object of emotion, pet species', the market-potential of all these categories remains big enough. Industry definitely would lap up if competitively priced product pipeline are available. For that matter, the veterinary 'recombinant and diagnostic antibody industry' cannot afford to have short-cut. Thrust of innovation has to be synergisitc with academia and institutes partnering with the industry. The approach of such innovations should not only be restricted to 'techno-business' efforts of the industry but relevant policy support by the government of states and at the Centre, is a must.

The issue of site selection for creating 'antibody/protein engineering hub' in the country has already been raised. With schemes such as 'Special Economic Zone' and 'Biotechnology Park or Knowledge Park or Immunobiological Park' getting favourable policy support, the 'veterinary immunobiological industry' has to join hands with 'academia' in 'creating favourable climate' for evolution of supportive measure at the country level. These efforts at the beginning decade of 21st Century have special importance as the animal-product consumption has been growing at a pace unmatched by 'production levels' in the country. Eventual deficit for all edible-animal-driven products

(even for liquid milk) has become cause of worry as serious repercussions not only in terms of availability of 'balanced diet' and 'food security'; but 'mediocre technologies' are driving cost of production of animal products very high. With those higher cost of production, herdsmen would be driven out of profession in livestock product and production industry. Therefore, central to all efforts is the affordability of veterinary immunobiologicals and production solutions capable of providing top-range productivity to the herdsmen (such as cheaper feed and fodder solutions, cheaper insemination, cheaper embryo transfer, cheaper food safety certification etc). Right in that spirit, this Symposium (XV Annual Convention and National Symposium of Indian Society of Veterinary Immunology and Biotechnology being organised from February 26-28, 2009 at Chaudhary Charan Singh Haryana Agricultural University, Hisar) has dedicated its plenary session to 'Globalising Indian R&D in Veterinary Immunology and Biotechnology for Affordable Animal Healthcare and Production Solutions'.

2.2. **Progressive development of antibody research at Hisar**:

The saga of graduation of antibody technology from polyclonal to recombinant and then, to one of the most advanced 'technology platform', has been written over the period of more than 23 years at the Section of Immunology of the Department of Veterinary Microbiology at Hisar. Antibody research was stared by Prof M C Goel at least 25 years ago. From conventional polyclonal antibody technologies, it progressed to monoclonal antibody technology by the advent of 1990s in which Dr Arvind Kumar played a crucial role. Progression to modern technology platform was spearheaded by a team led by Prof Ajit Singh in 2005 and by 2008, we had success in establishment of 'Phage Display Antibody Technology Platform' for production of 'single domain camelid antibodies'. This though is a laboratory scale achievement but this milestone assumes significance as it could provide spur to policy support efforts of institutionalizing the scale-up of 'academia-industrial partnership' in the field of 'antibody and/or protein engineering' at Hisar. Hisar has been promised to have a 'Knowledge Park' in which the author also played key role.

These developments are not by chance as Hisar has built up its claim to become a most important of the 'bioregions' of the country as it dedicated more than 50, 00 Acres of its land to development of various institutions of 'animal health care and production'. Progressive development of the region could be tracked to addition of capabilities of the region in consistent fashion right from the 19th Century , a time when such a mammoth land mass was dedicated for meeting 'animal health and production' needs of the country. With national institutions such as 'Central Institute of Research on Buffaloes', 'National Research Centre on Equines' and 'Equine Breeding Stud', accompanied by development of various state level farms dedicated for research and conservation of various farm animal species, Hisar could be favourite in housing a 'Special Economic Zone' on 'Protein Engineering and Veterinary Immunobiologicals'. The proposition has been discussed by the author at various levels in the Government of India and at the time of celebration of 'Natioanl Science Day on 28 February, 2009' during this Symposium, it is high time that claim of the city for establishment of such a facility is met with announcements of creation of a 'Working Group'. Further, it augurs so well that Guru Jambeshwar University of Science and Technology, another universityalso located at Hisar, is currently being headed by Lt General (Retired) D S Sandhu as Vice-chancellor, who before this assignment was Director General of Indian Ordnance of the Government of India. On the other hand, Hisar also has one of the largest Cantonment Area and is also located strategically in North West Region of India. Globalisation of Indian R&D in Veterinary Immunology and Biotechnology could only be possible when it gets institutionalized through supportive instruments of policy such as 'Special Economic Zone' getting housed in 171-Acre 'Knowledge Park', where participation of industry could be facilitated by generation and/or availability of affordable technologies from India and abroad. At least in South Asian countries and in Africa, there is tremendous opportunity for 'veterinary immunobiological' industry as has been spelt out clearly at various levels in recent past.

Coming to techno-business aspects of this robust technology platform, it is important that various other aspects of 'recombinant antibody and/or protein research, development and commercialisation' has to

contend with various means including collaboration with synergistic teams in India and abroad. Technology platforms perfected in academic settings require serious professional support as tackling the issue of 'technology transfer' is mired with problems of 'series of patents' and 'patent pool', good enough to seriously jeopardise entry of small and medium players in the business. Therefore, serious evaluation of 'techno-business capabilities' of the industry in India is required at this stage through 'recombinant industry specific case studies and reports', which could be done by professional agencies and/or Business Schools. These kind of reports should be seen as supportive measure for emerging 'regional parks/technology parks/knowledge parks' etc.

Taking an antibody product from 'proof of concept stage' to 'commercialisation stage', needs to be a serious work of design of a pipeline with time-sequence of activities and milestone dedicated for the domains of research, development and innovation. Research, development and innovation efforts for the pipeline could not bear fruit without supportive policies of the universities implementing or partnering with such 'parks' as they could have wherewithal to attract strong industries in the region. In addition, universities could have long-term commitment of its scientists in the 'selected platform technology area' with arrangement or access not only to liberal funding but supportive policies of supply of quality human resource to their laboratories. High-tech innovations require 'knowledge intensive process development', for which too much reliance is on 'top of the range research professionals'. Therefore, strategy of 'human resource growth' of the 'talent capital' needs to be made free from the shackles of the bureaucracy of the university and such laboratories should be provided 'autonomous status' so as they could provide enough incentive to retain highly talented individuals in their laboratories. Such individuals only could create 'multiplier effect'.

3. CONCLUSION

3.1. Challenging situation of international terror has enjoined upon countries of the world to create a system of mutual cooperation, collaboration, communication and coordination. The vulnerability of 'food and water' system of various countries are issues of challenge to the local-governance agencies. Local Self Government system in India have yet to get full powers in most of the states relating to funds, functions and functionaries, which means a lot of 'weakness' in administrative structures are evident and needs to be addressed. The issue of strengthening of 'having prepared formal administrative machinery with back-ups of preventative and curative tools' is at the heart of reformative agenda. The debate of having an FBI type set up and having a stringent legal power to the enforcement agencies notwithstanding the issue of 'strong system of command and undistorted intelligence' has to have shortest time-period in communication and operationalisation of 'command' originating from the Central level and finishing at the 'scene of possible hazards or threat'. Similarly, upward communication of the message should be with a minimum time elapse of time. The distrust amongst central, state and local policing system could be at the heart of all such structures, therefore, District Level Committees for 'biopreparedness', need to be given wideranging representation of the cross-section of the citizens as well as power to take decisions in a Jury like manner. The novel situation calls for novel solutions. As the country is grappling with terror events of various nature at regular intervals in the recent past, the issue of 'internal security or civil defence' need to be made 'zero tolerant' to any of such threats. And, that has to mean first of all, the system at local levels have to have wherewithal and preparedness machinery to detect these threats in a minimum time-period.

3.2. I have proposed the need for conducting a Study on 'TERROR-PANDEMIC-FOOD SPOILAGE-FOOD ADULTERATION-ENVIRONMENTAL HEALTH MONITORING: DESIGN OF THE PUBLIC PREPAREDNESS SYSTEM IN NORTH WEST OF NATIONAL CAPITAL REGION'.

3.3. In addition, I have also proposed to shift current focus on 'prevention' as a 'broader policy objective' of 'internal security to 'preparedness' should be a 'policy option'. For implementation, a high-powered DISTRICT ECONOMICAL AND ECOLGICAL COUNCIL (DEEC) wherein the issues of food chain safety and other linkages to terror, should be continuously monitored at the district level through preventative and/or preparedness teams for various kind of biological and chemical threats. There is a

progressive development in this direction as during a Conference of Gaushala Sangh held on 7 December, 2008 at Fatehabad, President of Sangh, Acharya Baldev Ji went forward to work in deployment of his volunteers to stop illegal trafficking of progeny of cows, which then taken to Bangladesh in lacs per year, has been shown to be a confirmed a source of providing money to 'cow smugglers', who also have been reported to have links with terror organisaitons as per reports of Union Ministry of Home.

3.4. There is a need for creation of a 'National Consortium on Animal Pathogen-based Hazards, Bioterrorism, Food Safety and International Trade' which could undertake various aspects of a coordinated support system to the public health authorities in the country. This Consortium should have techno-business-policy relationship with various institutions, organizations, government departments and a system of collaboration with global agencies and institutions. Various veterinary institutes including veterinary colleges and state and private sector immunobiological production agencies, would have to create specific 'expertise' in a 'given set of knowledge, service and production system'. The areas of expertise and institutionalization or strengthening of 'product industry or institutes' could be 'coordinated' with a Central Disease Control type of organizational structure. That of system is important as per advice of Lonnie King, DVM, senior veterinarian at the Centers for Disease Control and Prevention and director of the National Center for Zoonotic, Vector-Borne and Enteric Diseases, " in the last 20 or 25 years, approximately 75 percent of the new human diseases that have emerged are zoonotic, and of the 1,461 human pathogens that we know about today, about 60 percent are what we would term "multihost pathogens. In other words, they don't reside just in people by themselves". Contacts through animals or animal products -even plants- are actually responsible for multi-host diseases. To the credit of city, A National Repository of Veterinary Pathogens, has already been commissioned at Hisar at National Research Centre on Equines in last year (2008).

3.5. An Indo-US Working Group was proposed by the author at VII Annual Conference of Veterinary Public Health Specialists at Pant Nagar from November 7-9, 2008 on 'Animal Pathogen-based Hazards, Bioterrorism, Food Safety and International Trade'. Same is proposed here and I hope these recommendations should be forwarded to the Union Ministries of External Affairs, Biotechnology, Animal Husbandry, Health, Agriculture, Environment and Forest, Home, Food Processing and to some organisaitons like 'Export Inspection Council' of the Union Ministry of Commerce and also to APEDA so that suitable projects are developed and industries wishing to go-ahead could get policy support. Additionally, effective suitable institutions should be able to organize a system in which broader administrative mechanism of 'biopreparedness' could be studied by this Working Group and it may lead to implementation of the findings of the Group in the North West of the National Capital Region, as suggested or at some alternative location.

3.6. Efforts are already underway to further these efforts and various individual departments of the state, have been contacted so as to formalized the collaborations. An important dimension has been to contact important functionaries in the relevant Departments at the Government of India and Government of Haryana. Additionally, local self government authority at Hisar along with various other government and other stakeholder agencies, including Hisar Improvement Trust and Chambers of Industry, need to work in unison in building a case for Hisar. As Member of the Board of Governors of the Haryana School of Business, the author has been trying to contribute maximum towards this and would welcome any kind of collaboration and cooperation.

RELEVANT TECHNOLOGICAL SOLUTIONS FOR BUFFALO AND CATTLE BREEDERS OF HARYANA STATE

*Minakshi**

Department of Animal Biotechnology
CCS Haryana Agricultural University, Hisar
**Applied for GADVASU Best Women Researcher Award*

The DNA based diagnostic assays were developed for confirmatory diagnosis of 'Office International Des Epizooties (OIE)-notified disease pathogens' in semen samples. This was needed to facilitate the breeding programme of premium Murrah breed of buffalo of Haryana and Hariana breed of cattle of Haryana, which figures prominently in the policy mandate of the Government of Haryana as listed in 'Haryana Act no. 6 of 2001'. Relevant to breeders of the state for export of the semen and germplasm is the fact that Artificial Insemination (AI)-based spread of pathogenic agents poses a real threat as well as a single bull may yield up to 1000 doses of semen in a single ejaculation. Even the freezing conditions used for preservation of semen enables several infectious agents to survive. The earlier technology based on serological tests have limitations such as lack of sensitivity and specificity. Therefore, more sensitive and specific newer diagnostic procedures based upon DNA-based technologies were developed and some more are being developed at my lab for screening of the animals as well as their germplasm. I went on to identify that three was a dire need to develop methodologies for diseases caused by Bluetongue and BHV-1 viruses. The project was undertaken with stated objective to develop rapid and sensitive diagnostic methodologies for detection of BHV-1 and bluetongue virus in semen samples. Protocols for isolation of BTV specific viral RNA and BHV-1 specific viral DNA were developed, validated and are being applied as services to State Animal Husbandry Department for screening of semen samples of breeding bulls. Technology developed and its impact would be part of my presentation.

SESSION – VII
BIOTECHNOLOGY EDUCATION

Chairman, Dr. M.L. Sangwan
Co-chairman, Dr. N. Ahmad
Rapporteur , Dr. Minakshi Prasad

Date
27 Febraury, 2009, 11.30 AM -1.00 PM

Venue
SEMINAR ROOM
Department of Animal Biotechnology

BIOTECHNOLOGY EDUCATION, TRAINING AND INDUSTRY IN HARYANA: EVOLVING AN INTEGRATED RESPONSE

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Biotechnology Policy in Haryana was evolved in 2001. With a strong contribution of agriculture and animal husbandry in the economy of the state, it was natural these sectors got highlighted in the document. During the intervening period, state has witnessd some of the very significant developments in terms of opening up of number of universities and engineering colleges. In many of the newly opened institutions, programmes of undergraduate and post-graduate edcuation in this sector were launched. Speciality courses like B Tech and M Tech in biotechnology were started in the institutions of engineering. During this period, however, commensurate number of industries were not established to absorb the manpower. The issue of employability of the manpower generated during all these years remain a point of serious study as matter of quality of human resources, has been raised by the industry in India in general. The OECD (G-8) Report on Biotechnology has highlighted that the quality manpower of the developing countries generally heads to western economies as against information technology sector due to the fact that interdisciplinary research institutions along with a strong back up of industrial linkages are yet not being found commonly, which leads to the challenge of not only evolving academic-industry linkages but to create a necessary ecosystem for such linkages to take root and grow. This type of ecosystem could be established once state and region specific selection of appropriate biotechnology education and training is aligned with activve collaboration of industry. The paper shall discuss how the current pace of progress in the human resource management in various sub-sector of biotechnology could be fine-tuned to the need of all stakeholders.

THE FIELD VETERINARIANS' PERSPECTIVE ON WHAT FURTHER BEST COULD BE DONE IN VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY EDUCATION IN HARYANA

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Newly introduced discipline of animal biotechnology in Haryana has provided a fresh angle to the development of human resources in this advanced area of research, education and development for the students in the College of Veterianry Sciences. Being an area which might also change certain ways of production of livestock in times to come through effective utilisaiton of technologies such as embryo transfer and diagnostics etc, it is yet not very much part of the conventional curriculum of Veterinary Council of India. Therefore, how best to integrate it at the time of Continuous Professional Development of the field vets is the challenging issue for not only the academia at the college but it requires a good deal of dialogue with the field vets. This article explores various assumptions and views of the field vets and then, takes those views and assumptions for further discussion for the participants of the Symposium. On the other hand, Veterinary Immunology being a distinct and distinguished discipline since 1985, is also a subject handled sparsely during the BVSc &AH degree but has implications of great importance in diagnostics and preventive medicine, especially in the backdrop of heavy importance given to immunisation. The paper will discuss several issues through a field vet's perspective and would also reconcile that perspective with some chiseling of the academic vets.

A CASE FOR BIOTECHNOLOGY FINISHING SCHOOL IN NORTH INDIA

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Requirement for finishing schools in information technology industry was considered important in the recent past and some of such schools have been useful for the industry. Biotechnology sector has hit a road-block in terms of expectations of employers from the industry due to skill-sets and knowledge repertoire of the manpower being developed in the academia in recent past. A model for a Biotechnology Finishing School has to contend with so many challenging issues to encompass expectations of the industry as well as the resource position of the many of the institutions imparting education in various aspects of biotechnology. The recommendations of the curriculum and structure of various modules for different kind of training for such a School shall be presented for discussion.

SESSION – VIII
POSTERS

Chairman, Dr. Santosh Dhillon
Co-chairmen, Dr. Ravindra Sharma
Rapporteur, Dr. S.K. Kadian

Date
27 February, 2009, 5.15 - 6.30 PM

Venue
CORRIDOR
Department of Veterinary Animal Husbandry Extention

ANTIVIRAL EFFECT OF *O.SANCTUM* AGAINST NEW CASTLE DISEASE VIRUS (NDV)

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Ocimum sanctum (Tulsi) possess wide spectrum medicinal values viz: anabolic hypotensial, cardiac depressant muscle relaxant, expectant, anticancer, anthelmintic, antimalaria, analgesic and antimicrobial properties. To asses antiviral effect of extract of *O. sanctum* leaves, NCD virus, cultivated in embryonated chickens eggs by allantoic cavity route, was used as a challenge to the chicken embryo fibroblast (CEF) culture. Haemagglutination test was used for titration of virus. The nontoxic dose of *O. sanctum* was determined on 24 hours grown uniform CEF monolayer culture. The extract was diluted so as to contain 100, 50, 20, 10, 5, 2.5, and 1.25 mg/ml of extract in maintenance medium. 1 ml of each dilution was inoculated to CEF culture and incubated at 37 ^{0} in 5% CO_2. The toxic effect of each concentration was observed under microscope at 12 hrs intervals up to 48 hrs. Highest dilution showing any degenerative changes/CPE in cell culture was considered as cytotoxic dose of the extracts. Antiviral effects of *O. sanctum* extract were studied on the basis of CPE produced in CEF monolayer culture. Three different concentration of *O. sanctum* leaves extract 2.5mg, 5mg and 10mg/ml of aqueous extract were used to study the antiviral effect against NDV. NDV having 0.512 HA units was added in CEF culture with and without extract. Growth of fibroblast was monitored and supernatant was collected at different intervals viz: 12, 24, 36, 48, 60 and 72 hrs. The 10mg/ml of leaves extract of *O. sanctum* prevented the viral multiplication as evidenced by low grade CPE in NDV infected cell culture. Decline in multiplication of NDV was determined by HA titer and titer of virus in the culture supernatant was significantly lower at 16 with 10.0 mg/ml of extract of *O. sanctum* leaves as compared to 1024 with virus control. Over all there was a lowering of HA titer of NDV harvested from aqueous extract treated cell culture. The investigation further revealed that antiviral effect as evidenced on the basis of HA titer was also dose dependent and higher concentration of extract inhibited the NDV replication in higher extent.

SOLUTIONS TO SOME OF THE TOUGH PROBLEMS IN REALM OF HEALTH FOR RURALAREAS

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We report here a continual work of many years with rural institutions such as Gaushalas in promotion of formal research relevant to field conditions employing approved validational methods. Work at Ladwa Gaushala in Hisar district was initiated with the help of Late Vaidya Ram Niwas Jatasara by the first author in year 1999 and continued utilising various capabilities of the resources available at the Gaushala, including its animals and using a traditional system of indigenous medicine, named Panchgavya, we went on to report lots of positive benefits in healing support to the patients with AIDS. The methodology developed here is now called GAUPATHY and since then has attracted loads of formal interest during the intervening years. Case studies shall be reported. Additionally, work on usage of various other natural resources of Gaushala in promoting animal biotechnology and immunology research shall be cited. In the last, Gaushala could very well be reservoir for conservational efforts of endangered breeds of cattle and such issues as 'resistance to diseases', could be studied in a much rigourous fashion, the importance of which has already been presented and understood at highest levels in the Government of India in the recent past.

FACS ANALYSIS FOR DETECTION OF CD4/ CD8 RATIO IN MICE IMMUNIZED WITH BICISTRONIC CONSTRUCT CONTAINING AN N- TERMINAL PPE GENE ENCODING 34.9 KDA PROTEIN OF *MYCOBACTERIUM AVIUM SUBSPP PARATUBERCULOSIS* AND A MURINE GAMMA INTERFERON GENE

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The PPE family of protein of *Mycobacterium paratuberculosis* which are Proline Proline Glutamic acid rich have been hypothesized to play an important role in eliciting T-cell responses. Recently some of the PPE proteins of *M. tuberculosis* and *M.a.paratuberculosis* have been reported to be potent T cell and B cell antigens. The present work envisaged keeping in view the role of a PPE antigen of *M. a. paratuberculosis* and the concept of bicistronic DNA constructs using an immunostimulatory molecule IFNγ to potentiate immune response in mice. *M.a.paratuberculosis* 3737 is a putative hypothetical PPE family protein composed of 1515 bp ORF encoding 49.39 kDa protein. Among that 1080 bp encoding 34.9 kDa protein was choosen for expression and immunological studies. In the present study the gene fragment encoding PPE protein was amplified and cloned into the A frame of the plasmid pIRES (designated as *pIR PPE*) and simultaneously into the frame B the PCR amplified product of IFNã gene was cloned in the same vector (designated as *pIR PPE/ IFN)*.Four groups of mice were immunized with TE buffer control, mock plasmid pIRES control , recombinant plasmid pIR PPE and pIR PPE/IFN respectively at 0 and booster at 35th day. Splenocytes collected from randomly selected mice were subjected to flow cytometric analysis after labeling with CD4 and CD8 specific monoclonal antibodies conjugated with appropriate fluorescent tags. There was a significant decrease in the CD4/CD8 ratio in the mice group immunized with plasmid construct pIR PPE/IFN in comparison to pIR PPE and control groups. The reduction in the ratio may be correlated to the increase in the CD8+ count due to CMI mode of immunity ,as the bacteria is an intracellular pathogen and interferon gamma role may be emphasized to be an important costimulatory molecule.

EFFECT OF DIFFERENT REPRODUCTIVE STATUS ON MONTHLY MILK PRODUCTION IN PURANATHADI BUFFALOES

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The milk records of 20 Puranathadi Buffaloes from Buffalo Unit, PGIVAS, Akola were taken from April-2008 to December-2008. The monthly milk yield of each cow was calculated and subsequently classified according to their reproductive status in the particular month. The statistical analysis to ascertain the effect of reproductive stress on monthly milk production was carried out.

The lactating buffaloes were classified into four groups as G_1 (Non-pregnant), G_2 (Early pregnant), G_3 (Mid pregnant) and G_4 (Late pregnant) as per their reproductive status in a particular month.

The average monthly milk yield found to be 117.53 ± 5.84, 104.86 ± 17.49, 53.8 ± 9.73 and 1.5 ± 0 kg in G_1, G_2, G_3 and G_4 respectively. The percentages of buffaloes in each group were found to be 77.08% whereas, pregnant were 22.92%.

The analysis of variances indicated that the variation in average monthly milk yield found to be statistically significant between the groups. The critical difference indicated that the monthly milk yield per buffalo found to be significantly higher in G_1 followed by G_2, G_3 and significantly lowest in G_4. However, the differences between G_1 X G_2 and G_3 X G_4 are non-significant. This significant differences between the groups indicated that the variation in average monthly milk yield might be due to the advancement in pregnancy which resulted the stress condition on buffaloes causing significant reduction in milk yield particularly in middle and late pregnancy.

COMPARATIVE EFFICACY OF DIFFERENT ASSAYS FOR DETECTION OF GROUP A ROTAVIRUS FROM FAECAL SAMPLES OF BUFFALO CALVES

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Group A rotaviruses play an important role in causing gastroenteritis and mortality in buffalo calves. A number of assays like RNA-PAGE, ELISA, RT-PCR and virus isolation have been employed for rotavirus diagnosis. We evaluated the comparative efficacy of different assays for detection of group A rotavirus in buffalo calves. A total of 455 faecal samples collected from five organized farms in northern India were screened by monoclonal antibody based ELISA, 33 samples were positive for group A rotavirus. The per cent positivity ranged from 3.22 to 28% in different organized farms with an overall average of 7.25%. The same samples were also tested by RNA-PAGE which revealed classical 11 segments with 4:2:3:2 migration patterns in 14 faecal samples showing 3.08% positivity. However, all the PAGE positive samples could not be amplified by RT-PCR. Only 15 samples yielded a specific product of 864bp and 1011bp for VP4 and VP7 genes respectively. The sensitivity of ELISA, RNA-PAGE, and RT-PCR was found to be 100%, 66.67%, 71.43% while and specificity was 100%, 98.63%, 98.43% respectively, considering virus isolation as standard test. Being simple, fast and sensitive diagnostic assay, ELISA can be used as routine laboratory test for the diagnosis of BRV and field epidemiological studies.

CORRELATION OF DETECTION OF *PASTEURELLA MULTOCIDA* B: 2 ISOLATES BY PCR AND BY CONVENTIONAL METHODS

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A total of 150 samples comprising of nasal swabs and peripheral blood from live cattle and buffaloes (apparently healthy and diseased) and heart blood, nasopharyngeal swabs and tracheal swabs from dead animals were processed for isolation of *P. multocida*. The nine isolates obtained from these samples were classified as *Pasteurella multocida* subspecies *multoçida* on the basis of biotyping. All the isolates were positive both by isolation as well as by direct PCR detection on nasal swabs, nasopharyngeal swabs, tracheal swabs, blood and bacterial colony. Multiplex PCR was performed using the species specific (KMT1SP6-KMT1T7) and type specific (KTSP61-KTT72) primers that amplified 460 bp and 590 bp fragments, respectively. PCR correlated well with the conventional methods of isolation and was less time consuming. The antibiotic sensitivity pattern of the isolates revealed that the isolates were sensitive to chloramphenicol, enrofloxacin, gentamicin, ofloxacin, and pefloxacin (100% each), oxytetracycline (77.77%), ceftriaxone (66.66%), cefotaxime (66.66%) and erythromycin (66.66%). The isolates were resistant to ampicillin, amoxycillin and streptomycin (66.66% each).

EFFECT OF PASTEURIZATION ON ENDOSULFAN LEVELS IN BUFFALO MILK

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The soil-crop-animal-man flow route of food chain dynamics make it impossible to remove the xenobiotics from the processed animal products used in animal and human food industry. There is non-essential demarcation between the principals and xenobiotic for the end consumer that could be poultry, livestock or man. Number of reports exists regarding presence of endosulfan, an organo-cholrine acaricide used in crop protection across north-west India in milk. There is no dearth of pharmacological studies, but the removal of the molecule from the food chain is impractical. It is pertinent to study the fate of molecule as milk is processed in the dairy industry. Out of many food recourses for the Indian kitchen besides raw milk, the pasteurized milk supplied by public, private co-operative dairy unions is an important source. So it is important to note the effect of pasteurization of raw milk on endosulfan level. The present study aims this.

Study was on sixty samples of buffalo milk procured from different sources from the state of Haryana. Endosulfan @ 150 ppm was spiked to assayed samples, which were pasteurized by LTLT method. The gas-liquid chromatogram studies revealed that there was statistically significant difference ($p<0.05$) in concentration of endosulfan in control & pasteurized milk samples.

STRATEGIC ISSUES IN CONSERVATION AND DEVELOPMENT OF PREMIUM GENOME OF MURRAH BUFFALO IN HARYANA

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Loss of premium genome of the best breed of the Water buffalo in the world, named Murrah (the buffalo with supercoiled horns) has been facing a curious threat. On one hand, the breed occupies premium brand value at global level, on the other hand, policy support efforts by the state government since 2001 to conserve and improve the premium genome have, paradoxically resulted in further depletion of these resources! At the heart is severe shortage of milk in the country due to poor productivity levels of various milch animals and also, buffalo milk being a favourite of majority of consumers in India. Touted as best source of Mozarella cheese in the world, the milk of this breed, rich in fat, is somehow an addiction to buds of many Indians. These factors have endeared the milch Murrah at dairies of Metro cities such as Mumbai, Hyderabad and Pune to supply the milk. These dairies guided by sheer market reasons, purchase best yielding Murrah from Haryana. The temptation to have quick big-buck is spurred by the fact that insurance cover to the premium animals does not go up to market value of the animal, which have run more than one lacs in recent times. Another problem in recent years has been relating to premium Murrah bulls, which have been sold to exhorbitant prices of Rs 90, 000 even at the age of 6 months in Meham region. With these kind of market onslaughts in place, strategy to combat this onslaughts utilising tools of modern technoloies for tracking animals along with that of reproduction and production have been clubbed with need for appropriate policy reforms to make 'Premium Genome Clusters' in some regions of the state wherein a complete 'enterprise based approach' could be supported in not only producing animals through conventional technologies but with the help of Artificial Insemination as well.

EVALUATION OF DIFFERENT METHODS OF DNA EXTRACTION FROM SEMEN OF BUFFALO (*BUBALUS BUBALIS*) BULLS

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Microbes excreted in the semen of infected or carrier bulls can be disseminated to susceptible animals through artificial insemination. Polymerase chain reaction (PCR) has been employed successfully to detect infectious agents in tissues and body fluids. PCR inhibitors present in the semen pose serious problems in detection of microorganisms by inhibiting the amplification of the target DNA template. These inhibitors need to be removed completely during DNA extraction to amplify the target sequences in semen by PCR. DNA from semen of buffalo bulls was extracted using seven different protocols and the quantity and quality was evaluated spectrophotometrically. Chelex-100 and Qiagen modified methods for extraction of DNA from semen were found to be superior qualitatively as compared to the other methods. In qiagen modified protocol, the semen was treated with two extra buffers containing EDTA to chelate the metals. Additional treatment of semen with proteinase K was included to completely degrade cellular proteins. DNA extracted by Phenol-chloroform and CTAB methods yielded high value of residual RNA and other contaminants. Chelex-100 method has a potential advantage of requiring less volume of semen and to extract good quality of DNA.

RESURGENCE OF EQUINE INFLUENZA IN INDIA

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An outbreak of equine influenza among equines was reported in the country in 2008. The niche of the outbreak of equine influenza of 2008 was detected in the northern state of Jammu & Kashmir in the months of June -July, 2008 in mules, ponies and horses serving for transport of man and material for pilgrimage to a Hindu shrine near Katra (District Udhampur). The clinical signs comprising sudden onset of high fever (rectal temperature 104-105°F), watery to mucopurulent nasal discharge and dry cough were noticed in large number (approx. 70%) of 15000 animals in a gap of three to four days. Virus could be isolated in 10-11 days old embryonated hen eggs from nasal swabs from animals showing clinical signs and typing using reference sera indicated the virus to be of H3N8 subtype. Of 118 serum samples collected from sick and in-contact apparently healthy animals tested for antibodies (against both H3N8 and H7N7) by HAI assay, 85 samples (72.03%) revealed antibodies to equine influenza (H3N8) by HAI. Later the clinical signs simulating to those of equine influenza were reported from equines from Delhi and Haryana. The disease could be confirmed on the basis of rise in the titre in the paired serum samples. Serum samples and nasal swabs from various other states of the country *viz.* Uttar Pradesh, Himachal Pradesh, Rajasthan, Uttranchal, West Bengal, Maharashtra and Karnataka were tested at the centre and the presence of equine influenza could be ascertained. A total of 1175 serum samples from equines of unorganized sector were examined from various states of the country and out of these, 265 (22.53%) samples were positive for antibodies against equine influenza infection in horses. Similarly, serum samples of 376 horses of organized sector were tested for EI antibody and 163 (43.35%) horses were found positive for equine influenza. Virus was isolated from nasal swabs collected by NRCE team from Katra, Jammu & Kashmir (n=5) and from Mysore, Karnataka (n=2). Genetic characterization studies on the haemagglutinin (HA) gene of the equine influenza virus isolated from Katra indicate it to be belonging to American lineage.

DIFFERENT TISSUES OF BUFFALO *(BUBALUS BUBALIS)* EXHIBIT VARIABLE GENE EXPRESSION OF TOLL LIKE RECEPTOR 9

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Toll-like receptors (TLR9) detect unmethylated CpG dinucleotides in bacterial or viral DNA, and can be stimulated for therapeutic application with synthetic oligodeoxynucleotides containing immune stimulatory "CpG motifs". Proper targeting of CpG ODN for the induction of innate immune defenses requires an understanding of immune compartments which express high abundance of TLR9. This may inform decisions regarding appropriate formulation and delivery of CpG ODN. In the present study, transcriptional expression of TLR9 was seen in a variety of lymphoid tissues (mesenteric lymphnode (MLN), tonsil and spleen), blood and mucosal tissues (lung, intestine) of buffalo calves. All the RNA samples of tissues and peripheral blood mononuclear cells (PBMCs) exhibited mRNA expression of TLR9. This is the first report of transcriptional expression of TLR9 in buffalo (*Bubalus bubalis*). The abundance of TLR9 in different tissues was also determined by normalizing the integrated density values (IDV) of TLR9 expression of samples by that of housekeeping gene, GapdH. The variable expression of mRNA for TLR9 was observed in six tissue samples and PBMCs by RT-PCR. Lymphoid tissues (spleen, tonsil, MLN) expressed higher TLR9 than blood and mucosal tissues. The functional analysis of TLR9 may provide the first step to the development of new adjuvants, vaccines or therapeutics.

PHENOTYPIC CHARACTERIZATION OF VIRULENT *CHOE* GENE POSITIVE *RHODOCOCCUS EQUI* ISOLATES

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Rhodococcus equi strains were isolated from post-mortem lung samples obtained from three foals suspected of death due to Rhodococcal infection. *Rhodococcus equi* were isolated from all 3 samples. The isolates exhibited typical shovel-shaped synergistic hemolysis (CAMP-like) reaction using a hemolysine positive equine *Staphylococcus aureus* isolate. The Gram-positive isolates showed characteristic rod-cocci growth cycle morphology. The colony morphology of 3 isolates revealed typical salmon-pink colonies in two strains; however, colonies of third strain were orange colored. The isolates were subjected to biochemical characterization and antibiotic sensitivity profiling. Strains were late-urease positive. They were resistant to penicillins, quinolones and first generation cephalosporins, however, isolates were sensitive to azithromycin and ofloxacin. The isolates were confirmed using species specific primers detecting a 700 bp fragment of chromosomal DNA. Isolates were also positive for presence of the gene that encodes the virulence associated cholesterol oxidase gene (*cho*E), using PCR, giving a 959 bp amplicon. The results indicated that *cho*E positive virulent *R. equi* causing bronchopneumonia strains are prevalent. The CAMP-like reaction can be used as phenotypic marker for rapid presumptive identification of *R. equi*. Research efforts are needed for epidemiological investigations of strains of Rhodococci prevalent in farm environment in India.

PRESENCE OF UNUSUAL G3P[3] GENOTYPE OF GROUP A ROTAVIRUS IN DIARRHOEIC BUFFALO CALVES: AN EVIDENCE FOR INTER- SPECIES TRANSMISSION IN NORTHERN INDIA

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Rotaviruses, members of the family *Reoviridae*, are the major etiologic agents of severe, acute dehydrating diarrhea in the young ones of many mammalian species, including humans, calves and foals. Rotaviruses causing severe diarrhea in buffalo calves on animal farm in Haryana (northern India), during the period from 2003 to 2004, were characterized by electropherotyping, genotyping, and sequence analysis of the genes encoding the outer capsid proteins. Of 85 specimens, 11 (13%) were positive for group A rotavirus and exhibited long electropherotype. Analysis of the full length vp7 gene sequence of B29 buffalo rotavirus (BuRV) strain revealed maximum identities 81.0- 99.0% and 89.0-98.0% at nucleotide level and amino acid level respectively with G3 strains (Most prevalent in human), while partial vp4 gene sequence analysis revealed maximum identities 77.0- 84.0% and 90.0- 93.0% at nucleotide level and amino acid level respectively with P[3] rotavirus strains of bovine, human, caprine, equine and porcine from different countries. The B29 strain is the first reported rotavirus isolate with a G3P[3] genotypic combination in buffalo calves in this part of the country. The detection of unexpected buffalo rotavirus-derived G3P[3] may be a reassortant strain in the farm reveals an interesting epidemiological situation and diversity of bovine rotaviruses in India. The discovery and surveillance of novel bovine and non bovine rotavirus G or P types or of novel G&P combinations is essential for the design of future rotavirus vaccines and for our understanding of rotavirus diversity and evolution. Presenting Author: Hari Mohan for best Poster Award?[1]

SCOPE OF STEM CELLS FOR REGENERATIVE THERAPY OF MASTITIS IN BUFFALOES - ITS PROSPECTS, CONSTRAINS AND ROLE OF INDUSTRY

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Buffaloes are backbone of Indian dairy industry and research pertaining to stem cells in this species is still in primitive stage, with no stem cell lines available till date. This futuristic technology has potential to provide novel therapeutic approaches for mastitis through regenerative medicine and production of transgenic animals secreting therapeutic proteins in milk which is a multi million industry. Before application of these pluripotent, self renewing and undifferentiated cells for generation of specialize cells of mammary gland secreting milk, we have to address some constrains. In this species extensive efforts have been done to isolate embryonic stem cells which has drawbacks like ethical constrains, rejection due to non autologus source and tumor generation after therapy. Adult stem cells can be an alternative source but has limitations like they are difficult to isolate, have limited differentiation potential, and have to standardize differentiation protocol in vivo. The best possible source can be mammary gland tissue from adult animal. They have ability to replenish the mammary gland through different stages of life cycle of animal. We have to develop protocol and markers for isolation, culture and therapy for buffaloes. Industry can play a vital role by conduction collaborative research with state veterinary universities and research institutes working in this area.

EFFECT OF DIFFERENT MEDIA SUPPLEMENTS ON MATURATION OF BUFFALO OOCYTE

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Buffalo oocytes were matured in three groups. Group-I represents oocytes matured in TCM-199 supplemented with 0.4% BSA , group-II oocytes with 10% FCS and Group III oocytes with 5% neonatal bovine serum (NBS). Total 948 ovaries were taken from which 775 oocytes were aspirated with recovery rate of 81.75%. Maturation rate was assessed in respective groups which resulted in 234 matured out of 317 oocytes (74.12±4.2%) in Group I and 164 matured out of 185 oocytes (89.1±3.5%) in Group II and 210 matured out of 273 oocytes (77±2.75) in Group III. The difference in success rate of maturation may be due to factors like time taken for collection of oocytes, physiological and nutritional status of slaughtered animals, time taken during transportation of ovaries and media supplements. From this study it can be concluded that a supplementation of TCM-199 with 10% FCS give better result as compared to two other groups for *in vitro* maturation of buffalo oocytes.

A SOLID PHASE IMMUNOASSAY TO DIAGNOSE CANINE LEPTOSPIROSIS

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Leptospirosis in dogs is an important zoonotic disease which besides an acute disease may commonly be occurring as sub-clinical infection. The isolation of organism from clinical cases is tedious and time consuming and require dark field microscopy to view the organisms. A solid phase immunoassay (ImmunoComb diagnostic kit of Biogal Galed Labs, Kibbuts Galed, Israel) for Dog IgG antibody response to *Leptospira interrogans* was used in the present study to diagnose suspected cases of Leptospirosis in dogs. A dog came to Vety. Clinics, GADVASU, Ludhiana with anorexia and vomition. The dog was examined clinically and had temperature of 103°F. The serum sample was collected and subjected to analysis by ImmunoComb diagnostic kit. The dog showed low positive response graded as S2 as per the kit. Another dog also having the history of anorexia, vomition and fever showed positive response graded as S3. The grade S1 and S2 denote low positive, S3 and S4 positive and S5 and S6 highly positive reactions depending on the visual comparison with standard reference positive colored spot developed along with the test proper. The kit can detect antibodies against pathogenic serovars of *Leptospira* namely icterrohaemorrhagiae, canicola, pomona and grippotyphosa and most widespread variants found in dogs. The kit is claimed to be more sensitive than microagglutination test (MAT), however, it can not distinguish between the serotypes. The kit is very useful in clinical cases as the results are available within half an hour to diagnose leptospirosis. The kit is also useful to diagnose even sub-clinical cases of leptospirosis.

IN SILICO DESIGN OF VHH ANTIBODIES AGAINST LUNG CANCER BY USING IMMUNOINFORMATIC TOOLS

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Various tumor cells are characterized by a disregulation of EGF-recepotor (EGFR) signaling due to receptor over expression.The gene sequence of Variable Heavy Chain antibody of camelid origin (Vhh sequence) which binds to EGFR and / or homologues of the polypeptide, has been already worked out in the literature.The sequence obtained through various interventions in the literature displaying 90% amino acid sequence homology to human VH framework region was further studied by us by following some novel means of interpretation of the data available. The homologues sequence was searched by putting the sequence in various tools such as BLAST and FASTA.The sequence in FASTA format were aligned.The most similar sequence was taken and a hypothetical site for mutation was also designated and compared with the one reported in literature.The structure with highest identity with that reported in literature, was selected for comparative studies and a 3D structure was predicted.The result will be shownWe believe that the Vhh prediction using immunoinformatic tools might be useful to efficacy of the antibody but also to avoid problems of 'adverse immunogenicity associated reactions' to the patients where the antibody is administered as a biopharmaceutical new product.

APPLICATIONS OF GENETIC ENGINEERING IN ANIMAL BREEDING

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Genetic engineering is the name of a group of techniques used to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms. Genetic engineering has number of applications in animal breeding such as: 1) Marker-assisted selection (MAS). The objective of this technology is to improve productivity, product quality and disease resistance in farm animals by adding information of DNA markers to phenotypes and genealogies for selection decisions. Other very important applications of genetic markers in animal improvement include the optimization of mating strategies for non-additive genetic effects (estimation and managing of inbreeding and heterosis), parentage determination, genetic characterization of diverse animal breeds and populations using studies of between and within population (breeds) diversity and marker-assisted introgression of particular alleles. 2) Transgenesis, the direct transfer of specific genes/alleles between individuals, species, or even Kingdoms, in order to change their phenotypic expression in the recipients. Transgenic animals with genetic modifications has potential use in studying mechanisms of gene function, changing attributes of the animal in order to synthesize proteins of high value, create models for human disease or to improve productivity or disease resistance in animals. 3. Cloning an animal is the production of a genetically identical individual, by transferring the nucleus of differentiated adult cells into an oocyte from which the nucleus has been removed. This is known as Nuclear Transfer and is how the Dolly sheep was produced. Use of cloning in animal genetic improvement may increase the rates of selection progress in certain cases, particularly in situations where artificial insemination is not possible, such as in pastoral systems with ruminants. Currently, high costs of cloning are one of the main factors limiting their use as a technique in practical animal breeding. 4. Sexed semen, will contribute to increased profitability in farm animals in a variety of ways. It could be used to produce offspring of the desired sex from particular mating to take advantage of differences in value of males and females for specific marketing purposes.

UTILIZATION OF GENE MAPPING INFORMATION IN LIVESTOCK ANIMALS

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Great progress has been made in the field of genomics in the last few years, and new technologies indicate that this rate of progress will increase in the near future. Genetic information provided by genomics research will dramatically change livestock selection practices. Genetic linkage maps have been developed for a number of livestock species including cattle, sheep & pigs. These maps allow scientists to identify chromosomal regions that influence traits of economic importance. This information will lead to improved genetic selection practices by identifying animals with superior copies of the chromosomal regions that affect the selected trait. This mapping information will also be used to identify the genes controlling the trait. A number of genomic regions or loci have already been reported that affect production, carcass or disease traits, and in few cases, a specific gene has been identified. Production of transgenic animals with sequence changes in these genes may be beneficial for evaluating the effect of the gene upon the selected trait and more specifically the effect of certain polymorphisms within genes.

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