

INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY

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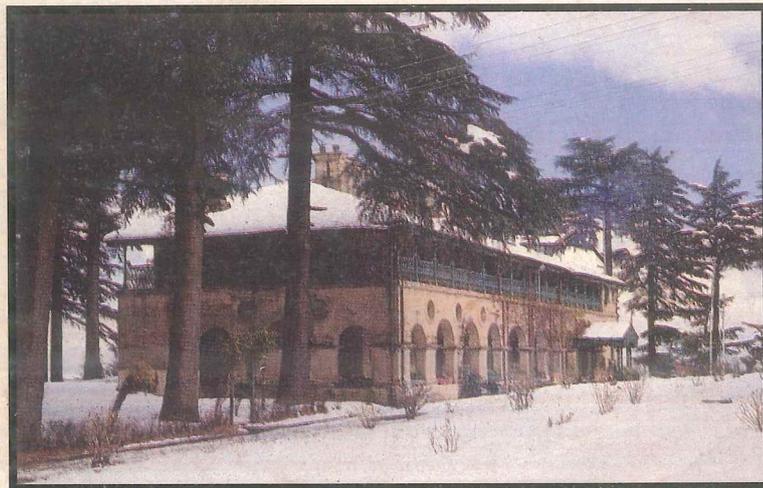
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**The XI Annual Convention of ISVIB
and National Symposium
on**

**Alternate Strategies for Livestock Production and Protection :
Role of Biotechnology and Immunology**

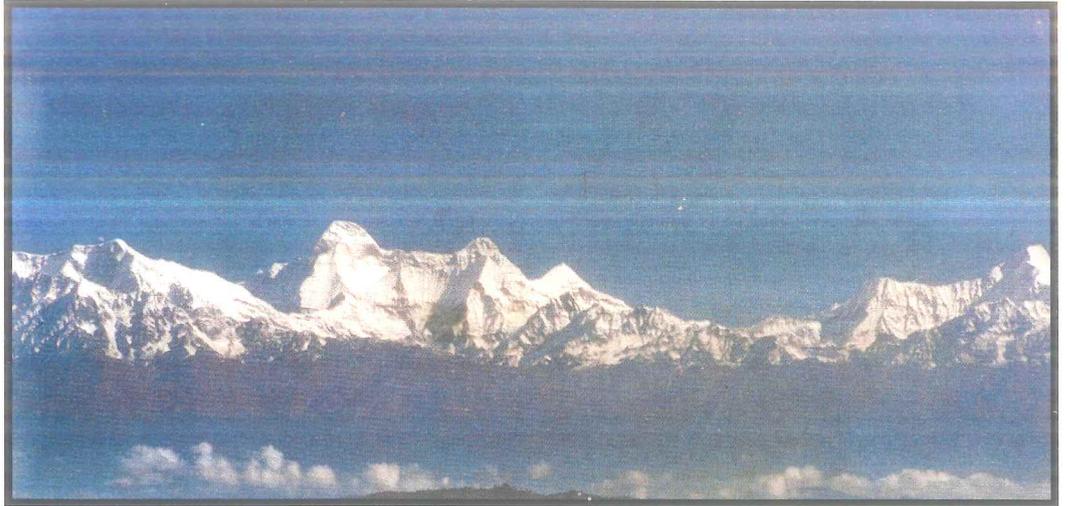
November 3-4, 2004

ABSTRACTS



Organized By :
Indian Veterinary Research Institute
Campus Mukteswar- 263138
Nainital (Uttaranchal) India





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Alternate Strategies for Livestock Production and Protection : Role of Biotechnology & Immunology

XI CONVENTION
OF
INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY
AND
NATIONAL SYMPOSIUM
ON
**ALTERNATE STRATEGIES FOR LIVESTOCK PRODUCTION AND PROTECTION:
ROLE OF BIOTECHNOLOGY AND IMMUNOLOGY**

[November 3-4, 2004]

IVRI, MUKTESWAR CAMPUS, UTTRANCHAL-263 138

● — **PROGRAMME** — ●

WEDNESDAY NOVEMBER 3, 2004

8.00 AM (onwards)	:	REGISTRATION
10.30 AM to 12.05 PM	:	INAUGURAL SESSION
12.05 to 12.30 PM	:	TEA
12.30 to 1.15 PM	:	RICHARD MASSILAMONY ORATION — Dr. B.U.Rao
1.15 to 2.15 PM	:	LUNCH

XI Convention of ISVIB & National Symposium - Abstracts

2.30 to 3.00 PM

**SATELLITE SYMPOSIUM ON RNA THERAPEUTICS
AND ITS POTENTIAL IN ANIMAL SCIENCES**

CHAIRMAN : Dr. M.P. Yadav
CO-CHAIRMAN : Dr. K.S. Palaniswamy
RAPPORTEUR : Dr. M. Hosamani / Dr. B. Mondal

INVITED SPEAKER

RTA-IS-1: RNA therapeutics - An overview
T.J. Rasool

3.00 to 4.15 PM : Scientific Session - I

**GENOMICS AND PROTEOMICS: ROLE IN
LIVESTOCK PRODUCTIVITY**

CHAIRMAN : Dr. Nem Singh
CO-CHAIRMAN : Dr. Deepak Sharma
RAPPORTEUR : Dr. P. Saravanan / Dr. V. Bhanuprakash

INVITED SPEAKERS

GPP-IS-1 Transgenic chicken through sperm mediated gene transfer
Deepak Sharma and R.R.Churchil

GPP-IS-2 Can genetic selection influence nucleotide sequences of candidate
genes for immuneresponsiveness
V.K. Saxena

ORAL PRESENTATIONS

- GPP-O-1 **Species non-specific tumor markers, conformational epitope(s) in immune complex and loss of differentiation antigens in plasma of canines and bovines with tumors**
Raman Chopra and Hari Mohan Saxena
- GPP-O-2 **16S-23S rRNA Intergenic space polymorphism in *Escherichia coli* isolates of cattle**
Shuchismita Chatterjee, S.K. Ghorui and S.K. Kashyap
- GPP-O-3 **Genetic Polymorphism of growth hormone gene in Indian buffalo breeds**
M. Muhagheh, S. L. Goswami, S. De, T.K. Datta, Paras Yadav
- GPP-O-4 **ERIC-PCR as a tool for molecular typing of avian *E.coli* isolates**
R. Amita, L. Gomes, B. Muniyappa, Prakash and S. Isloor
- GPP-O-5 **GFBP-3 gene polymorphism and its effect on serum IgG level of cattle**
Vivek Choudhary, Pushpendra Kumar, V.K. Saxena, T.K. Bhattacharya, Bharat Bhushan, Arjava Sharma and A.K. Ahmed
- GPP-O-6 **Identification and cDNA characterization of three novel CC-chemokines from domestic duck (*Anas platyrhynchos*)**
E. Sreekumar, Avinash Premraj, D.S. Arathy, T.J.Rasool
- GPP-O-7 **Heterodimeric expression of functional recombinant Interleukin12 of buffalo (*Bubalus bubalis*) in HEK293T cells**
Avinash Premraj, E. Sreekumar, Mamta Jain, T.J. Rasool
- GPP-O-8 **Polymorphism in buffalo growth hormone second exon gene**
U. Sarkar, A. K Gupta, S. De, T. K Datta, S. L. Goswami, M. D. Muhagheh, V. S. Raina, T. K. Mohanty, V. Sarkar, K. Yogesh and P. Yadav
- GPP-O-9 **Synthetic peptide strategies in evaluating reactive epitope of monoclonal antibody specific to haemagglutinin protein of PPR virus**
Sameer Shrivastava, Satish Kumar and R.K. Singh
- GPP-O-10 **Cloning of mature E2 gene of swine fever vaccine virus**
P. Dhar, V. Upamanyu and R. Verma

GPP-O-11 **Recent molecular epidemiology of *peste-des-petits* ruminants virus in India**

Rajeev Kaul, M.P. Yadav, R.P. Singh, Bikas Sahay, B.P. Sreenivasa, P. Dhar, S.K. Bandyopadhyaya and R.K. Singh

4.15 to 4.30 PM : TEA

4.30 to 5.30 PM : Scientific Session - II

NEW DIMENSIONS AND DEVELOPMENTS IN ANIMAL DISEASE DIAGNOSTICS

CHAIRMAN : Dr. G. Butchiah
CO-CHAIRMAN : Dr. V. Thiagarajan
RAPPORTEUR : Dr. C. Ramakrishna/ Dr. Hira Ram

INVITED SPEAKERS

ADD-IS-1 **Recent trends in diagnosis and immunoprophylaxis of Capripox viral infections**

R.K. Singh

ADD-IS-2 **Current trends in Animal disease diagnosis**

Bhasker Sharma

ORAL PRESENTATIONS

ADD-O-1 **Detection of avian infectious bronchitis virus (IBV) in field samples by reverse transcription-polymerase chain reaction (RT-PCR)**

S. Arthur Sylvester, J. M. Kataria, K. Dhama, S. Rahul and S. Tomar

- ADD-O-2 **Evaluation of monoclonal antibody based immunoassay in the detection of infectious bursal disease virus**
N. Vengadabady.A. Koteeswaran. K.S. Palaniswami, and R. Govindarajan
- ADD-O-3 **Diagnosis of Infectious Bursal Disease in Poultry in field outbreak using RT-PCR**
V. Umapathi, M. Nagalingam, Deena Khan, S.K. Shukla and B.D. Lakhchaura
- ADD-O-4 **Development and standardization of single serum dilution ELISA kit for quantification of antibodies against newcastle disease virus**
J. John Kirubaharan, S. Suresh Babu, M.P.M Latha, A. Thangavelu,
N. Daniel Joy Chandran
- ADD-O-5 **Development of 3M KCl solubilized Rous Sarcoma extract or transformed fibroblast antigen (TfAg) as a diagnostic antigen for ALS/ V infections in poultry**
Anita P, Alka Tomar and B. Singh
- ADD-O-6 **Cloning and Sequencing of Type 4 fimbrial gene of *Pasteurella multocida* Serogroup B:2(strain P52)**
Joseph Siju, A.A.Kumar, S.B.Shivachandra, P.Chaudhury, S.K.Srivastava and V.P.Singh
- ADD-O-7 **Comparative efficacy of different enrichment broths, PCR, ELISA and MAT for diagnosis of salmonellosis in equines**
N. Babu, B.R. Singh, Harishankar, Ravi kant Agrawal, Mudit Chandra, T.V.Vijo, S.K. Srivastava and M.P. Yadav
- ADD-O-8 **Monoclonal antibody and outer membrane protein based single dilution ELISA for antibodies against Haemorrhagic septicaemia in serum of cattle and buffaloes**
Arvind Kumar, Gita Dahiya and N.K.Kakker

5.30 to 7.00 PM : GENERAL BODY MEETING
7.15 to 9.00 PM : CULTURAL PROGRAMME
9.00 PM : DINNER

THURSDAY NOVEMBER 4, 2004

9.30 AM to 11.00 AM : Scientific Session - III

**NOVEL DEVELOPMENTS IN ANIMAL
DISEASE PROPHYLACTICS**

CHAIRMAN : Dr. M.S. Oberoi
CO-CHAIRMAN : Dr. J.M. Kataria
RAPPORTEUR : Dr. A. Sen / Dr. V. Balamurugan

INVITED SPEAKERS

ADP-IS-1 **Molecular Biology of Bird Flu- diagnosis and control measures**
J.M. Kataria

ORAL PRESENTATIONS

ADP-O-1 **Studies on immunizing property of *E.coli* pili vaccine in chickens**
Manju Pal and S.D.Singh.

ADP-O-2 **Propylactic vaccination against anthrax in endemic area - an investigation**
M D. Vankatesh B R. Harish , C. Renuka prasad, S R. Jaykumar, and G. Krishnappa

ADP-O-3 **Immunogenicity of aerobic and anaerobic harvests of *Pasteurella multocida* P52 in murine model**
S. Sudha Rani,, V.K.Chaturvedi., K.D.Pandey. and P.K.Gupta

ADP-O-4 **A combined vaccine against goat pox and *Peste des petits ruminants* induces protective immune response in goats**
Madhusudan Hosamani, S.K. Singh, Arnab Sen, B. Mondal and R.K. Singh

- ADP-O-5 **Peste des petits ruminants (PPR) in small ruminants**
V. Purushothaman, S. Hemalatha, G.Ravikumar, R. Govindarajan, N. Sundar and
A. Koteeswaran
- ADP-O-6 **Development of molecular diagnostics, prophylactics and therapeutics
against bacterial and viral agents**
R.K. Singh
- ADP-O-7 **Preparation and standardization of oral Pellet vaccine to control
Newcastle Disease in village chickens**
J. John Kirubaharan, K.S.Palaniswamy, D. Indira, P. Prbhakar, N. DanielJoy Chandran
- ADP-O-8 **Antigenic and genetic analyses of foot-and-mouth disease virus type
A isolates from India for selection of candidate vaccine strain**
R.K. Jangra, C. Tosh, A. Sanyal, D. Hemadri, and S.K. Bandyopadhyay
- ADP-O-9 **Kinetics of humoral immune response in pigs following FMD vaccination**
Maitri Sharma, S.K. Kadian, , R. Sharma and T. Rana
- ADP-O-10 **Immuno-reactivity of Foot and Mouth disease virus serotype 'A'
encapsulated in poly (DL lactide-co glycolide) micro sphere**
U. K. Chaudhari, S. Kishore, V.V.S. Suryanarayana, M. GaJendragaud, S.
Chowdhury. G. Nagrajan
- ADP-O-11 **Immuno-reactive protein gene construct of Foot and Mouth disease
virus serotype 'A' as DNA vaccine**
S. Chowdhury, G.R.Reddy, V.V.S.Suryanarayana, H.J.Dechamma, P.Ravi Kumar,
C.Ashok Kumar, U.K.Chaudhari
- ADP-O-12 **Induction of systemic and mucosal immune responses in poultry by
intranasal immunization with microencapsulated EDS-76 virus**
S. Dandapat, D. Banerjee, Rajan, J.M. Kataria and G.C. Ram

11.00 AM to 11.15 AM : TEA

11.15 AM to 12.30 PM

Poster Session

- GPP-P-1 **Molecular characterization of Indian isolates of avian reovirus (ARV) isolated from different disease manifestations**
Nitin Bhardwaj, J.M. Kataria, S. Arthur Sylvester, K. Dhama and N. Senthilkumar
- GPP-P-2 **Molecular epidemiological studies of fowl adenoviruses (FAVs) associated with inclusion body hepatitis- hydropericardium syndrome (IBH-HPS) in India**
S. Rahul, J.M. Kataria, N. Senthilkumar, K. Dhama, S. Arthur Sylvester and B.B. Dash
- GPP-P-3 **Restriction enzyme analysis of tissue culture adapted velogenic Newcastle Disease virus**
C.Madhan Mohan and Sohini Dey
- GPP-P-4 **Detection of telomerase activity in spontaneously occurring canine neoplasms**
B.C. Nair, O.P.Paliwal, G.Saikumar, V.K.Singh, and S.K.Maiti
- GPP-P-5 **DNA polymorphism of BoLA-DRB3 exon 2 in Tharparkar Cattle (*Bos indicus*)**
Bharat Bhushan, B. N. Patra, Pushpendra Kumar and Arjava Sharma
- GPP-P-6 **Characterization of AB 330: a novel CC-chemokine from domestic duck (*Anas platyrhynchos*)**
D.S.Arathy, E. Sreekumar and T.J. Rasool
- GPP-P-7 **A Novel Cathelidicin Congener from *Bubalus bubalis***
Hemen Das and Ashok kumar
- GPP-P-8 **Identification of five new *Salmonella* serovars haunting poultry birds**
Saud Hassan, Pallavi, Manupriya, Babu, N., Akshay, A. S. Yadav, M.Z. Siddiqui and B.R. Singh

- ADD-P-1 **Recombinant antigen-based latex agglutination test for diagnosis of leptospirosis**
Sohini Dey and C. Madhan Mohan
- ADD-P-2 **Detection of adhesive curli gene (crl and csga) in avian pathogenic *E.coli* using polymerase chain reaction**
Manju Pal , S.D.singh and J.M.Kataria
- ADD-P-3 **Micro agglutination Test for monitoring of immune status against *Haemorrhagic Septicaemia***
S. Sudha Rani, V.K. Chaturvedi, K.D. Pandey, and B.C. Nair
- ADD-P-4 **Nested PCR for the diagnosis of Tetanus**
S. Sudha Rani, V.K. Chaturvedi, P.K.Gupta and K.D. Pandey
- ADD-P-5 **Application of Random Amplification of Polymorphic DNA (RAPD)-PCR for avian *E.coli* serotype differentiation**
Amita R. Gomes, L. MuniYappa, B. Prakash and S. Isloor
- ADD-P-6 **Detection of Salmonella in chicken meat and eggs using Polymerase Chain Reaction**
K. Nagappa, Brajmadhuri, Shantanu Tamuly, Mumtash Kumar Saxena, Sandeep K Singh, L.S. Girish and S.P. Singh
- ADD-P-7 **Antigen competitive ELISA (AC-ELISA) to detect Newcastle disease virus from tissue samples**
R. Ananth, J. John Kirubaharan, S. Suresh Babu and N. Daniel Joy Chandran
- ADD-P-8 **Development of Indirect ELISA for the diagnosis of *Haemonchus contortus* infection in goats**
Hira Ram, V. Balamurugan, A.K.Sharma and T.J. Rasool
- ADD-P-9 **Production and characterization of neutralizing monoclonal antibodies against *peste des petits ruminants* (PPR) virus**
S. Saravanan, R.P. Singh, A. Sen, R.K Singh, V. Balamurugan and P. Saravanan
- ADD-P-10 **A simple Dot-ELISA for diagnosis of *Peste des petits ruminants***
P.Saravanan, Arnab Sen, V.Balamurugan and R.K.Singh

- ADD-P-11 **Development of an one-step multiplex RT-PCR assay for the detection of *peste des petits ruminants* (PPR) virus in clinical samples**
V. Balamurugan, A. Sen, P. Saravanan, R.P. Singh, R.K. Singh
and S.K. Bandyopadhyay
- ADD-P-12 **Cloning of peste des petits ruminants (PPR) virus haemagglutinin (H) gene in heterologous expression system**
V. Balamurugan, P. Saravanan, A. Sen, R.K. Singh and T. J. Rasool
- ADD-P-13 **Comparison of monoclonal antibody based ELISA with that of polyclonal antibody based ELISA for serodiagnosis of infectious bursal disease in poultry**
V.I. Sabarish, Rajesh Chhabra, S.K. Batra and S.K. Kalra
- ADD-P-14 **Development and application of an indirect ELISA for the serodiagnosis of bluetongue virus infection**
V Bhanuprakash, B Mondal, M Hosamani, R K Singh and Nem Singh
- ADD-P-15 **Potential of recombinant VP7 as an antigen for detection of bluetongue antibody in sera**
Kunj B Pathak, V. Bhanuprakash, M. Hosamani, R. K. Singh, Nem Singh
and B. Mondal
- ADD-P-16 **Usefulness of urinary enzymes in diagnosis of canine nephritis**
V. Mrudula, V. Titus George, C. Balachandran and V. Muralimanohar
- LPP-P-1 ***In-vitro* immunotoxic studies of endosulfan in avian lymphocytes**
P.V. Ravindra. Deena Khan, Gunjan Shukla, N.Ahmed and R.S. Chauhan
- LPP-P-2 **Efficacy of PGF₂α in induction of estrus in non-descript hilly goats**
S. K. Singh, Ram Naresh, Hira Ram, A.K. Sharma and T. J. Rasool
- LPP-P-3 **Influence of enrofloxacin on phagocytic activity and phagocytic index of milk polymorphonuclear leukocytes in bovine sub clinical mastitis**
Reena Mukherjee and P K Dash

12.30 to 1.30 PM : Scientific Session- IV

**BIOTECHNOLOGICAL ADVANCES IN THE VALUE
ADDITION OF LIVESTOCK PRODUCTS AND
PROCESSES AND OTHERS**

CHAIRMAN : Dr. M. Rajashekar
CO-CHAIRMAN : Dr. A.S.R. Anjaneyelu
RAPPORTEUR : Dr. S.K. Singh / H. R. Meena

INVITED SPEAKER

LPP-IS-1 **Quality assurance of recombinant veterinary diagnostics**
M. Rajashekar

ORAL PRESENTATIONS

- LPP-O-1 **Rodenticide poisoning in cattle and buffaloes-an investigation**
MD.Vankatesh, S R. Jaykumar, C. Renuka prasad and G. Krishnappa
- LPP-O-2 **Morbili virus receptors : New avenues for understanding pathogenesis**
Arnab Sen, V. Balamurugan, J. Sarkar, K.K. Rajak, B. Sahay, P. Saravanan and
R.K. Singh
- LPP-O-3 **Pathogenesis of Hydropericardium syndrome virus in experimental
infection of broilers**
Jyotsna Dubey, Sanjay Shakya, K.C.P. Singh and S.D. Hirpurkar
- LPP-O-4 **Experimental studies on immunosuppressive effects of *peste des petits
ruminants (PPR) virus in goats***
K.K.Rajak, B.P.Sreenivasa, M.Hosamani, R.P.Singh, S.K.Singh
and S.K.Bandyopadhyay
- LPP-O-5 **Production of goat kids using *in vitro* fertilization and embryos transfer
through laparoscopy technique**
Dhruba Malakar, S. K. Das, S. L. Goswami

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NATIONAL SYMPOSIUM AND XI CONVENTION**

OF

INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY & BIOTECHNOLOGY

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| 2. Dr. H.R. Meena | 4. Sri N.S. Bora |



**The XI Annual Convention of
Indian Society for Veterinary immunology and Biotechnology
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**Alternate Strategies for Livestock Production and Protection :
Role of Biotechnology and Immunology**

November 3-4, 2004

A B S T R A C T S



**Indian Veterinary Research Institute
Campus Mukteswar- 263138
Nainital (Uttaranchal) India**



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Scientific Session-I

GENOMICS AND PROTEOMICS : ROLE IN LIVESTOCK PRODUCTIVITY

CHAIRMAN : Dr. Nem Singh

CO-CHAIRMAN : Dr. Deepak Sharma

RAPPORTEUR : Dr. P. Saravanan / Dr. V. Bhanuprakash

PAGE - 1 to 12

GPP-IS-1:

Transgenic chicken through sperm mediated gene transfer

Deepak Sharma and R. R. Churchil

Central Avian Research Institute, Izatnagar, UP-243 122

Transgenic chicken hold great promise in basic biological research and in industrial applications. Beyond their intrinsic value as model systems in biological research, transgenic chickens hold promise in many commercial and industrial applications. Chickens deposit large amounts of proteins into their eggs. A transgenic chicken could conceivably function as a bioreactor for the production of commercially or pharmaceutically important proteins, which are difficult or impossible to produce economically in prokaryotic systems. Gene transfer technology may also prove to be a useful complement to traditional poultry breeding methods in the improvement of commercially important production traits such as growth and disease resistance. There are several methods for producing transgenic chicken such as retrovirus-mediated Gene Transfer, microinjection, artificial chromosomes, chimeric intermediates, nuclear transfer, testis mediated gene transfer and sperm mediated gene transfer. Each one have its own merit and demerits.

Sperm-mediated gene transfer (SMGT) using spermatozoa as the vector for introducing exogenous genes offers an attractive alternative that can ensure a large scale of efficient and reproducible results with experiments that do not require expensive equipment. Several groups have reported that sperm cells from a variety of different species were permeable to exogenous DNA and the transgenic embryos or fetus were produced from oocytes fertilized by spermatozoa that had been exposed to exogenous DNA. Most of the reports have shown higher proportion of incorporation of exogenous DNA into the spermatozoa, but the proportion of transgenic fetuses/progenies were highly variable and more towards lower side. There are different methods of sperm manipulation to assist the DNA uptake such as electroporation, restriction enzyme-mediated integration (REMI), receptor-mediated gene transfer, Intracytoplasmic sperm injection (ICSI) and lipofection. The REMI technique is currently being used to produce transgenic *Xenopus* sp. and recently was applied successfully to cattle and pig. This technique holds promises for production of transgenic chicken.

GPP-O-1:

Species non-specific tumor markers, conformational epitope(s) in immune complex and loss of differentiation antigens in plasma of canines and bovines with tumors

Raman Chopra and Hari Mohan Saxena

*Department of Veterinary Microbiology, College of Veterinary Science,
Punjab Agricultural University, Ludhiana - 141004*

The SDS PAGE analysis of plasma from 70 tumor bearing (61 dogs, 3 cattle and 6 buffaloes) and 14 healthy (7 dogs and 7 cattle) animals revealed new protein bands in tumor bearing animals. Proteins p71 / 72 , p54.5, p53, p51, p49, p46.5, p42, p30.5, p26 / 27, and p19 detected in tumor affected animals were absent in normal healthy animals. New proteins p51, p53, and p46.5 were detected in majority of tumor bearing animals. Proteins p71 / 72, p53, and p51 were detected in bovines with tumors. Proteins p51 and p53 may be possible species non-specific tumor markers. An increase in the plasma levels of p58.5 in 81.20% cases and p35 in 75.40% cases was found in tumor bearing dogs. Proteins p77.5 and p74 present in the plasma of normal animals were absent in tumor bearing animals and could be possible differentiation - associated antigens lost during tumorigenesis. A high reactivity of antiserum to intact whole immune complex (from pooled plasma of mammary tumor bearing dogs) against plasma from tumor bearing dogs, cattle and buffaloes was found by Dot ELISA. Such putative conformational epitope(s) in intact whole immune complex may be possible tumor associated antigen(s) in canines and bovines with tumors. A highly significant increase in total plasma proteins was found in buffaloes with tumors. The plasma Globulin levels were significantly high in tumor bearing buffaloes and dogs with mammary tumors. Plasma Albumin levels were significantly low in dogs with mammary tumors and transmissible venereal tumors. The plasma Albumin : Globulin (A:G) ratio was significantly low in dogs with mammary tumors and venereal tumor, and buffaloes with tumors.

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GPP-O-2:

16S-23S rRNA Intergenic space polymorphism in *Escherichia coli* isolates of cattle

Shuchismita Chatterjee, S.K. Ghorui and S.K. Kashyap

*Department of Veterinary Microbiology,
College of Veterinary and Animal Science, Bikaner (Rajasthan)-334001*

Escherichia coli is the most extensively studied model organism with respect to genetic variations. It is considered as the most abundant commensal organism albeit with some strains having pathogenic potential. It thus becomes important to discriminate between different isolates by various typing systems. Ribotyping is the most reliable method to distinguish between organisms upto generic and species level. In the present investigation different strains of *E.coli* could be discriminated on the basis of polymorphism in their intergenic space between 16S- 23S ribosomal RNA transcription units.

###

GPP-O-3:

Genetic Polymorphism of growth hormone gene in Indian buffalo breeds

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The major thrust area of research in animal genetics is the identification of genes (major genes), which have an important influence on the expression of quantitative traits. One of the potential major genes is the Growth Hormone (GH) gene. Several studies have indicated the associations between polymorphism of the bovine growth hormone gene and production traits. Perusal of literature has indicated the paucity of information on buffalo GH gene.

The present investigation was undertaken to explore any DNA polymorphism in Buffalo Growth Hormone gene by Single Strand Conformation Polymorphism (SSCP) of six amplified fragments covering the entire buffalo Growth Hormone gene. A total of 250 unrelated animals were included in the present study from five different Indian buffalo breeds (*Murrah, Nagpuri, Mehsana, Nili-Ravi* and *Surti*). The PCR amplification parameters were optimized to amplify six different fragments (GH1, GH2, GH3, GH4, GH5, and GH6) in Buffalo Growth Hormone Gene corresponding to bovine GH gene sequence (M57764). The PCR products were denatured and subjected to 7-8% acrylamide: bis-acryamide (49:1) gel. Electrophoresis was performed at 7-15 W for 4-8 hr at room temperature to detect sequence variation by SSCP. Polymorphism was detected in one of these fragments (GH2). Frequency of genotypes varied in different buffalo breeds. The results of our study may be used in research on the association between different SSCP patterns and buffalo performance traits.

###

GPP-O-4:

ERIC-PCR as a tool for molecular typing of avian *E.coli* isolates

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Escherichia coli is one of the common and important bacterial avian pathogen causing wide variety of disease syndromes in farmed birds. Various tools that have been employed to characterize isolates of *E.coli* and to aid in identification of pathogenic strains are often limited to a few select reference laboratories and are not sensitive enough to further differentiate bacterial isolates. A molecular typing method based on the analysis of the enterobacterial repetitive inter-genic consensus (ERIC) sequences has been used to find out the genetic diversity among *E.coli* isolates. In all, fifteen *E.coli* serotypes were subjected to ERIC-PCR, which generated a total of twenty six bands. By plotting dendrograms, these serotypes were grouped into two major clusters with a maximum linkage distance of 25.5 units. Furthermore,

the principal Component analysis (PCA) also revealed similar clustering as in the dendrogram. In conclusion, ERIC-PCR based molecular typing and dendrogram based analysis indicated genetic diversity among *E.coli* serotypes.

###

GPP-O-5:

IGFBP-3 Gene polymorphism and its effect on serum IgG level of cattle

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Insulin-like growth factor binding protein-3 (IGFBP-3) is a protein that binds to majority of IGFs in circulation for regulation of its action on growth and metabolism of the animals. They are also involved in the immune function of animals and thus, are candidate genes for the study of association with immune functions. PCR-RFLP was done to screen crossbred (Holstein Friesian x Haryana) female calves of one year of age. A 651 bp fragment of IGFBP-3 gene spanning over a part of exon 2, complete intron 2, exon 3 and a part of intron 3 was amplified using a set of forward and reverse primers. The amplicons were digested with *HaeIII* restriction enzymes and three genotypes (AA, AB and BB) were observed. The serum IgG level was estimated using SRID assay. The mean level of serum IgG was 28.83 ± 2.73 mg/ml. The effect of these identified genotypes on serum IgG level of calves at one year of age was analysed using least squares analysis. The *HaeIII* RFLP-AB genotype had significantly ($P < 0.05$) higher serum IgG level (31.86 ± 3.05) than the *HaeIII* RFLP-AA (25.62 ± 2.96) genotype. The present results indicated role of IGFBP-3 on serum IgG level of cattle calves.

###

GPP-O-6:

Identification and cDNA characterization of three novel CC-chemokines from domestic duck (*Anas platyrhynchos*)

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In order to understand the duck immunology better and to identify molecules that will play role in the immune response, a subtracted cDNA library was made from mitogen stimulated duck splenic lymphocytes. The library was enriched for differentially expressed sequences by one round of subtraction with cDNA from unstimulated cells. The clones were screened for novel cytokine-like and other immunomodulatory proteins by random sequencing and comparative sequence analyses. Among the three chemokine clones identified, two clones showed significant identity to chicken homologue of RANTES and MIP- β , respectively, The third clone showed low to medium level of identity to a large number of chemokines, which belonged to MIP- α and MCP group of proteins. Initial characterization of these molecules by sequence analysis and bioinformatics approach will be presented.

###

GPP-O-7:

Heterodimeric expression of functional recombinant Interleukin-12 of buffalo (*Bubalus bubalis*) in HEK293Tcells

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Interleukin-12 is an early cytokine produced mainly by antigen presenting cells like dendritic cells and macrophages. IL-12 is a heterodimeric cytokine consisting of p35 and p40

subunits linked together by a disulphide bond. Co-expression of both the p35 and p40 subunits of the IL-12 heterodimer is necessary for the formation of the bioactive IL-12. Moreover, equal expression of the subunits is critical, since over expression of the p40 subunit leads to the formation of p40 homodimers, which may antagonize the IL-12 action. The cDNAs-encoding buffalo IL-12p35 and p40 subunits were amplified by RT-PCR from Concanavalin A stimulated splenocytes. A recombinant buffalo IL-12 expression construct expression both the subunits was made by utilizing the autocleavage properties of the 2A polypeptide of the Foot and Mouth Disease virus (FMDV), which was used as a linker between the two subunits. Western blot with IL-12 specific antibody of 293T supernatants transfected with the expression construct, revealed expression of IL-12 heterodimer. Furthermore, supernatants showed a dose dependent induction of NO production in bovine splenocytes confirming the bioactivity of the recombinant buffalo IL-12.

###

GPP-O-8:

Polymorphism in buffalo growth hormone second exon gene

U. Sarkar, A. K. Gupta, S.De, T. K. Datta, S.L. Goswami, M.D. Muhaghegh, V.S. Raina, T.K. Mohanty, V. Sarkar, K. Yogesh and P. Yadav

Animal Biotechnology Centre, N.D.R.I., Karnal

The growth hormone (GH) plays a vital role in growth, lactation and mammary development in dairy animals and it could be used as a useful candidate gene for milk production and reproduction. Polymorphism identified at exon sequence level for this important gene have been reported to indicate variation in production and reproduction traits in cattle and other livestock species. In the present study genomic DNA of 93 Murrah buffaloes available at NDRI herd were subjected for amplification of growth hormone exon 2 gene and Single-strand conformation polymorphism (SSCP) technique was used to identify polymorphism pattern in the same exon. The oligonucleotide primer set used to amplify the buffalo gene was designed from a corresponding orthologous bovine sequence information (M57764). A 510 base pair region including the second exon and portions of flanking introns was selected for PCR amplification. The optimized PCR condition of initial denaturation at 94°C for 4 min followed by

30 cycles of denaturation at 94° C for 45 sec, annealing at 59°C for 30 seconds and extension at 72°C for 25 second yielded a specific amplification of ~ 460 bp fragment. After denaturation of the amplified PCR product at 95°C for 5 minutes followed by snap cooling and resolving on 7% acrylamide gel for 6 hours (70 Volts) revealed two types of band patterns indicating a polymorphisms at nucleotide sequence level. The frequency of two polymorphism patterns was 0.23 and 0.77 among the animals studied.

###

GPP-O-9:

Synthetic peptide strategies in evaluating reactive epitope of monoclonal antibody specific to haemagglutinin protein of PPR virus

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Peste des petits ruminants (PPR), a highly contagious viral disease of small ruminants is caused by PPR virus, which is a member of genus *Morbillivirus* under the family Paramyxoviridae. Several outbreaks of PPR have been reported from almost every part of the country resulting into severe economic losses owing to high mortality and morbidity in infected small ruminants. Out of the two surface glycoproteins of PPR virus, haemagglutinin (H) protein recognizes the host cell receptor to initiate the infection. Monoclonal antibodies have been widely used for diagnosis of this disease and also to determine the protein domains responsible for attachment of virus to host cells. Epitope mapping of monoclonal antibodies using synthetic peptides is a valuable technique that provides information on region responsible for antigen-antibody recognition.

We have used two prong strategies using computer modeling for epitope mapping and synthetic peptide approach to identify the epitope of anti-PPR monoclonal antibody raised against H protein of PPR virus. Twenty-two different immunogenic regions identified using

antigenic modeling were synthesized as bead bound peptides, varying in length from 7mer to 25mer using manual solid phase peptide synthesis employing Fmoc chemistry. The peptides were made to react with anti-H monoclonal antibody by performing indirect ELISA. It was found that the binding site of monoclonal antibody is fully contained within a short continuous peptide sequence of the protein, thereby depicting that the antibody recognizes a linear epitope on H protein. Interestingly, this sequence is unique to PPRV and is conserved. These peptides were also evaluated using anti-RPV hyperimmune serum to establish their specificity. The identified specific epitope can be used for differential diagnosis of PPR and RP. Further studies are being carried out for identifying the precise length for this peptide sequence to determine the specific residues that are important for epitope recognition.

###

GPP-O-10:

Cloning of mature E2 gene of swine fever vaccine virus

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A 1110 bp PCR product corresponding to E2 glycoprotein gene was amplified from a classical swine fever vaccine virus genome, with a start and stop codon. The PCR product was ligated into pGEMT vector. Subsequently a 1032 bp product amplified from the recombinant pGEMT/E2 plasmid was ligated into pTarget mammalian expression vector with start and stop codons in the insert. This removed the transmembrane region in order to express a mature E2 protein on the surface of the cells following transfection with recombinant plasmid.

###

GPP-O-11:

Recent molecular epidemiology of *peste-des-petits ruminants* virus in India

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Peste des petits ruminants (PPR) is an economically important viral disease of goats and sheep first described in West Africa in the 1940s. PPR was first reported in India in 1987 from Arasur village in Tamil Nadu and 1994 onwards disease has become endemic throughout the country. In order to elucidate the recent epidemiological status of PPR in India, seven isolates (2000-2003) of PPRV from different states were employed in the present study for phylogenetic & molecular epidemiological studies. These isolates represented all the geographical regions of India. To study the genetic relationship between isolates, a selected region (322 bp) of the fusion (F) protein gene of various isolates of PPRV were amplified using RT/PCR and the resulting DNA product sequenced for phylogenetic analysis. In addition to these, the sequence data of 19 other isolates from India and other countries available in GenBank were also used in analysis.

The phylogenetic relationship between isolates was studied using MEGA software. On the basis of *F* gene sequences, all the isolates included in present study grouped along with all other Indian isolates in lineage-4. Two groups of 2 isolates each (PPRV-TN-Cbe-Dhinakar, PPRV-TN-Ara-Dhinakar) and (Ind-IN-2002-G, Ind-TN-2002-S2) from Tamilnadu and one group of 2 isolates from Himachal Pradesh (PPRV-Sungri-96 and PPRV-HP-Uri-99) discerned on the basis of geographical relatedness in the phylogenetic tree. However, the analysis on the basis of state of origin of isolates showed that isolates from Rajasthan and Gujarat formed a separate phylogenetic group. The isolates from Andhra Pradesh and Jammu & Kashmir also branched separately. Isolates from all other states were closely related. The isolates from Eastern region showed the maximum variation (1.90 %) in amino acid sequences. The isolates from North and South did not show any variation at all

at amino acid level, whereas those from East and West did show some variation (0.63 % and 1.43 %).

The present study confirms the presence of a single lineage (lineage 4) of PPR virus in India and also indicates that isolates from eastern region differ from others. The close relatedness of isolates from different regions indicate spatial mixing of virus types which could be because of highly infectious nature of PPRV combined with geographical spread due to movement of animals from one part of country to others, sometimes over very long distances.

Scientific Session-II

NEW DIMENSIONS AND DEVELOPMENTS IN ANIMAL DISEASE DIAGNOSTICS

CHAIRMAN : Dr. G. Butchiah

CO-CHAIRMAN : Dr. V. Thiagarajan

RAPPORTEUR : Dr. C. Ramakrishna/ Dr. Hira Ram

PAGE - 13 to 20

ADD-O-1:

Detection of avian infectious bronchitis virus (IBV) in field samples by reverse transcription-polymerase chain reaction (RT-PCR)

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A total number of 12 field samples suspected for avian infectious bronchitis virus (IBV) were received from different states of India viz. Maharashtra (5 isolates), Uttar Pradesh (2 isolates), Rajasthan (1) and Haryana (4 isolates). All the samples were passaged in allantoic cavity of 9 day old specific pathogen free (SPF) embryonated chicken eggs (ECE). At 48 hours of incubation, 5 ECE for each sample were chilled and the allantoic fluid harvested for the detection of IBV by reverse transcription-polymerase chain reaction (RT-PCR). Rest 5 of the ECE was incubated for 7 days till the characteristic stunting, curling and dwarfing was observed. The presence of urates and thinning of chorioallantoic membrane (CAM) were also noticed. Since IB in chicks can be confused with other similar respiratory disease conditions, therefore many a times it goes unnoticed in field because of the masking of pathognomic symptoms by secondary/mixed infections, which is commonly observed. Therefore, the present study was formulated to detect IBV field infection by testing for the specific nucleic acid by employing the recent rapid and confirmatory molecular biological technique like reverse transcription - polymerase chain reaction (RT-PCR). Viral RNA was extracted from the harvested 250 μ l of the pooled allantoic fluid for each sample separately. Reverse transcription (RT) was performed using avian myeloblastosis virus reverse transcriptase and the cDNA generated was used for PCR amplification employing 7 sets of primers. The primers used in the present study were specifically designed to amplify the S1 (spike) gene (5 sets) and the untranslated region (UTR) (2 sets) of IBV. Using these primers the expected virus specific amplified product size corresponding with the primer sets used were obtained with 4 field isolates from Maharashtra, 1 from Uttar Pradesh, 1 from Rajasthan, and 2 from Haryana. Thus confirming the presence of IBV infection in 8 out of 12 field samples examined. Further research works on the field IBV isolates by pathogenicity testing, restriction enzyme analysis and sequencing works are going on in the laboratory for further characterization of the IBV field isolates.

###

ADD-O-2:

Evaluation of monoclonal antibody based immunoassay in the detection of infectious bursal disease virus

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and R. Govindarajan**

Tamil Nadu University of Animal and Fishery Sciences, Chennai

Laboratory Confirmation of the incidence of infectious bursal disease (IBD) could be made by antigen or antibody demonstration and virus isolation. There are a few commercial antibody demonstration kits available, but none for antigen detection. Monoclonal antibody based antigen capture ELISA (AC-ELISA) and Dot-ELISA were carried out for the detection of IBD and to study their suitability for routine laboratory use. Bursal samples from 120 flocks suspected for IBD was screened for infectious bursal disease virus (IBDV) antigen by these two methods and also by agar gel immunodiffusion (AGID) test. These three tests were compared for their efficiency in the detection of IBD. Out of 120 samples AC-ELISA detected 74 (62%) as positive, Dot-ELISA detected 70 (58%) and AGID test detected only 53 (40%) as positive for IBDV antigen. AC-ELISA was found to be more sensitive followed by Dot-ELISA than AGID test in the detection of IBDV antigen from field specimens.

###

ADD-O-3:

Diagnosis of infectious bursal disease in poultry in field outbreak using RT-PCR

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Infectious Bursal Disease (IBD) caused by a birna virus, has been an economically significant and widely distributed condition affecting immature chickens since 1960 world

wide and from 1971 in India. Recently there have been several reports, which indicate that outbreaks of the disease have occurred in vaccinated flocks. In order to diagnose the disease quickly with high sensitivity, an RT-PCR was standardized for the detection of the virus in field samples. The total cellular RNA was isolated by guanidium method from bursa of Fabricius, which was collected from the dead birds suspected for IBD. The isolated RNA was subjected to RT-PCR using VP-2 gene specific primers. The test was carried out using Invitrogen(Life Technologies, India) RT-PCR kit following the manufacturer's protocol. Out of six samples analyzed, all gave the positive tests. The results were authenticated by comparing the size of the RT-PCR product with molecular weight markers and also by the standard agar gel precipitation test (AGPT) using the hyperimmune serum raised against IBDV.

###

ADD-O-4:

Development and standardization of single serum dilution ELISA kit for quantification of antibodies against Newcastle disease virus

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A Single serum dilution ELISA kit has been developed for quantifying antibodies against Newcastle disease. The kit was developed for predicting the antibody titre based on linear regression ($y=ax+b$). Using negative samples a positive negative threshold line (PNT) was developed. The formula for prediction of titre was developed using 191 quality serum samples with varying antibody levels. The ELISA titre against these samples was estimated first and the observed titre (OT) was compared with the optical density values of varying serum dilutions. The dilution (1:1000) that gives maximum correlation coefficient value with OT was chosen as dilution for predicting the antibody titre - predicted titre (PT). This technique has been extensively in-house validated with QC samples following guidelines specified in

OIE Manual - Standards for Vaccines and Diagnostics - 2000. The kit was checked for its repeatability (one quality control (QC) sample at four time points and twenty QC samples at four replicates, reproducibility (between ELISA plates and between Operators), Diagnostic Sensitivity (DSn), Diagnostic Specificity (DSp), Correlation with other tests, Kappa Statistics (estimation of k value), receiver operating characteristics (ROC) etc. The kit developed performed well and the results obtained were found to be repeatable, reproducible, correlates with other diagnostic tests and have high diagnostic sensitivity and specificity. The antigen used for coating was also checked for purity and found to be pure with no cross-reaction to infectious bronchitis, infectious bursal disease etc. The antigen-coated plates were found to have shelf life of fourteen days. The efficacy of the kit has been checked with more than 3000 field samples and the results were found to be reliable. The kit was also found to be suitable in estimating the egg yolk antibodies (IgY). The kit also picked up rise and fall in antibody titre in a controlled vaccination trial with virulent virus challenge. The kit was found to be suitable in quantification of antibodies against NDV in ducks and turkeys (with respective conjugates). The kit was also successfully validated in other laboratories. The kit was also checked with international reference serum.

###

ADD-O-5:

Development of 3M KCl solubilized Rous Sarcoma extract or transformed fibroblast antigen (TfAg) as a diagnostic antigen for AL/SV infections in poultry

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Immunology Section

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Avian leucosis / Sarcoma infections constitute a chronic economic problem for the poultry industry, as only few AL/SV infected flocks develop typical clinical lesions, others remain as carriers and shedders. 3M KCl solubilized Rous Sarcoma extract or TfAg appeared to provide a convenient alternate antigen to detect AL/SV infections in commercial chickens

in view of the lack of differentiation of exogenous and endogenous AL/SV infections by routine serological assays. Twenty hatchmate chicks were infected on DOH 21 with RSV @ 2000 pk.f.u. / 0.2 ml / chick, s/c in the right wing web and reared separately from control uninfected chicks. Standardization of indirect ELISA was done by employing TfAg as the standard antigen, chicken anti - TfAg hyperimmune serum as reference positive and pooled RSV uninfected chicken serum collected on day of hatch 17 as reference negative serum. The results indicated that RSV infected chicks mean serum anti - TfAg antibody levels were significantly higher as compared to RSV uninfected chicks which confirmed establishment of RSV infection following experimental infection. Further, the results indicated that the RSV uninfected chicks were also getting similar kind of exposure to infection from the environment, equivalent to experimental RSV infection in RSV infected chicks. Thus, the observations in this study indicated that the TfAg could detect the infective status of the commercial chickens resulting from exposure to environment as with RSV uninfected chicks, and from experimental inoculation as in the RSV infected chicks.

###

ADD-O-6:

Cloning and sequencing of Type 4 fimbrial gene of *Pasteurella multocida* serogroup B:2(strain P₅₂)

**Joseph Siju, A.A.Kumar, S.B.Shivachandra, P.Chaudhury, S.K.Srivastava
and V.P.Singh**

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Pasteurella multocida B:2 is the causative agent of Haemorrhagic septicaemia, a fatal disease in cattle and buffaloes. The mechanisms involved in the pathogenesis of the disease are poorly understood. Type 4 fimbriae are considered to be one of the surface structures which favour the adhesion of the organism to the host respiratory tract. In the present study efforts were made to see the differences in the Type 4 fimbrial gene (ptfA) of *Pasteurella multocida* B:2 which causes septicaemic form of the disease and that of *Pasteurella multocida* A:1

causing fowl cholera, a localized form of disease in poultry. The sequence analysis revealed that there exists only 78.4% similarity for the fimbrial gene (ptfA) between these two serogroups. Further the differences in the ptfA gene of *Pasteurella multocida* B:2 are present only at the 3' end of the gene, encoding a 100% conserved N-terminal end and a markedly differing C-terminal end. Further study will have to be carried out to confirm whether these differences contribute to the differences in pathogenesis among various serogroups of *Pasteurella multocida*.

###

ADD-O-7:

Comparative efficacy of different enrichment broths, PCR, ELISA and MAT for diagnosis of salmonellosis in equines

N. Babu, B.R. Singh, Harishankar, Ravi kant Agrawal, Mudit Chandra, T.V.Vijo, S.K. Srivastava and M.P. Yadav

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In a cross-sectional study on 245 equines, *Salmonella* was isolated from faecal samples of 35 animals (14.2%), eight of these were found to be excreting *Salmonella* belonging to more than one serovar. A total of 43 isolates were serotyped into 10 serovars viz. *S. I. 4, 5; 12, 27: r i: 1, 5* (13), *S. Drogana* (9), *S. Lagos* (6), *S. Kottbus* (4), *S. Bovismorbificans* (4), *S. Dumfries* (3), *S. Tshiongwe* (1), *S. I. 3, 10, 15: r:-* (1), *S. I. 6,7: y: lz28* (1) and *S. enterica ssp salamae* 6,7:g t: z42 (1). Maximum isolations could be made on enrichment of faecal samples in tetrathionate broth (TTB) while other enrichment media viz., selenite cystine (SC) broth, Rappaport Vassiliadis (RV) broth and *Salmonella* enrichment broth (SEB) yielded variable results. A total of 63 out of 188 faecal samples screened by *Salmonella* specific PCR using *his J* gene primers yielded a specific product of 496 bp. However, *Salmonella* could be isolated from only 50.8% of PCR positive samples. Prevalence of *Salmonella* antibodies in serum of

equines with ELISA (using cytotoxin I), MAT-H (Microagglutination test using flagellar antigens) and MAT-O (Microagglutination test using somatic antigens) was 66.3%, 90.05% and 47.4%, respectively. The results of different tests had little or no correlation among themselves and with isolation of *Salmonella* from faecal samples.

###

ADD-O-8:

Monoclonal antibody and outer membrane protein based single dilution ELISA for antibodies against Haemorrhagic septicaemia in serum of cattle and buffaloes

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An ELISA using outer membrane protein (OMP) of *Pasteurella multocida* (B:2) as antigen and monoclonal antibody raised against buffalo IgG2 was developed to estimate antibody levels in serum of cattle and buffaloes. The test is sensitive to detect as little as 25ng/ml of the antibodies and diagnostic sensitivity and specificity are 99.6% and 98.1% respectively. To have the test more specificity, cut off value favoring diagnostic specificity was chosen. In repeatability studies, the coefficient of variation was less than 20% for interplate as well as between the runs of the test. To test more number of samples speedily as well with economy, the test has been converted in to single dilution ELISA with the capacity of an ELISA plate to test 88 samples. The test has also been converted in to user friendly Kit. Based on a monoclonal antibody that cross reacts equally well with both cattle and buffalo IgG, the test is suitable to estimate anti OMP antibodies levels in serum samples of both cattle and buffalo. Development of the test, its conversion in to the single dilution ELISA and about the kit are described.

###

Scientific Session-III

NOVEL DEVELOPMENTS IN ANIMAL DISEASE PROPHYLACTICS

CHAIRMAN : Dr. M.S. Oberoi

CO-CHAIRMAN : Dr. J.M. Kataria

RAPPORTEUR : Dr. A. Sen / Dr. V. Balamurugan

PAGE - 21 to 32

ADP-O-1:

Studies on immunizing property of *E.coli* pili vaccine in chickens

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Attachment/adhesion of *Escherichia coli* to host cell surfaces especially mucosal surfaces is an essential prerequisite for the initiation of infection. Adhesion is mediated by adhesions on the bacterium and complementary receptors on the host cell membranes. Avian pathogenic *E. coli* serotype 078 and O2 possess somatic pili which serve as adhesions. The adhesion of infectious agents to host cell surfaces can be prevented by antipili antibodies. In present study we have evaluated the immunogenicity of an oil emulsified *E. coli* (serotype 078) pili vaccine against colibacillosis in chickens. Challenge study, prevention of mucosal colonization of bacteria and histopathological study of tissues were the parameters to evaluate the vaccine response.

For assessment of ability of pilus vaccine to prevent mucosal colonization to tracheal epithelial cell, adhesion index (AI) and adhesion ratio (AR) were calculated. The vaccinated birds had AI = 2.87, A.R. = 11.57% for homologous serotype 078 and AI=2.58 and AR = 15.91% for heterologous serotype O2 whereas control birds had AI =5.79 and AR= 59% for serotype 078 and AI= 5.86 and AR= 59% for serotype O2. In challenge study, there was 100% protection upto 4 wpv for homologous challenge while 100% protection in 2 wpv which was reduced to 71% and than 57.14% during successive challenges with heterologous serotypes. The gross lesions in the air sacs, pericardial sacs and livers in vaccinated chickens challenged with homologous serotype were significantly different (P/0.05) and mild when compared with severe serofibrinous airsacculitis, pericarditis of control groups. Heterologous challenged birds had moderate lesions in the airsac and pericardium which were not significantly different (P 0.05), from unvaccinated chickens. The reisolation of *E. coli* from heart blood indicates that vaccinated birds eliminated *E. coli* more efficiently than their unvaccinated counterparts.

###

ADP-O-2:

Propylactic vaccination against anthrax in endemic area- an investigation

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and G. Krishnappa**

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Anthrax continues to be problem in many parts of the country. There are many endemic *loci* for anthrax in the state like Kolar, Hassan, Bangalore rural, etc. and preventive vaccinations are being carried out to control the infection. The disease outbreaks are being reported in these endemic areas in spite of preventive vaccinations. Investigations carried out had brought important issues to be considered for control of infection. Improper carcass disposal, lack of awareness among farmers, poverty and continuous draught have further complicated the situation. Results of testing of soil samples, presence of antibody titres in street dog, man, and Post vaccinal antibodies in vaccinated animals will be discussed during the presentation.

###

ADP-O-3

Immunogenicity of aerobic and anaerobic harvests of *Pasteurella multocida* P52 in murine model

S. Sudha Rani, V.K.Chaturvedi., K.D.Pandey. and P.K.Gupta

Indian Veterinary Research Institute, Izatnagar

The comparative immunogenicity of aerobically and anaerobically grown *Pasteurella multocida* was ascertained by immunization of mice with oil adjuvant vaccines prepared from aerobic and anaerobic harvests. One group of mice was subjected to single immunization on day first of the experiment while the other was subjected to double immunization on day first

and fourteenth. The sera collected at different time intervals were analyzed for humoral immune response by employing MAT, ELISA and IHA. The cell mediated immune response was assessed by Macrophage Disappearance Test. Challenge studies were conducted with 10⁴ LD₅₀ of *Pasteurella multocida* on 21st and 75th day post immunization. MAT, ELISA and IHA titres followed a similar trend of immune response. MAT and IHA titres were shown to be having good correlation with protection. Anaerobic vaccine was found to give more protective immune response compared to aerobic vaccine.

###

ADP-O-4:

A combined vaccine against goat pox and Peste des Petits ruminants induces protective immune response in goats

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Safety and immunogenicity of an experimental combined vaccine comprising attenuated strains of *Peste des Petits ruminants virus* (PPRV) and goat poxvirus (GPV) was investigated in goats. Goats immunized subcutaneously with .1ml of vaccine consisting of 10³ TCID₅₀ of each of PPRV and GPV in 1.0 ml dose were monitored for clinical and serological responses for a period of 4 weeks post immunization (PI) and following challenge. Specific antibodies directed to both GPV and PPRV could be demonstrated by indirect ELISA/SNT and competitive ELISA, respectively following immunization. All the immunized animals resisted challenge with virulent strains of either GPV or PPRV on day 28 PI, while control animals developed characteristic signs of disease. Specific antigen could be detected in all the unvaccinated control animals after challenge but not from any of the immunized goats. The combined vaccine protected goats against both PPR and goat pox and did not show any reactogenicity.

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ADP-O-5:

Peste des petits ruminants (PPR) in small ruminants

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Pests des petits ruminants (PPR), an acute viral disease of goats and sheep in characterized by Pyrexia, catarrhal inflammation of ocular and nasal membrane, erosive stomatitis, gastroenteritis and pneumonia. In India, PPR was first reported in Tamil Nadu (Purushothaman *et al.*, 1987) and subsequently reported from other parts of the country (Kulkarni *et al.*, 1998; Singh *et al.*, 1999). The disease has emerged as a major constraint to extensive small ruminant farming in recent years. The present paper reports an outbreak of PPR among sheep and goats in an organised farm. The haematology, Immunohistochemistry, Molecular Characterization was discussed. Modern trends in diagnosis and control of Peste des Petits Ruminants (PPR) was described in this context.

###

ADP-O-6:

Development of molecular diagnostics, prophylactics and therapeutics against bacterial and viral agents

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The work reported here encompasses the research done for development of molecular diagnostics, prophylactics (vaccines) and therapeutics against bacterial and viral agents. Development of molecular diagnostics includes PCR, PCR-restriction enzyme analysis (PCR-

REA), multiplex PCR for detection and differentiation of goatpox virus, sheeppox virus and buffalopox virus. Similarly, vaccine development includes a combined vaccine for goatpox and PPR, a DNA vaccine for goatpox, STM-generated *Salmonella Abortus equi* mutants as vaccine candidate for equine abortion and a Vero cell adapted buffalopox vaccine. The genome analysis of MHC class II DRB 3 exon 2 genes of cattle and buffalo as well as sheep, goats and deer revealed interesting information. A total of 48 accession numbers were added to genome sequence database. Molecular analysis of buffalopox virus genome and proteins also revealed interesting data. Towards therapeutics against viruses, RNA Interference (RNAi) approach was used against PPR virus and found to be effective in *In vitro* system reducing the virus titre by 1.4 log (25-fold). The information generated out of the work has been presented in the compilation (enclosed) and will also be discussed while presenting the work.

###

ADP-O-7

Preparation and standardization of oral pellet vaccine to control newcastle disease in village chickens

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Newcastle disease viruses (NDV) of varying intracerebral pathogenicity index (ICPI) were isolated from village chickens that were apparently normal and were never vaccinated against ND. Nearly 160 cloacal swabs were screened for NDV, and 16 isolates were obtained. The ICPI values ranged from 0.14 to 1.74. It was observed that though village chicken had NDV with high ICPI, they did not show any symptoms and it is attributed village chicken harbor ND and possible incidence of ND in village chicken may be as a result of immunosuppression caused by other agents. The virulence of NDV isolates was further confirmed by the DNA sequencing of F protein cleavage site (FPCS). The amino

acid sequence between 111-119th position strongly correlated with ICPI values and the results substantiated the fact that village chicken harbor NDV without any characteristic symptoms and shed them. One such isolate (D58) with low ICPI (0.14) was purified by triple terminal dilution and thermostabilized by exposing it at temperature cycles of 25°C for 36 days; 37°C for 29 days and 56°C for 6 hours. After exposure to each cycle the virus was passaged in embryonated eggs. The thermostability was also confirmed by stability of haemagglutinin at 56°C after every passage in embryonated eggs. Oral pellet vaccine was prepared by following lactose starch pellet technology. The vaccines were standardized for safety, purity, potency as per the guidelines for live ND vaccine specified in OIE Manual - Standards for Vaccines and Diagnostics - 2000 and the vaccine grains were found to be safe, pure and potent. Three different types of controlled laboratory trials were carried out at Biosafety laboratory Level-II. The three trials were (1) The grains were given to maternal antibody free chicks (2) The grains were given to chicks that received the D58 isolate as priming vaccine instead of F strain (3) The grains were given to birds at 12th week, which were earlier vaccinated with F strain and LaSota. In all the three trials good sero-conversion was noticed. With convincing results, controlled field trials were carried out in village chickens. The vaccine grains were given as feed is given to birds. The vaccine grains were well taken by birds and good sero-conversion was noticed as evident from Haemagglutination inhibition (HI) and enzyme linked immunosorbant assay (ELISA) titres 21 days post vaccination. No harmful post-vaccinal reactions were reported so far in field trials. The D58 isolate, which was thermostabilised was also used as priming vaccine for commercial broiler chicks instead of regular F strain. Good sero-conversion was noticed, which was evident from Haemagglutination inhibition (HI) and enzyme linked immunosorbant assay (ELISA) titres 21 days post vaccination. The birds also had protective immunity till the birds were sold (45-50 days).

###

ADP-O-8:

Antigenic and genetic analyses of foot-and-mouth disease virus: type A isolates from India for selection of candidate vaccine strain

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Previous analyses indicated two antigenic and genetic groups (genotypes VI and VII) of FMDV type A in India that are divergent from the current vaccine strains. In order to choose a suitable field isolate as candidate vaccine strain, 4 each genotype (VI and VII)-specific anti-sera against representative isolates were tested in neutralization assay. Between the two reference strains from both the genotypes that outperformed the others, IND 81/00 (genotype VII)-specific anti-sera gave a better antigenic coverage (r-value >0.40 with 79% of isolates of 2002-03) than IND 258/99 (genotype VI) (r-value >0.40 with 42% of 2002-03 isolates). In the capsid-based phylogeny, all the recent isolates (2001-2003) belonged to genotype VII and none were grouped with genotype VI, with emergence of a new variant virus with amino acid deletion at an antigenically critical residue (VP3 59), indicating a major evolutionary jump probably due to immune selection. Though very limited in its extent, this data indicates an apparent dominance of genotype VII over genotype VI and underscores the need to continue further molecular epidemiological investigations to substantiate this finding.

###

ADP-O-9:

Kinetics of humoral immune response in pigs following FMD vaccination

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The present investigation was carried out to study the virus-specific humoral immune response in pigs, following vaccination with inactivated oil adjuvanted polyvalent FMD vaccine, upto 90 days post vaccination (dpv). For this, 40 LWY pigs (20, one-year old gilts and 20, three-month old piglets) were vaccinated @ 2 ml/animal, subcutaneously. Sera samples were collected at fortnight interval from all the animals. Half of the gilts (Group A) and half of the piglets (Group D) were bled on odd weeks post vaccination, whereas remaining gilts (Group B) and piglets (Group C) were bled on even weeks post vaccination. Sera samples so obtained were analyzed by SNT to determine serum neutralizing antibody titres in order to study the humoral immune response (HIR).

The log₁₀SN₅₀ antibody titers against all the serotypes (Type O, A & Asia 1) were detected in both gilts and piglets from day 7 to 90 dpv indicating the occurrence of HIR upto the last day of sampling. The maximum SN antibody titres were observed on 28 dpv, thereafter, titres started declining, but were still present on 90 dpv against all the three FMDV serotypes. HIR was more pronounced in piglets in comparison to gilts as group mean SN antibody titres against all the FMDV serotypes were significantly ($P < 0.05$) more in piglets as compared to gilts. Therefore, from these findings, it can be concluded that HIR was generated in pigs and piglets upto 90 dpv following FMD vaccination. The peak response was observed on 28 dpv. Piglets exhibited a significantly ($P < 0.001$) higher HIR as compared to gilts.

###

ADP-O-10:

Immuno-reactivity of Foot and Mouth disease virus serotype 'A' encapsulated in poly (DL lactide-co glycolide) micro sphere

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Foot and Mouth disease (FMD) is a highly contagious and economically most important disease of cloven-footed animals. It is of immense concern to the world livestock industry as it is endemic in several countries including India. Vaccination against disease is achieved by parenteral administration of inactivated virus adjuvanted with alhydrogel and metabolizable oils. These vaccines are effective with high virus neutralizing titres, but for short duration of time. As initial multiplication of virus takes place in upper respiratory tract, mucosal immunity against virus bears immense significance to prevent the initial infection and subsequent shedding of virus in the environment, which may help to build herd immunity. Based on this approach, inactivated whole virus and C-terminal half of VP1 purified protein of FMDV serotype 'A' was encapsulated in poly (DL Lactide-co-Glycolide)[PLG], which was found to be an effective antigen delivery system for mucosal as well as parental immunization. Virus and purified protein encapsulated microspheres were prepared by solvent evaporation method and characterized by SEM, antigen loading and release study by dot-ELISA. Guinea pigs were immunized with microencapsulated antigens (Virus / Protein) by intranasal and subcutaneous routes found protective levels of virus neutralizing antibodies in sera and secretory immunoglobulin-A (SIgA) in nasal washes. SIgA responses were found better in intranasal immunized guinea pigs, cell mediated immune response also observed by PLG encapsulated antigens in guinea pigs.

###

ADP-O-11:

Immuno-reactive protein gene construct of Foot and Mouth disease virus serotype 'A' as DNA vaccine

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Foot and mouth disease (FMD) is the major constraint to international trade in livestock and animal products. FMD is one of the highly contagious and economically important viral diseases of cloven-hoofed animals such as cattle, buffaloes, sheep, goats and pigs. Though conventional vaccines i.e. tissue culture inactivated vaccine has shown to provide adequate protection, it has several limitations like, short duration of immunity and poor cell mediated immune response. DNA vaccines are believed to induce both cell mediated as well as humoral immune response. Present work involves the synthesis of a candidate DNA vaccine construct with VP1 gene and IRES of FMDV serotype 'A' and preliminarily studying the efficacy in guinea pigs.

VP1 of serotype 'A' was cloned into pcDNA vector containing IRES. The construct (pcDNA-IRES-VP1) was transfected into BHK-21 cells and suitably expressed. DNA vaccine constructs (pcDNA-IRES-VP1) were injected in guinea pigs to study the induction of immunity. The protective ELISA titers obtained at 28th day post vaccination were 1:20 and 1:40 for naked DNA and DNA adjuvanted with PLGA respectively. Whereas SNT titers ranged between 1:8 with naked DNA and 1:16 with PLGA- DNA combination. Challenge result in guinea pigs indicated approx. 83% protection with PLGA adjuvanted DNA construct in contrast with 50% protection with naked DNA administered alone.

###

ADP-O-12:

Induction of systemic and mucosal immune responses in poultry by intranasal immunization with microencapsulated EDS-76 virus

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Formalin inactivated EDS-76 virus was encapsulated in the poly-lactide-co-glycolide (PLG) microspheres by solvent evaporation technique. White Leghorn layer chicks were immunized with a single intranasal dose of 75 µg of the microencapsulated EDS-76 virus and then both systemic and mucosal immune responses were evaluated. It was found that the microencapsulated EDS-76 virus could induce a considerable level of virus specific circulating IgG with a peak titre of (\log_{10}) 3.77 at the 3rd wk post immunization (PI) as measured by indirect ELISA. However the IgG level was comparatively less than that induced by the conventional oil adjuvanted EDS-76 vaccine, which showed the peak IgG titre of (\log_{10}) 3.90. Similarly, the haemagglutination inhibition (HI) titre in the serum of the immunized chicks was found to be (\log_2) 8.0, whereas that induced by the conventional vaccine was (\log_2) 9.0. In the intestinal and tracheal washings of the immunized chicks, considerably higher levels of virus specific IgA were detected with the peak titres of 32 and 40, respectively at 3rd wk PI as compared to the conventional inactivated EDS-76 vaccine, which showed very negligible IgA level (titre <2.0). The cell-mediated immune responses induced at mucosal sites were evaluated by assessing the lymphoproliferative responses of the intestinal intraepithelial lymphocytes (IEL), which were significantly higher ($p < 0.05$) with a peak stimulation index value of (1.375 ± 0.012) at 3rd wk PI as compared to the microsphere alone (control) and the conventional EDS-76 vaccine. From this study it can be concluded that intranasal immunization with the microencapsulated EDS-76 virus was able to induce both systemic as well as the mucosal immune responses in poultry.

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Poster Session

PAGE - 33 to 58

GPP-P-1

Molecular characterization of Indian isolates of avian reovirus (ARV) isolated from different disease manifestations

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The present study was conducted to ascertain the differences at genome level of the Indian isolates of avian reovirus (ARV) isolated from different disease manifestations. The four ARV isolates (VA-1, 547/AD/01, 604/AD/02 and MS-6) were passaged in the chicken embryo fibroblast (CEF) cell culture and the viral RNA extracted by TRIzol method, was subjected to reverse transcriptase - polymerase chain reaction (RT-PCR). A 422 bp fragment containing partial overlapping open reading frames (ORFs) of p10 and p17 genes of the S1 region could be amplified from the extracted RNA of all the isolates under study. The restriction endonuclease enzyme (RE) analysis of the amplified 422 bp PCR product of all the isolates, using three enzymes viz. *Sac* I, *Hpa* II and *Dde* I, revealed no differences. Further, the amplified products of all the ARV isolates were cloned in the pGEM-T PCR cloning vector. The recombinant clones were screened by PCR amplification and digestion with *Apa* I and *Pst* I enzymes for the presence of insert. The cloned part of S1 gene segments were sequenced and aligned with the published sequences of ARVs. Sequence analysis of the 171 bp sequence containing partial coding region of p10 gene revealed homology except one nucleotide substitution and its deduced two amino acids (A.A.) revealed variation among the Indian isolates 547/AD/01 and MS-6. Isolate VA-1 revealed 100% identity with 604/AD/02 and 99.4% identity with isolates 547/AD/01 and MS-6. Phylogenetic analysis of 171 bp partial p10 nucleotide and deduced A.A. sequence revealed lineages with the American isolates. The sequence analysis of 284 bp containing partial coding region of p17 also revealed homology among the Indian isolates, with variations in two nucleotides and its corresponding two deduced A.A. in the isolate VA-1. All the Indian isolates revealed 100% sequence homology with each other except VA-1, which showed 99.3% sequence identity with rest of the three isolates. Phylogenetic analysis of 284

bp partial p17 nucleotide and deduced A.A. sequences revealed similar results as found with p10 gene. The interesting finding among the Indian isolates was that two amino acid substitutions in the deduced A.A. sequence, where proline (P) was replaced by histidine (H) and histidine (H) by threonine (T) at position 93 and 94, respectively, could serve as molecular markers for differentiating Indian ARV isolates from the isolates of foreign origin.

###

GPP-P-2:

Molecular epidemiological studies of fowl adenoviruses (FAVs) associated with inclusion body hepatitis- hydropericardium syndrome (IBH-HPS) in India

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Fowl adenoviruses (FAVs) recovered from the outbreaks of IBH-HPS occurring in different parts of the country have been typed earlier as serotype 4 by serum neutralization test. During the present study eight FAV isolates obtained from different geographical regions of the country viz. 383/AD/97, 387/AD/97, 421/AD/98, 488/AD/98, 507/AD/01, 528/AD/01, 608/AD/02 and 617/AD/02 were characterized to detect differences at genomic level, if any. PCR amplification of the hypervariable region of hexon gene of all the isolates and the subsequent RE analysis using eight different enzymes revealed two types of RE profiles. All the isolates, except 421/AD/98 and 617/AD/02 could be grouped together and belonged to FAV-4 while isolate 617/AD/02 was distinct from that of all other isolates, with all the enzymes used in the study. Isolate 421/AD/98 showed a mixture of bands of both of the above mentioned patterns indicating it to be a mixture of FAV's. The gel eluted hexon gene segments of FAV isolates 507/AD/01, 528/AD/01, and 617/AD/02 were cloned, sequenced and aligned with other published sequences

of FAVs. Sequence analysis from isolates 507/AD/01 and 528/AD/02 revealed 100% identity, and phylogenetic analysis confirmed the grouping of these isolates in FAV serotype-4. Isolate 617/AD/02, on phylogenetic analysis was grouped with FAV-12 and closely related to FAV-2, while it maintained a distant relation with FAV-11 strain X11 or FAV-4 isolates. Serotyping results also confirmed that 617/AD/02 isolate belonged to FAV-12. The present study revealed that IBH/HPS in the Indian poultry flocks is the outcome of the involvement of more than one serotype of FAV. It may be due to FAV-4 or FAV-12 or the association of both the serotypes. Therefore this urges a need for a thorough revamping of disease monitoring, surveillance and effective control strategies of IBH-HPS in Indian poultry flocks.

###

GPP-P-3:

Restriction enzyme analysis of tissue culture adapted velogenic Newcastle Disease virus

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A velogenic Newcastle disease virus (NDV) isolate typed to belong to C1 group by monoclonal antibody typing was adapted 50 times in chicken embryo fibroblast (CEF) cell culture and 60 times in Vero cells. At every 10th passage the virus was characterized based on mean death time, intra cerebral pathogenicity indices and viral titration studies. A gradual reduction in the virulence of the virus was noticed as the passage level increased. RT - PCR of a 254 bp region of the fusion gene encompassing the fusion protein cleavage site was carried out for the virulent as well as cell culture adapted viruses at every 10th passage level. The amplicons were subsequently digested with three restriction enzymes *viz.*, *AluI*, *HaeIII* and *PstI*. It was found out that there was difference in banding patterns between the virulent and adapted viruses indicating nucleotide substitutions in the virulent virus when it was sequentially passaged onto cell culture systems.

###

GPP-P-4:

Detection of telomerase activity in spontaneously occurring canine neoplasms

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Telomerase enzyme, owing to its reverse transcriptase activity, plays a vital role in circumventing the telomeric instability in tumor cells. The frequency of expression of telomerase across the whole spectrum of tumors has stimulated considerable interest in its potential as a diagnostic and prognostic marker. Majority of human cancer tissues have shown to possess telomerase activity with little or no activity detectable in normal somatic tissues excepting lymphocytes. The present study was envisaged to assess the telomerase activity in spontaneously occurring canine neoplasms. The estimation of telomerase activity in tissue lysates was done using the PCR based telomeric repeat amplification protocol assay (TRAP assay). Twenty-seven suspected tumors were collected and cryo-preserved for the same. Out of twenty-five histologically confirmed neoplasms (25 cases), 22 cases (88%) were found positive for telomerase activity irrespective of benign or malignant nature. Curiously, some of the tumor cases mainly melanoma, histiocytoma and trichoepithelioma failed to demonstrate telomerase activity. The reasons for these exceptions are not known yet. Two samples were histopathologically diagnosed as non-neoplastic conditions; mainly inflammatory condition of skin and gingival hyperplasia and both these cases revealed no telomerase activity on TRAP assay. The results obtained indicated that telomerase activity could be used as potential marker for a variety of tumors in dogs.

###

GPP-P-5:

DNA polymorphism of BoLA-DRB3 exon 2 in Tharparkar Cattle (*Bos indicus*)

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Genes controlling most of the immune functions are either linked to or actually mapped within a group of genes called major histocompatibility complex (MHC). MHC of cattle is known as bovine lymphocyte antigen (BoLA) and is located on chromosome 23, which comprised of four regions i.e. class I, class IIa, class IIb and class III. DRB3 gene of class IIa complex is major expressed and highly polymorphic locus. Hence, the polymorphism of BoLA-DRB3 exon 2 was studied by PCR-RFLP using 3 restriction enzymes namely *HaeIII*, *RsaI* and *PstI* in 36 Tharparkar cattle. The restriction enzyme digestion of amplified 284 bp BoLA-DRB3.2 gene with *HaeIII* yielded two patterns (*HaeIII*-aa and *HaeIII*-ab). The frequencies of these PCR-RFLP patterns were 0.833 and 0.167 with major fragment sizes as 167, 52 and 65 and 219, 167, 52, 65 respectively. The percentage of homozygous and heterozygous cattle was 83.3% and 16.7%, respectively. The frequency of allele 'a' was higher (0.9165) as compared to the allele 'b' (0.0835), which indicated the presence of allele 'a' in more than 91% of the Tharparkar cattle population. Digestion with *RsaI* revealed 10 different patterns (I, II, III, IV, V, I/II, I/III, II/III, III/? and I/?) and out of these 5 alleles were new and all new alleles were in heterozygous conditions. The frequencies of these patterns were 0.031, 0.156, 0.281, 0.125, 0.156, 0.063, 0.031, 0.063, 0.063 and 0.031, respectively. The results demonstrated that the 75% of the total animals were homozygous for *RsaI* enzyme site, whereas the percentage of heterozygous animals was only 25%. Digestion of 284 bp PCR products with *PstI* did not reveal any restriction site. These results revealed that there is lose of polymorphism of DRB3 haplotypes, which may be due to decreasing the herd size of Tharparkar cattle. Extensive crossbreeding of this breed with exotic breeds of cattle and intensification of agricultural practices due to mechanization led to serious problem for decreasing the

number of animals of this breed. Hence, there are chances of diminishing important genes related to disease resistance and production traits. Therefore, it is suggested that the future breeding plans must be based on establishing or maintaining the effective population size of this breed and limiting the crossbreeding programme so that reservoir of important genes of indigenous cattle can be preserved.

###

GPP-P-6:

Characterization of AB 330: a novel CC-chemokine from domestic duck (*Anas platyrhynchos*)

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Chemokines are low molecular weight peptides secreted by the immune system and are capable of producing wide range of biological functions. We report the further characterization of a novel CC chemokine cDNA (AB330) identified from an enriched cDNA library of mitogen activated duck (*Anas platyrhynchos*) splenic mononuclear cells. Exon-intron organization, promoter structure and tissue expression profiles of the molecule was analysed based on the cDNA sequence information. Exon-intron organization revealed presence of four exons and three introns in the 1165 bp gene. Promoter structure by genome walking could identify the presence of several major transcription factor binding sites in the immediate upstream sequences of 1500 bp. This is the first report of chemokine molecules in domestic duck and of any avian chemokine promoter sequence.

###

GPP-P-7:

A Novel Cathelicidin Congener from *Bubalus bubalis*

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Cathelicidins constitutes a group of endogenous antimicrobial peptides present in variety of mammalian species and is a key component of peptide -based innate host defense. They are synthesized as prepropeptides, store in neutrophil granules as propeptide and processed by enzymatic cleavage to release the mature biologically active peptide. In paucity of information, we investigated the presence of this group of peptide in *Bubalis bubalis* using RT-PCR based approach, a cDNA was cloned from buffalo myeloid RNA and was found to encode a 144- residues peptide precursor, which is composed of signal sequence (N- terminal 29 residues), propeptide (101 residues) and mature peptide (C-terminal 14 residues). When the deduced amino acid sequence of this peptide was aligned with other reported sequences of cathelicidin-derived peptides, it shown the characteristic feature of cathelicidin family describing high conservation at N-terminal region and extensive variation at C- terminal region. This novel peptide shares the highest degree of sequence identity with cathelicidin-4 (Indolicidin) of *Bos taurus* with only a few divergent residues. Thus our findings indicate the existence of cathelicidin group of peptide in *Bubalis bubalis*.

###

GPP-P-8:

Identification of five new *Salmonella* serovars haunting poultry birds

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During the study on prevalence of salmonellosis in poultry birds commercially marketed for meat purpose, during May 04 to October 04, a total of 410 samples comprising of 385 of ileo-caecal contents from the slaughtered birds (75 from Tripur-Tamilnadu, 75 from Baraut-UP and 235 from Bareilly-UP) and 25 egg samples (Bareilly) were collected and processed for detection of *Salmonella*. A total of 20 samples of ileo-caecal contents (10 Baraut, 3 Tripur and 7 Bareilly) and one egg sample were detected positive for *Salmonella* and isolates belonged to eight different serovars namely, *S. Kentucky* (5, Baraut), *S. Virchow* (4, Bareilly, 1 from egg and 3 from ileo-caecal contents), *S. Winneba* (4, Bareilly), *S. Stockholm* (1, Baraut), *S. Warnow* (1, Baraut), *S. Amager* (1, Baraut), *S. II. 4,12:g,m,s,t:1,5* (1, Baraut) and *S. II. 6,7:g,m,s,t:1,5* (3, Tripur). The study revealed that poultry birds from different places carry *Salmonella*, however serovars and prevalence of *Salmonella* may vary. All isolates were resistant to multiple drugs and contained virulence plasmid. Of the eight serovars, five viz., *S. Winneba*, *S. Warnow*, *S. Amager*, *S. II. 4,12:g,m,s,t:1,5* and *S. II. 6,7:g,m,s,t:1,5* have been identified for the first time in India from any of the sources. It may be either due to gradual drift in antigenic structure of some of the established strains of *Salmonella* in poultry or import of poultry meat and its products might be having some role. Though cause may be any, it needs a detailed molecular investigation as emergence of new serovars in poultry birds is of immense public health concern because poultry products have often played the source of *Salmonella* infection for numerous outbreaks of salmonellosis in human population.

###

ADD-P-1:

Recombinant antigen-based latex agglutination test for diagnosis of leptospirosis

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Leptospirosis is a zoonosis of worldwide significance, caused by infection with pathogenic *Leptospira* species. There is an urgent need for the development of new diagnostic strategies for leptospirosis. Clinical recognition is difficult because leptospires can affect many different organ systems, resulting in a wide variety of clinical presentations. Laboratory confirmation is of utmost importance and the standard serologic test; the microscopic agglutination test (MAT) requires the detailed knowledge of the locally occurring strains, as the predominant serovars have to be selected for use as antigens. MAT is inadequate for rapid case identification, as it requires analysis of paired sera to achieve sufficient sensitivity. Hence, a rapid (2 - 5 min) and simple recombinant LipL32 antigen - based Latex agglutination test (rLAT) kit was developed for the first time for canine and human serodiagnosis in the developing countries. The expression of the antigen is highly conserved to the pathogenic leptospires. Positive results were read on a +1 to +4 scales depending on the extent of agglutination and time taken for the development of agglutination. A relatively high sensitivity and specificity between rLAT and MAT indicated that the test measured similar trends in exposure to leptospirosis. The recombinant antigen coated latex beads could detect the anti-leptospiral antibodies in the acute phase of illness.

###

ADD-P-2

Detection of adhesive curli gene (*crl* and *csgA*) in avian pathogenic *E.coli* using polymerase chain reaction

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Avian pathogenic *E.coli*, the causative agent of colibacillosis, harbors several putative virulence genes. In this study, we examined the presence of temperature regulated adhesin curli gene (*Crl* and *CsgA*) by Polymerase chain reaction (PCR). The main adhesins identified in APEC are F1 fimbriae, whose ability to agglutinate fowl erythrocytes is abolished in a medium containing added mannose and are thus called as mannose-sensitive haemagglutination. Other adhesins were also detected in APEC such as curli fibers, thin aggregative surface fibers. In present study different *E.coli* isolates were screened for the presence of curli genes (*Crl* and *CsgA*). 12 *E.coli* isolates showing MSHA were grown in 1 ml BHI broth at 37°C. 100 µl of this culture was heated for 10 min. in boiling water and immediately transferred to ice and supernatant were used for PCR. Amplification of bacterial DNA for detection of '*Crl*' and '*CsgA*' gene was done in total volume of 25 µl reaction mixture using specific set of primers. Out of 12 isolates tested, 7 *E.coli* isolates were positive for curli regulatory gene '*Crl*' and 5 were positive for '*CsgA*' a curli structural gene. The curli genes '*Crl*' and '*CsgA*' were present in most of the avian *E.coli* showing their ubiquity among commensals, clinical as well as nonclinical isolates.

###

ADD-P-3:

Micro agglutination Test for monitoring of immune status against Haemorrhagic Septicaemia

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Major requirement for success of immunization against Haemorrhagic Septicaemia is monitoring of immunological status of vaccinated animals. A series of tests like ELISA, IHA and PMPT are being used for the purpose. But these tests are of limited field applicability in the Indian context. With the prime objective to develop a farmer friendly, sensitive and economic test, efforts were made to unveil suitability of micro agglutination test. Colored antigen of *Pasteurella multocida* P52 prepared using basic dye was checked for its agglutinability in micro-titre plates using serial dilutions of sera of mice immunized with different HS oil adjuvant vaccine preparations. The test was found to be sensitive and a definite pattern of immune response was observed.

###

ADD-P-4:

Nested PCR for the diagnosis of Tetanus

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Tetanus is a disease characterized by neurological signs like clonic spasm, respiratory distress and invariably results in death. The diagnosis of the disease is difficult due to the requirement of complete anaerobiasis for isolation of the organism and the mere demonstration of tennis racket shaped organism cannot be taken as suggestive of *Clostridium tetani*. Therefore

molecular strategy is to be adopted. For this purpose soil samples, fecal samples and clinical materials were collected and inoculated in thioglycollate broth after the heat treatment. DNA extraction was done and C-fragment gene was amplified with reported primers. A 1.4 Kb product was obtained in many of the field samples but only few samples were positive for the 780 bp product with our designed nested primers. These positive samples when inoculated in mice showed typical signs of tetanus and from these animals, *Clostridium tetani* was isolated. The colony morphology was typical showing spider like appearance. Morphologically the organisms were having drumstick appearance. This strategy may also be followed for selective isolation of *Clostridium tetani* from soil samples.

###

ADD-P-5:

Application of Random Amplification of Polymorphic DNA (RAPD)-PCR for avian *E.coli* serotype differentiation

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Random Amplification of Polymorphic DNA (RAPD)-PCR is a simple rapid technique that uses arbitrary oligonucleotide primers to produce a set of amplified DNA products that can be analysed for identification of bacterial strains. The following are the results of RAPD-PCR method used for identifying genomic polymorphism and inturn for serotype differentiation.

In all, fifteen serotypes of *E.coli* isolated from poultry were subjected to RAPD-PCR using eight oligonucleotide random primers. Amplification with each of the primers resulted in generation of different DNA fingerprinting profile with varied number of bands. Dendrogram and Principal Component Anaysis based generation of clustering of *E.coli* serotypes showed either two major clusters or random placement of serotypes or combination of both with different primers. The polymorphic amplicons served as markers for *E.coli* serotypes. RAPD

based fingerprinting provided a rapid means of discriminating *E.coli* serotypes and is a valuable tool for typing in epidemiological studies. The details of primers used, species / serotype specific markers and intricacies involved in the technique will be discussed.

###

ADD-P-6:

Detection of Salmonella in chicken meat and eggs using Polymerase Chain Reaction

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Sandeep K Singh, L.S. Girish and S.P. Singh**

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Salmonellosis is a zoonotic disease which also causes heavy economic loss to the poultry industry. The present study was undertaken to detect the presence of Salmonellae in chicken meat and eggs using polymerase chain reaction technique in and around Panthagar. A total of 100 samples consisting of 50 each of chicken meat and eggs were collected from the retail outlets and processed for the isolation and identification of Salmonellae. The genomic DNA was isolated from putative colonies using stab method. The template DNA was amplified using the Inva Gene Primer. The amplified products were electrophoresed and the appearance of DNA fragment of 284 Bp was documented in 4 Samples. These results were in corroboration with the conventional cultural method. The present study suggest that PCR based methods can efficiently be used for rapid detection of Salmonellae in food of animal origin.

###

ADD-P-7:

Antigen competitive ELISA (AC-ELISA) to detect Newcastle disease virus from tissue samples

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Antigen competitive ELISA (AC-ELISA) technique was developed and standardized to detect Newcastle disease virus from tissue samples and amnioallantoic fluid. This method was found to be as sensitive as PCR and better than double antibody sandwich ELISA (S-ELISA) in detection of NDV antigen in tissue. This technique also eliminates the need for using antibodies from two sources as needed for S-ELISA. This technique is combination of solid and liquid phase ELISA techniques. In this method the mean optical density values of duplicate test samples were converted into inhibition per centage (IP), which was calculated as follows; $IP = 100 - [(OD \text{ of test sample} / OD \text{ of Negative controls}) * 100]$. The test samples were classified as positive when the IP value was more than 15%, which is three times the value of negative samples. This was arrived using infected and uninfected tissue samples and infected allantoic fluid. Nearly 100 field samples from suspected ND outbreaks were collected. The samples included trachea, lungs, ileo-caecal junctio, proventriculus etc. To compare the efficacy of this test S-ELISA also performed on samples. On select samples RT-PCR was also performed. AC-ELISA was found to be as sensitive as RT-PCR in identifying NDV. This technique was found to be as sensitive as RT-PCR in identifying NDV from allantoic fluid. The samples that were detected as positive by AC-ELISA were also identified as positive in RT-PCR. Further, when this test was performed on tissue samples, this test identified NDV in nearly 30% and 20% per cent of samples, which were detected as negative by S-ELISA and HA/HI respectively. These results were also confirmed in RT-PCR. The results confirmed the superiority of AC-ELISA technique over S-ELISA. Further it is also comparable to RT-PCR and takes same time as RT-PCR.

###

ADD-P-8:

Development of indirect ELISA for the diagnosis of *Haemonchus contortus* infection in goats

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Haemonchosis is a major parasitic disease of small ruminants in tropical, sub tropical and temperate regions of the country. Traditional way of diagnosis is based on fecal egg count, which is unable to differentiate acute and chronic infections and procedure needs intensive labor to screen a flock of animals. However, arrested development of larvae in the host during extreme climates is also limiting use of test in such periods. Considering these problems an attempt was made to develop a rapid, sensitive and easy alternative for diagnosis of *H. contortus* infection. An indirect ELISA test was standardized and used for the diagnosis of moderate level of *H. contortus* infection (EPG range 300-600 for positive samples). Somatic antigen of *H. contortus* was prepared and positive and negative blood samples collected from goats were used in the assay. Optimum concentration of antigen, antibody and conjugate were assessed by checkerboard titration and test was employed to differentiate the positive and negative samples in first instance. Marked differentiation was noticed in the positive and negative samples in terms of absorbance values (recorded at OD492 nm in ELISA reader) at different serum dilutions. The present preliminary study concluded that serum samples of high, moderate and low-grade infection carrying animals would be used for better understanding of test applicability, before field trial.

###

ADD-P-9:

Production and characterization of neutralizing monoclonal antibodies against *peste des petits ruminants* (PPR) virus

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PPR is an infectious viral disease of small ruminants, caused by a morbillivirus of family Paramyxoviridae. PPR is antigenically related to other morbilliviruses, closer being the rinderpest virus. Since PPR and rinderpest virus can cause similar disease in small ruminants, they need to be differentiated serologically. In the present study attempt were made to produce neutralizing MAbs. A set of nine PPR positive MAbs which showed complete / or partial neutralization were selected. Out of nine three were associated with complete neutralization of virus. These three MAbs were studied extensively for PPR diagnosis using enzyme immunoassay and they were compared with already existing one (4B11). It was found that MAb 5C3 produced during present study can be a better alternative to MAb 4B11 in terms of specificity in enzyme immunoassay.

###

ADD-P-10:

A simple Dot-ELISA for diagnosis of *Peste des petits ruminants* (PPR)

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Peste des petits ruminants (PPR) is a highly contagious viral disease of goats and sheep, characterized by pyrexia, mucopurulent nasal and ocular discharges, necrotizing and

erosive stomatitis, enteritis and pneumonia. Diagnosis of the disease in field condition is important to launch control measures. Although various techniques have been applied for the diagnosis of PPR in clinical samples, no tests could be performed under field condition. A simple dot-enzyme linked immunosorbent assay (Dot-ELISA) using nitrocellulose membrane as solid support was standardized and developed for the detection of PPR viral antigen in caprine tissues. A dot of 5 µl of tissue materials prepared as 10% suspension in PBS was applied on the nitrocellulose strip to enable PPRV antigen to bind and then incubated with purified monoclonal antibody (PPRV specific anti-nucleocapsid protein monoclonal antibody). The antigen-antibody reaction was detected with horse raddish peroxidase-conjugated anti-mouse immunoglobulin G and the enzyme substrate, 4-choloro-1-naphthol. The blue colour dots were visually assessed and scored. The relative sensitivity and specificity of this test was found to be comparable to that the routinely used sandwich-ELISA for the diagnosis of PPR using 450 clinical field specimens. Our results showed that the Dot-ELISA could serve as simple, easy to perform preliminary test to screen clinical samples from goats suffering from PPR disease under field conditions and is also suitable for field laboratories.

###

ADD-P-11:

Development of an one-step multiplex RT-PCR assay for the detection of *peste des petits ruminants* (PPR) virus in clinical samples

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Peste des petits ruminants (PPR) is an acute highly contagious and economically important viral disease of small ruminants, especially goats and sheep. Both PPR and rinderpest viruses have been found to cause a similar disease in small ruminants, namely sheep and goats, which cannot be differentiated clinically. For effective control of the disease the development of vaccines as well as rapid, specific and sensitive methods for the diagnosis of both viruses and for their differentiation is highly imperative. In this direction, a single-tube

one-step multiplex RT-PCR was standardized to amplify both 337 bp and 191 bp fragments of N and M genes of PPRV, respectively and only a 337 bp fragment of N gene of Rinderpest virus (RPV). In the assay the Qiagen One-step RT-PCR kit containing the Ominiscript and Sensiscript reverse transcriptase and Hot star *Taq* DNA polymerase was utilized. The RT-PCR using purified viral RNA was easily adopted for direct detection of PPRV in clinical field samples and its differentiation from RPV. The amplified N and M gene products were confirmed to be PPRV and RPV-specific by their size in 1.5% agarose gel and restriction analysis. The sensitivity of the assay was found to be 100 fg of PPRV RNA. The one-step assay as compared with a two-step assay is easier and time saving as it requires just a single buffer for both reactions, reverse transcription (RT) and PCR. In experimentally infected goats, PPRV was detectable by the one-step RT-PCR in nasal and ocular swabs between 7 and 17 days post infection (p.i.) and in oral swabs between 7 and 15 days p.i. Out of 32 clinical field samples tested 18 were found positive by sandwich ELISA, while 22 were found positive by the one-step RT-PCR.

###

ADD-P-12:

Cloning of *peste des petits ruminants* (PPR) virus haemagglutinin (H) gene in heterologous expression system

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Peste des petits ruminants (PPR) virus is the aetiological agent of an acute, highly contagious and economically important viral disease of goats and sheep. The currently employed test for sero-surveillance and sero-monitoring of PPR throughout the country is a monoclonal antibody (Mab) based competitive-ELISA developed at IVRI, Mukteswar campus, which uses the tissue culture PPR virus and anti haemagglutinin Mab. The main hurdle to the production of the kits is obtaining large quantities of cell culture antigens. To circumvent the difficulties associated with the antigen production, we have planned for the production of recombinant H protein in heterologous system (mammalian/ yeast) using rDNA approaches and to replace

the whole virus particles as the supply of later involves considerable risks. Development of a potent, safe and cost-effective antigen with long stability as diagnostic agent is of considerable importance for controlling the disease and assay would be of immense value in last stage of disease elimination or eradication.

In the present study, as a preliminary work, the cloning of PPR *virus* H gene in heterologous system was carried out. The gene coding for the H protein of PPRV from Vaccine virus (Sungri-94 isolates) was amplified using virus specific primers in RT-PCR reaction from viral RNA extracted directly from PPRV infected Vero cell culture. The purified amplicon (1827bp) was cloned downstream to the highly inducible AOX I promoter in yeast transfer vector (pPIC-9K) at Not I site and under the control of CMV promoter in pTargetTTM mammalian expression vector in proper reading frame for the expression. The recombinant colonies grown on Ampicillin LB agar plates were characterized by initially screening by PCR, using vector specific and insert specific primers, followed by restriction enzyme analysis of plasmid DNA. Further, the expression of the cloned gene will be carried out in eukaryotic system, for its potential utility as recombinant antigen.

###

ADD-P-13:

Comparison of monoclonal antibody based ELISA with that of polyclonal antibody based ELISA for serodiagnosis of infectious bursal disease in poultry

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Infectious bursal disease (IBD) is one of the most economically devastating disease of chicken which has been reported in more than 95 per cent member countries of OIE. The causative agent of the disease is an Avibirna virus of family *Birnaviridae* i.e. a small non enveloped bisegmented RNA virus. It affects chicken of 4 to 6 weeks of causing bursal atrophy and immunosuppression. The disease to some extent is prevented by vaccination of chicken

but level of maternal antibodies acquired through yolk at the time of vaccination interferes with successful intake of vaccination. The antibodies to IBD virus in serum therefore need to be measured to assess the immune status for proper vaccination. During the present studies a monoclonal antibody raised against the Georgia strain of IBD virus was used to standardize a double antibody sandwich ELISA for detection of anti-IBD virus antibodies in poultry sera. The applicability of monoclonal antibody based ELISA was compared with that of polyclonal antibody based ELISA and serum neutralization test. The monoclonal antibody based ELISA had a higher degree of correlation with polyclonal antibody based ELISA ($R^2=0.93$) and serum neutralization test ($R^2=0.84$).

###

ADD-P-14:

Development and application of an indirect ELISA for the serodiagnosis of bluetongue virus infection

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Bluetongue (BT) is an economically important arthropod-borne disease of domestic and wild ruminants, and also dogs and transmitted by midges of the genus *Culicoides spp.* The disease is caused by bluetongue virus (BTV), which is the prototype virus of the family *Reoviridae*. The disease has been grouped in *List A* of OIE. 24 (possibly 25) serotypes of the virus have been reported throughout the world and India is one of the endemic countries. One of the most commonly used test for screening the antibodies for BTV antibodies is AGPT. However, the test is not reliable owing to poor sensitivity. Hence, an indirect ELISA based on purified antigen of BTV serotype 23 has been standardized using polyclonal immune sera of sheep. Purified antigen and immune serum diluted 1:100 and 1:20 respectively were found optimum in chequre-board titration. A threshold (cut-off) value was set as twice the mean of negative population based on the distribution of known negative (SNT) sera samples in respect of BTV antibodies in the test. A total of 496 sera samples from sheep were screened by indirect ELISA, of which 288 (58.06%) samples were found positive and 208 (41.94%) samples negative. In the field sera, the assay could clearly differentiate the infected population with that

of uninfected. Further, the work is in progress to know the efficacy of the test in comparison with monoclonal antibody based c-ELISA involving large number of field samples.

###

ADD-P-15:

Potential of recombinant VP7 as an antigen for detection of bluetongue antibody in sera

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Bluetongue (BT) is a highly infectious, non-contagious arthropod borne viral disease of domestic and wild ruminants caused by bluetongue virus (BTV). Diagnosis of BT is complicated owing to the presence of 25 serotypes and cross reactivity to other related orbiviruses and hence development of specific diagnostic reagents is of paramount importance in bluetongue diagnosis. Among various proteins of BTV, VP7 is the most abundant soluble protein in the virion. The protein is serogroup-specific and most conserved among all the serotypes. A 559 bp segment of VP7 gene of BTV 23 was amplified which contained major antigenic sites. The segment was cloned in pET 32a prokaryotic vector and expressed as a 36 kDa fusion protein in *E. coli*. Prominent band of expressed protein was appreciated at 3 h post induction, which increased gradually up to 16 h. The recombinant VP7 strongly reacted with rabbit antisera raised against BTV serotypes 23 and 18 in sandwich ELISA and indirect ELISA. The recombinant protein also reacted with convalescent sheep and goat sera against these serotypes. Results were encouraging and further efforts for development of an ELISA based diagnostic test using this recombinant antigen is in progress.

###

ADD-P-16:

Usefulness of urinary enzymes in diagnosis of canine nephritis

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The study was conducted in 80 dogs presented at the small animal clinics of Madras Veterinary College with clinical signs suggestive of renal insufficiency. Eight apparently healthy dogs brought for general health check up formed the control group. Hematology, Serum biochemical estimation, Urine analysis and Urinary Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGT) estimation were performed in all the cases. There was a reduction in PCV, hemoglobin and total erythrocyte count in nephritis cases, whereas, erythrocyte indices were within the normal range indicating normocytic-normochromic anemia. Serum biochemical abnormalities include Uremia, hypoproteinemia, hypoalbuminemia and hyperphosphatemia. Serum potassium levels were normal. The consistent abnormality detected in urine analysis was proteinuria. Specific gravity ranged from 1.002 to 1.040 in nephritis cases. There was significant elevation in urinary ALP level and highly significant elevation in urinary GGT level in nephritis cases when compared to control group. Mortality of 43.3% was observed and histopathologically most of them showed chronic interstitial nephritis. Increased incidence of chronic nephritis in this study suggested that renal diseases are not detected in the early stages because of the low sensitivity of routinely used screening tests like BUN and serum creatinine. Urinary enzymes are found to be promising as early indicators of tubular damage. Hence these enzymes can be included in the panel of diagnostic tests for the early detection of renal dysfunction.

###

LPP-P-1:

***In-vitro* immunotoxic studies of endosulfan in avian lymphocytes**

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Environmental pollution is one of the chief concerns in today's hi-tech world. Various chemicals used by man are increasingly transforming into potent pollutants. Pesticides are being widely used in agriculture and public health operations. Indeed they have become indispensable items to enhance the agricultural output. Endosulfan is such widely used pesticide found to decrease the population of immune cells *in-vitro*. Lymphocytes harvested aseptically using histopaque-1077 were exposed to NOEL/103 dose of endosulfan for 60 and 120 min. *In-vitro* immunotoxic effect was evaluated by various assays including cell proliferation assay, electron microscopy, flow-cytometric analysis, annexin-V binding assay and DNA fragmentation assay. Cell proliferation assay revealed a significant reduction in mean delta OD when compared to control in presence of mitogens ConA and LPS. Condensation and margination of chromatin, formation of membrane blebs and their engulfment by macrophages was evident on electron microscopy. Flow cytometric analysis recorded cells showing increased side scatter (SSC) and decreased forward scatter (FSC) on dot plots. Annexin-V binding assay using immuno-peroxidase technique revealed cells with brown color on outer surface indicating specific binding of biotin conjugated Annexin-V to the exposed phosphatidyl serine, while the fragmentation assay detected typical ladder pattern of DNA on agarose gel electrophoresis. Based on the above findings it can be concluded that endosulfan even at minute dose executes immunotoxicity by inducing apoptotic death of immune cells leading to distorted T and B cell ratios and loss of regulatory cells in critical numbers causing perturbations in immune functions. Therefore necessary measures have to be taken to create awareness among farmers about its devastating effects on immune system and encouraging them to go for eco-friendly organic farming/ for its careful usage and disposal in agriculture, animal husbandry and public health operations.

###

LPP-P-2:

Efficacy of PGF₂α in induction of estrus in non-descript hilly goats

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The present study was aimed to induce estrus in hilly goats using PGF₂α to synchronize estrus as well as kidding. A total of 24 goats, aged > 1.5 years were selected. The PGF₂α injection (7.5 mg/ goats, I/M) was administered in all the goats and the behavioral estrus was observed. The non-responders were injected with another dose of PGF₂α 10-11 days after the first one. The first PGF₂α injection induced estrus in 9 goats (37.5%), however, second injection also induced estrus in the 9 (60%) non-responders to first one with an overall induction of estrus in 18 goats (75%). Most of the goats (11; 61.11%) exhibited estrus within 72 hours with an average of 108.22±27.39 and 95.77±22.26 hrs to first and second injection of PGF₂α, respectively. The goats observed in estrus were served to buck of high vigour on two occasions leading to pregnancy in sixteen goats (88.88%). The results indicate that PGF₂α could be used effectively to induce estrus in hilly goats with high fertility.

###

LPP-P-3:

Influence of enrofloxacin on phagocytic activity and phagocytic index of milk polymorphonuclear leukocytes in bovine sub clinical mastitis

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The influence of enrofloxacin on phagocytic activity and phagocytic index of milk polymorphonuclear (PMN) cells were evaluated in cows suffering from sub clinical

mastitis(SCM).Thirty cows were initially screened by California Mastitis Test (CMT), milk samples showing positive CMT reaction were considered as SCM.15 infected cows were infused with enrofloxacin by intramammary route. Isolation of the organism revealed hemolytic and non hemolytic colonies, mainly gram positive cocci. The observations were made up to 30 days post Treatment (PT).There was average drop (43.8%) in neutrophil count, whereas, lymphocyte count was enhanced 9.84%, on day 3 PT in Group II cows. The average phagocytic neutrophil enhanced by 36.3% and phagocytic index enhanced to 18.81%, on day 3 PT. The rise of lymphocyte percentage indicates the immunomodulatory potential of enrofloxacin in bovine SCM.

###

Scientific Session-IV

BIOTECHNOLOGICAL ADVANCES IN THE VALUE ADDITION OF LIVESTOCK PRODUCTS AND PROCESSES AND OTHERS

CHAIRMAN : Dr. M. Rajashekar

CO-CHAIRMAN : Dr. A.S.R. Anjaneyelu

RAPPORTEUR : Dr. S.K. Singh / H. R. Meena

PAGE - 59 to 68

LPP-O-1:

Rodenticide poisoning in cattle and buffaloes- an investigation

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Mortality in cattle and buffaloes were reported continuously over a period of more than eight months with clinical signs of nervous disorders, excessive salivation and not responding to treatment. Post mortem examination did not reveal the presence of infectious etiological agents. Laboratory investigation of liver tissue revealed the presence of zinc phosphide. Clinical signs, mortality rate, post mortem lesions and results of other laboratory analysis will be presented.

###

LPP-O-2:

Morbillivirus receptors: New avenues for understanding pathogenesis

**Arnab Sen, V. Balamurugan, J. Sarkar, K.K. Rajak, B. Sahay,
P. Saravanan and R.K. Singh**

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Virus receptors are key mediators of pathogenesis. They determine the cell tropism and mode of replication of viruses. The target cells are key factors in understanding the overall mechanisms of pathogenesis of viruses. Morbilliviruses are highly contagious pathogens and bear a lot of impact on the overall livestock health. They cause devastating animal and human viral diseases including Rinderpest, PPR, canine distemper, phocine distemper, and Dolphin distemper. Measles in humans is also caused by this group. The emerging morbilliviruses viz.

Hendra and Nipah viruses are also a cause of concern. Receptor tropism with regard to these viruses have been studied in detail for the human measles virus. On the basis of work done it is seen that two classes of receptors are mainly involved. The signalling lymphocyte adhesion molecule (SLAM also known as CD150) which is ubiquitously expressed in many species serves as the primary receptor. An alternative receptor viz. CD46 which is expressed on epithelial cells has also been identified. This paper deals with the receptor tropism of morbilliviruses and attempts to address possible implication on pathogenesis.

###

LPP-O-3

Pathogenesis of Hydropericardium syndrome virus in experimental infection of broilers

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Hydropericardium Syndrome (HPS), a disease primarily of broilers birds causes severe hydropericardium and has a great economic impact on poultry industry. To study the pathogenesis of HPS, a total of 60 broiler birds aged 3 weeks were divided into 2 groups. Infected group consisted of 50 broiler birds and each bird received 0.3 ml liver homogenate (20% v/v) by subcutaneous route whereas, 10 control birds received PBS in similar dose and route. The birds were sacrificed at 6, 12, 24, 36, 48, 60, 72 and 120 hrs post infection (PI). The tissue sections and impression smears from liver, heart, kidney and spleen were stained by immunoperoxidase technique (IPT) to study the distribution of virus. The results of IPT revealed presence of virus antigen in liver, kidney and spleen as early as 24 hrs PI. The IPT positivity in liver and kidney progressed to severe degree between 48 to 72 hrs and thereafter there was reduction in intensity and extent of IPT positive reaction. In case of spleen the IPT positive reaction remained mild through out the period of experiment. No virus antigen was demonstrated in heart. The significance of IPT to study the pathogenesis of HPSV has been limelighted.

###

LPP-O-4:

Experimental studies on immunosuppressive effects of *peste des petits ruminants*(PPR) virus in goats

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Effect of virulent and attenuated *peste des petits ruminants* (PPR) virus on the immune response to nonspecific antigen (ovalbumin) was investigated. Clinical and serological responses were monitored in goats administered with ovalbumin concurrently with either PPR vaccine or virulent virus. Study showed that PPR virulent virus causes a marked immunosuppression as evidenced by leucopenia, lymphopenia, and reduced early antibody response to both specific and nonspecific antigen. These observations were predominant particularly during acute phase of disease (4 -10 days post infection). On the other hand, the vaccine virus induced only a transient lymphopenia without significantly affecting the immune response to nonspecific antigen or to itself during this period. Further, the antibody levels to ovalbumin in the group administered with virulent PPRV increased significantly high between day 28-35 post infection in comparison to the titers in other two groups given with either ovalbumin alone or in combination with vaccine.

###

LPP-O-5:

Production of goat kids using *in vitro* fertilization and embryos transfer through laparoscopy technique

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The aim of this study was to produce *in vitro* goat embryos and their transfer to recipient using laparoscopy techniques. Ovaries were brought from slaughterhouse and oocytes were collected in oocyte collection medium. A and B grade oocytes were washed 4-5 times with maturation medium (Medium 199, 5 μ g/ml FSH, 10 μ g/ml LH, 1 μ g/ml estradiol-17 β , 10% estrus goat serum, 5% gentamycin and 3% BSA) and placed 15-20 oocytes in 100 μ l drop medium in 35 mm dish covered with mineral oil. Dishes were kept in 5% CO₂ incubator at 38.±5°C with maximum humidity for 27 h. Semen was collected from bucks with AV and processed for capacitation of spermatozoa. For this, 50- μ l semen was mixed in 5 ml of sperm TALP medium and centrifuged 2-3 times at 320 g for 5 minutes. The pellet was finally suspended in fertilization-TALP medium containing heparin (10- μ g/ml) and kept in incubator for 2 h. *In-vitro* fertilization was performed with 27 h matured oocytes and capacitated spermatozoa. Cleaved embryos were isolated after 24 h and cultured in embryo development medium with oviductal cells. In the present study, a total of 6,648 goat ovary samples were collected and 16,218 oocytes were isolated from those ovaries. The oocytes were graded as A (21.20%), B (23.52%), C (25.31%) and D (29.95%) types depending upon the layers of cumulus cells present around the oocytes and the visual appraisal of the cytoplasm. A total of 12% embryos and 43% morula were produced. Recipient goats were synchronized with two injection of PGF_{2a} (Lutalyse @ 15 mg /goat) 10 days apart. IVF goat embryos were transferred to uterotubal junction of recipient goats using laparoscopy technique. Total 3 recipients developed pregnancy out of 14 recipients. Four kids were born after completion of pregnancy. One recipient delivered twin kids (one male & other female) whereas other two recipients delivered single kid each. From the present study it could be concluded that the simple method of laparoscopy is useful for transferring IVF goat embryos to synchronize recipient goats and to produce offspring from the recipients.

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LPP-O-6:

Isolation and culture of embryonic stem cells from *in vitro* produced goat embryos

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Embryonic stem (ES) cells are pluripotent cells, which are derived from early embryos. These cells have the capability to undergo unlimited, undifferentiated proliferation on feeder layer and remains karyotypically normal and phenotypically stable. ES cell technology has revolutionized modern biology and provides us with unique opportunities to produce livestock by genetic manipulation and to understand the key events of developmental biology. Hence, the present project is aimed at exploring the ES cells in goat with two objectives viz., production of goat embryos *in vitro* and isolation and culture of goat embryonic stem cells on feeder layer. For developing the feeder layer of primary goat fetal fibroblasts, 50-60 days old goat fetal skin was treated with 0.5% trypsin in RPMI-1640 to get the cell suspension, which was plated in tissue culture flask containing RPMI-1640 + 3% BSA + 10% FCS and incubated in 5% CO₂ incubator at 38±5°C which formed confluent monolayer after 4-5 days. Caprine embryos were produced by *in vitro* maturation, fertilization and culture (IVMFC). Total 2884 oocytes were collected from slaughtered 1183 goat ovaries and A (15.75%) and B (19.10%) grade oocytes were matured *in vitro* with maturation medium (TCM 199 HEPES modification, 5 mg/ml FSH, 10 mg/ml LH, 1 mg/ml estradiol-17b, 10% estrus goat serum, 3 mg/ml BSA and 50 mg/ml gentamycin). After 18 h of co-incubation with capacitated sperm, oocytes were cultured in embryo development medium (TCM 199 HEPES modification, 100 mg/ml L-glutamine, 27 mg/ml sodium pyruvate, 10% FCS, 3 mg/ml BSA and 50 mg/ml gentamycin) with oviductal cells for the further development. Total 189 embryos were produced 28.04% reached morula stage on the 4th day. The embryonic cells were isolated by removing the zone pellucida mechanically and were plated on inactivated primary goat embryonic fibroblast feeder layer. The embryonic cells took 48 h for attachment on the feeder layer and subsequent proliferation and elongation was observed on 3rd and 4th day of plating. From the present study, it has been found that goat embryos could be produced *in vitro* and embryonic cells could be isolated and cultured successfully on goat fetal fibroblasts feeder layer for production of embryonic stem cell.

###

LPP-O-7:

***In-vitro* and *in-vivo* determination of biocompatibility of different biomaterials used for reconstructive surgery**

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The surgical reconstruction of soft tissues of injured diseased or otherwise lost tissue poses a therapeutic dilemma for the veterinary surgeons and required use of biomaterials in reconstructive surgery. The use of biomaterials has been increasing and it has been observed that after reconstructive surgery employing biomaterials, recovery is rapid and there is no weakness of patients structures as seen in cases of collection of autografts. Before *in-vivo* application of biomaterials *in-vitro* cytotoxicity of biocompatibility should be done. In the present study *in-vitro* cytotoxicity testing and *in-vivo* immunogenicity of five biomaterials of porcine origin viz. glutaraldehyde treated diaphragm, pericardium and acellular diaphragm, dermal and urinary bladder was done. The two types of cell systems i.e. peripheral blood leucocytes of rabbits and chicken embryo fibroblasts (CEF) cell culture were used. Saline extract of biomaterials were prepared after repeated washing of biomaterials. In the presence of highest concentration of biomaterials (1:20 dilution) no appreciable changes in cell morphology at 24 hrs of post incubation was recorded in leucocytes. In CEF cell culture the cell cytotoxicity was tested once the monolayer was completed. Simultaneous addition of biomaterial extract during seeding of CEF cells was not practiced as this can hamper the growth of mitotic cells to differentiate into fibroblastic appearance. Mild cytotoxicity was seen at 24 hrs of post incubation in glutaraldehyde treated grafts. For detecting immunogenicity the biomaterials were used for repair of abdominal wall in rabbits and the plasma was collected before implantation and on day 7, 14, 30, 60 and 90-post implantation. The absorbance values of ELISA were taken as a measure to compare the magnitude of immune response. In ELISA study a fixed dilution 1: 100 was undertaken all the tests. Higher the absorbance greater is the antibody response within that fixed dilution of plasma was considered criteria for decision making. The peak values in glutaraldehyde treated groups were seen at day 90 post implantation whereas; in acellular biomaterials the peak values were recorded on day 60 post implantation.

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LPP-O-8:

Development of new *in vitro* model for evaluation of pathogenicity of *Salmonella*: Germinating maize seed model

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In the study a new *in vitro* model for evaluation of pathogenicity of *Salmonella enterica* subspecies *enterica* strains was developed and evaluated using two reference pathogenic and one non-pathogenic strain each of the four serovars viz., Typhimurium, Paratyphi B, Gallinarum and Weltevreden. In the model, healthy certified Ganga cultivar seed (GB Pant University of Agriculture and Technology, Pantnagar) with 98% germination rate were inoculated with 4.5×10^5 cfu of the test strains along with Swiss albino mice to evaluate pathogenicity. Besides seeds were also exposed to cell culture supernatants and cell lysates of the strains to determine their cytotoxicity in Vero cell line assay. The study revealed a perfect correlation in results of germinating maize seeds model with mouse pathogenicity assay. However, the new model was about 3 times less sensitive than Vero cells in determining the cytotoxicity of *Salmonella* preparations and to inhibit germination three Verotoxic units/ml preparation were required to induce the inhibition of germination. Considering the simplicity, economy and requirements, the germinating seed model appears to be quite capable to replace mouse model and complicated Vero cell assays, at least for routine evaluation of different strains. The model involved soaking of ten seeds in 1 ml test solution for 1 h at 37°C and then washing the seeds twice with 10 ml of sterile distilled water and the seed were allowed to germinate in dark at 37°C for 24 h between six layers of wet sterile cheese cloth kept in a germination plate. The affected seeds did not give out any radicle while visible radicle were apparent on control seeds, in case of confusion further incubation for 24 h was carried out and germinated and nongerminated seeds were counted. A fifty percent reduction in germination was considered as one pathogenic unit or toxic unit. Further studies are under progress.

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LPP-O-9:

Comparison of superovulatory response (PMSG, FSH) and embryo recovery in Tellicherry goats

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Twelve tellicherry goats were used for surgical embryo transfer programme. All the twelve goats were oestrus synchronized with CIDR - G in combination with intra muscular injections of 2 ml of PGF 2 α and 500 IU of PMSG. Aporonised buck was used for oestrus detection in does. Six does were superovulated with single intramuscular injection of 1000 IU of PMSG and six does were superovulated with 200 mg of FSH in divided doses during the day 10 to day 12 of oestrous cycle. Two ml of PGF 2 α was given intramuscularly on the next day after the PMSG injection and on penultimate day after the last dose of FSH injection. All the superovulated does were observed for oestrus signs. The onset of superovulated oestrus and the duration of oestrus for PMSG and FSH regime was 47.09 hrs and 34. 13 hrs respectively. A proven buck was allowed for mating in the morning , evening and subsequent day morning. Five hundred IU of Chorulon was administered intramuscularly at the time of mating. Surgical embryo collection was done on third day after mating. Midventral laparotomy was performed under general anaesthesia (Ketamine and Xylazine combination) and the superovulatory response was assessed. In the PMSG superovulated does, 19 CL & 12 CL and 19 AOF & 15 AOF were noticed in the right and left ovaries respectively. In the FSH superovulated does, 38 CL & 35 CL and 13 AOF and 11 AOF were noticed in the right and left ovaries respectively. A total of 20 embryos (14 right and 6 left side of oviduct) were collected from PMSG superovulated does and a total of 49 embryos (26 right and 23 left side of oviduct) from FSH superovulated does were collected. A total of 33 good quality embryos were transferred to eight recipient does and one recipient doe delivered two kids. In conclusion, FSH treated group showed higher superovulatory response and number of embryos collected while number of anovulatory follicle were lower than PMSG treated groups.

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LPP-O-10:

Hazardous use of plastic packing before and after eighty decade and their usable scoring at the present time bioenvironment

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After the first invention of plastics by an American Chemist Leo Blackeland and their patency in 1909, the main theme of increased use of plastic is its economy and greatness in use in this century. Earthen pot for tea, sweets etc., bag made of cloth, jutes, paper, phenol bonded paper, glass, wooden, tin, asbestos, ceramic, were much useful items before eighty decades. These were replaced by the plastic chemicals. (polyethylene, polypropanol, polyvinylchlorire, polyethylene, terethylate, polycarbonate, polystyrene) in the form of plastics as packing-a bio-un-degradable toxic industrial waste products. Cadmium as colouring agent, CO₂, NO₂, and cyanide etc from plastic fire smoke and cadmium furans, dioxins, mercury from incinerator fire-smoke were very hazardous to human health. Near about 101 countries under the organization for Economic Cooperation and Development (OECD) had banned plastic import with awareness extension programmes of Green Peace (*w.e.f*1998). Himachal Pradesh as a pioneer in India, some part of Assam and Agartala and adjacent West District Area in Tripura had limited the use of plastic packing for fast food etc. By order, though people of the city and living nearby are making disposal of used packing. Disposal scoring for consecutive two years of 5±1 family numbers were recorded. Overall daily mean disposal score is 12.5 gram/day. Daily mean disposal scoring before and after implementation of amendment were 16.4 g/day and 4.9 g/day. Today's necessities are public awareness against the use of plastic and law enforcement for banning the sale of bags and packing made of plastic.

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Abstract of selected Invited papers

RNA Therapeutics-an overview

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RNA, Ribonucleic acid the key molecule involved in information transfer in the central dogma of molecular genetics, often credited with the origin of life, has also many other unique powers. The same RNA has adaptor molecule function as tRNA, structural molecule function as in ribosome or regulatory function as miRNA etc. The myriad of basic discoveries and technical development are now propelling RNA either as a therapeutic agent or as a therapeutic targets thus, occupying the forefront position in pharmaceutical industry. RNAs ability to induce immune response has also evinced keen interest among immunologists, its ability to down regulate or up regulate gene expression and interfere with the protein functioning has evoked interest among molecular geneticists and clinicians alike. Though these findings have made strategic advances in last few years there are many aspects to be standardized before the RNA reaches the shelf of Medical shops as drugs.

The emerging technologies involving RNA can be grouped into three, specially on the functional basis. Inhibition of the expression of a gene, that causes a disease directly or that help in the propagation of an agent that causes disease indirectly, is one of the most important avenues. The expression of such genes can be manipulated or suppressed by different means. Antisense approach is the oldest technology in this category. The different ribozymes such as Trans cleaving ribozymes, hammerhead ribozymes and hairpin ribozymes are the second category. However, the recent and most important category appears to be RNA interference mediated by small double stranded RNA of 21 to 23 nts long. At IVRI Mukteswar, research has been initiated using this technology, to suppress many of the viral genes there by developing alternate strategies for protection of livestock.

Quite often restoration of a gene function, can also help in preventing a disease here again trans splicing ribozymes, spliceosomes play a major role. RNA decoy and ligands such as aptamers, intramers and spiegelmers can play substantially for restoration of a gene expression through RNA mediation. This presentation deals with some of the promises of RNA in therapeutics in coming years in animal sciences.

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Current trends in diagnosis and prophylaxis of Capripox virus infections

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Capripox virus infections of sheep, goat and cattle result in significant economic loss. Prompt initiation of control measures such as slaughter; ring vaccination and restriction on movement of animals are needed to reduce the economic loss after diagnosis is made. The three virus members viz. goat pox virus (GPV), sheep pox virus (SPV) and lumpy skin disease virus (LSDV) of capripox virus genus are closely related and serologically indistinguishable. Pox disease in goats and sheep has usually been diagnosed on the basis of the clinical signs that constitute skin lesions, the gross pathology and the host species affected. However, it is not possible to unequivocally confirm the diagnosis of a disease as sheep pox or goat pox on the basis of merely clinical signs, the gross pathology and the host species affected. Serological tests like agar gel immuno-diffusion (AGID) test, counter-immuno-electrophoresis (CIE), or enzyme linked immunosorbent assays (ELISAs) have been of little value in laboratory confirmation of the disease due to serological cross-reactivity. The other existing methods that discriminate between capripox and other viruses are electron microscopy and virus neutralization test. Isolation of capripox virus is difficult as this virus grows slowly and requires many passages even if passaged in most sensitive cultured lamb testicle cells. However, none of these conventional techniques are able to unequivocally differentiate sheep pox and goat pox viruses. Most strains of poxviruses can cause disease in more than one animal species. Simultaneous infection of sheep and goats has been seen, at times, in a single flock where sheep and goats are reared in a mixed flock which is a general practice in goat and sheep rearing in most parts of the country. Such situations warrant development of a more reliable diagnostic assay that can differentiate between goat pox and sheep pox viruses. Although ELISA and virus isolation in cell culture are sensitive, they are tedious, time consuming and they also fail to detect virus particles that are bound to neutralizing antibody. The restriction fragment length polymorphism (RFLP) of the whole genomic DNA of virus could barely distinguish goat pox and sheep poxviruses. Of late, polymerase chain reaction (PCR) assays have been reported for detection of capripox viruses in sheep and goat skin biopsies and infected cell

culture fluids. However, none of these assays reported so far have been able to unequivocally differentiate between sheep pox and goat poxviruses which is essentially required in situations of simultaneous infection of sheep and goats in a flock with capripox virus. Restriction enzyme digestion of PCR amplified fragments, also known as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), has often been shown to be more reliable in differentiation of closely related virus isolates. The Indian Veterinary Research Institute (IVRI), over the last 2-3 years, had been successful in gaining some useful insights in molecular biology of poxvirus infections and as of now has taken a lead towards molecular epidemiology of capripox virus infections, differentiation of sheep pox and goat pox viruses as well as development of prophylactics and therapeutics.

###

Can genetic selection for immunological traits influence the nucleotide sequences of candidate genes for immune responsiveness

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White plumaged synthetic broiler dam line (SDL) maintained at experimental broiler farm of the institute has also been subjected to divergent selection for response to Sheep RBCs and PHA-P. Full sib mating plan was designed with 8 males randomly allotted to 6-8 females in each line to produce F1 generation of four divergent lines namely HSRBC and LSRBC (high and low lines for response to Sheep RBCs); HCMI and LCMI (high and low lines for response to PHA-P). The divergent lines showed significant differences in the mean values for respective immunological trait Mean titre (log₂ transformed) for response to sheep RBCs in HSRBC and LSRBC were 7.010 ± 0.216 and 6.423 ± 0.215 , respectively. Mean in vivo response to PHA-P in HCMI and LCMI lines were 47.76 ± 1.92 and 39.99 ± 1.83 , respectively. Candidate genes of MHC region and cytokines (IFN-g & IL-2 promoter and IL-2 Rg chain) were analysed in these divergent groups. PCR-RFLP analysis of BL-bII region with Taq I showed polymorphic variations with two genotypes AA and AC. The genotype frequencies of AA genotype in HSRBC, LSRBC, HCMI and LCMI lines were 0.9, 0.3, 0.1 and 1.0, respectively. Corresponding values for AC genotype were 0.1, 0.7, 0.9 and 0.0. IF-g gene promoter showed polymorphism with Tsp 509I enzyme. Genotype AA was observed at very high frequency in high responding HSRBC (1.0) and HCMI (1.0) lines as compared to low responding LSRBC (0.6) and LCMI (0.3) lines. Mnl I PCR-RFLP showed polymorphic pattern in IL-2 gene promoter. The genotype frequency of AA and AB genotype in HSRBC line were 0.2 and 0.8 and in LSRBC line 0.7 and 0.3, respectively. IL-2Rg chain showed polymorphism with Hph I enzyme with genotype frequency of AA and AB genotypes in HSRBC line as 0.80 and 0.2, respectively. Whereas, in LSRBC line all the individual were AB type with genotype frequency of 1.0. The frequencies of genotypes AA and AB in HCMI line were 0.50 and 0.50 respectively. Corresponding values in LCMI were 0.7 and 0.3. Cloning and sequencing of BL-b II region (of MHC) of SDL, Aseel and Turkey and IL-2 exon and IFN-g exon was performed in SDL individuals. The sequence analysis

of BL-bII gene revealed that SDL had 97% sequence homology with B21 haplotype thus was a variant of B21 haplotype. Aseel was B6 type. Turkey BL-bII therefore, was found to be a novel haplotype and the inclusion of its sequences has changed the topology phylogenetic tree of avian MHCs. Analysis of IL-2 exon sequenced showed that there were 6 nt differences and 2 nt differences from broiler. Out of the 6 nt variations in SDL IL-2 exon two were non-synonymous type. The sequence analysis of IFN-g revealed no sequence change as compared to published data.

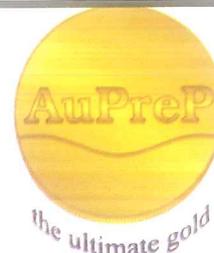
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Historic photograph taken in 1897 at Mukteswar on the eve of the visit by Robert Koch