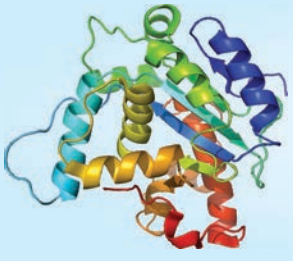
Indian Society for Veterinary Immunology and Biotechnology

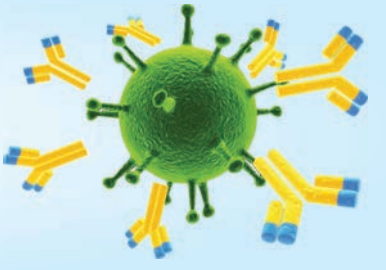
XXII Annual Convention
and
National Symposium
on
Immunomics and Proteogenomics in Livestock Health & Productivity

DECEMBER 17-19, 2015



VIBCON-2015

SOUVENIR & ABSTRACT



Organized by

ICAR-National Research Centre on Equines

Sirsa Road, Hisar-125001, Haryana India

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SOUVENIR & ABSTRACT BOOK

XXII Annual Convention and National Symposium on
"Immunomics and Proteogenomics in Livestock Health & Productivity"
December 17-19, 2015, ICAR-NRCE, Hisar (Haryana)

Organised by

ICAR-National Research Centre on Equines
Sirsa Road, Hisar-125001, Haryana India

Indian Society for Veterinary Immunology and Biotechnology

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Talluri Rao

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Dr. B.N. Tripathi
Chairman, Organizing Committee
VIBCON-2015

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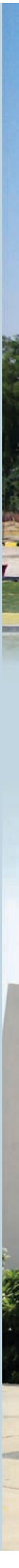

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Our Sponsors :

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Messages

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Message

The increasing human population, rapid urbanization, shrinking land for feed and fodder, increase in demand of protein rich diets and climatic changes are new challenges for the researchers and policy makers to cater to the needs and demands of the society. Science and technology are taking strides to contest these problems and new scientific frontiers are opening up with the rapid advancement and amalgamation of technologies. Fusion of new areas of technologies such as proteo-genomics and immunomics helps to open new vistas in understanding complex mechanisms of host-pathogen interactions, unlocking complex mechanisms of biological functions, devising strategies to discover potential targets for enhancing host immunity and developing next generation diagnostics and vaccines for diseases.

I am happy to know that Indian Society of Veterinary immunology and Biotechnology is organizing XXII Annual Convention - "VIBCON-2015" at National Research Centre on Equines, Hisar and have chosen a very apt theme - 'Immunomics and Proteogenomics in Livestock Health & Productivity' - for the National Symposium. This will certainly help the scientific fraternity to interact and deliberate with each other on the burning issues concerning the livestock health and productivity and to bring out new solutions for emerging problems through technological innovation and interventions.

The deliberations will bring out new collaborations between scientists working in various organizations / institutions traversing the disciplines. I expect that coherent support of scientific community will help examine transforming solutions to existing problems in the area of livestock health and production.

I wish the Programme, a great success.

Dated : the 7th December, 2015

Place : New Delhi

(S. AYYAPPAN)

Prof. K.M.L. Pathak
Deputy Director General
(Animal Science)
Indian Council of Agricultural Research
Krishi Bhawan, New Delhi-110 001

Message

I am glad to know that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) is organizing XXII Annual Convention (VIBCON-2015) and National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity" at National Research Centre on Equines, Hisar from 17-19 December, 2015.

In the current scenario of climate change leading to changes in the ecosystem exotic and emerging infectious diseases needs to be monitored and controlled effectively for better livestock health and production.

The VIBCON-2015 will provide a platform for interaction of biotechnologists, immunologists and animal health specialists from various disciplines on immunological advances in the field of proteomics and genomics for augmentation of livestock health and productivity. The collaboration and co-operation amongst researchers across the disciplines shall help in reviewing our current knowledge and technical acumen required to effectively manage the challenges being faced in enhancing the productivity of indigenous livestock.

I sincerely hope that efforts will be made by the researchers to use this opportunity to share their rich experiences in diversified fields of veterinary and animal sciences for sustainable animal husbandry through better health management & production.

I wish all success for this endeavour.

Date : 9th December, 2015

Place : New Delhi

(K.M.L. Pathak)

Dr. R. K. SINGH
Director & Vice-Chancellor
ICAR - Indian Veterinary Research Institute
Izatnagar-243 122, Bareilly, U.P.
President, ISVIB

Message

It gives me immense pleasure to share that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) is organizing its XXII Annual Convention & National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity" in collaboration with ICAR-National Research Centre on Equines, at Hisar.

Indian Society for Veterinary Immunology and Biotechnology (ISVIB) is the premier conglomerate organization of more than 1000 veterinary and animal scientists from the diverse disciplines with the objective of fostering the growth of veterinary immunology and biotechnology in the era of biological technology.

ICAR-NRCE, Hisar is a premier organization of ICAR working in frontier areas of Veterinary Sciences including immunology, biotechnology and infectious diseases. Recently, NRCE has been conferred with Sardar Patel Outstanding ICAR Institution Award - 2014 for its contributions in the area of equine health, production and management for the welfare of the society.

The theme of the conference has been aptly chosen to include the modern approaches to design strategies for combating livestock diseases and augment animal production. With the immense progress in the genomics and proteomics, scientists have been able to infer interrelationships between genes and/or proteins to better understand the immune system. The technological advances in proteo-genomics and immunomics have paved the way for understanding the genetic basis of host-pathogen interactions, which influence the host immune response. Innovations in genomic technologies have led to unraveling interactions of microorganisms with cells of the innate immune system. New proteomic approaches, including T-cell and B-cell-epitope mapping, have accelerated the pace at which scientists discover antibody-antigen relationships, aiding in the development of newer diagnostics and vaccines for infectious diseases.

The Convention will provide an excellent platform for scientists, academicians, students and industrialists pursuing research and developments in diverse areas of Veterinary Immunology and Biotechnology for sharing their scientific data and interacting with renowned scientists in the country.

I wish the conference a grand success.

(R. K. SINGH)

Dr. D. T. MOURYA
Director
National Institute of Virology
Pune, India

Message

I am pleased to know that ICAR-National Research Centre on Equines, Sirsa Road, Hisar and the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) are organizing XXII Annual Convention of ISVIB & National Symposium from December 17-19, 2015 at Hisar.

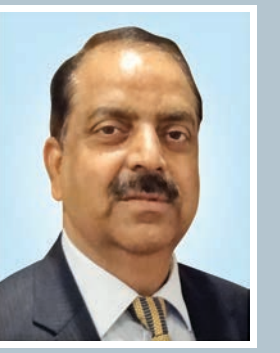
The ISVIB provides a perfect platform for the Immunologists and Biotechnologists from various disciplines of animal and human health for deliberations on issues related to improving livestock health and productivity, which in turn leads to better human health.

The theme of the Convention "Immunomics and Proteogenomics in Livestock Health & Productivity" is aptly chosen and will help in deliberation to design novel and effective strategies for combating the emerging diseases and augment animal production. I am sure that academicians and researchers across the disciplines will use this opportunity to share their rich experiences in diversified fields and will come out with roadmap for augmenting livestock health and productivity.

I wish all the success to the organization for the forthcoming event of VIBCON-2015.

(D. T. MOURYA)

Dr. B. N. TRIPATHI
Director
ICAR-National Research Centre on Equines,
Sirsa Road, Hisar-125001, Haryana
Chairman, VIBCON-2015



Message

I am extremely pleased that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) chose ICAR-National Research Centre on Equines (including Veterinary Type Culture Collection) at Hisar for organizing XXII Annual Convention & National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity" - VIBCON-2015.

Research in the area of genomics and proteomics has led us into the arena that holds promises to manipulate life through understanding the molecular mechanisms underlying the pathobiology of the diseases. It expands the horizons of modern medicine with unprecedented pace to offer cure of infectious diseases and complex oncology problems. In future, we expect a paradigm shift towards personalized targeted treatment, based on an individual's genome. Molecular diagnostic industry is also experiencing explosive growth and warrants further innovation and strategic planning to come to the rescue of our livestock population and thereby impact livestock productivity. Emerging descriptions of newly discovered immunological pathways and functional proteomics are leading to better understanding of perplexing nature of complex disease situations and carries new hope for development of immunoprophylactics and therapeutics. The systems immunology (immunogenomics) will further pave way to profile individual's immune system and assist in development of biomarkers for early diagnosis of the disease and development of disease resistant breeds. Genomics also brings hope for faster selection of traits of interest with better and reliable prediction than conventional testing programmes. Amongst a mingle of such upcoming technologies, we envisage an inter-disciplinary involvement of premiere universities, R & D institutions and industries via their most recent innovations and trends to be discussed at stretch in the upcoming conference.

NRCE Hisar has significantly contributed in the area of infectious diseases, veterinary immunology and biotechnology since its inception in 1985. The Institute is the recipient of Sardar Patel Outstanding ICAR Institution Award - 2014 for its contributions in the area of equine health, production and management for the welfare of the society. The institute is well equipped to perform high quality research and hence, it is most appropriate for us to host this very prestigious conference.

As Director, NRCE and the Chairman, Organizing Committee of VIBCON-2015, I heartily welcome guests and delegates to our institute and wish to provide the best hospitality and congenial environment for exchange of scientific intellect and hope for fruitful deliberations and discussions to take place during well crafted technical sessions.

Btripathi

(B.N. TRIPATHI)

PROF. PALANISWAMI K.S.

Vice President

ISVIB

Message

Greetings. It is my great pleasure to send the message though Chennai is in great distress due to Nature's fury possibly related to El Nino changes. Our Honourable Prime Minister has taken Chennai defect to Paris debate. The current rise of 1°C itself causes catastrophic changes and the climate change negotiations will almost certainly get the world deal but not one that may inhibit 2°C in world temperature . The livestock sector marginally contribute to this climate change but the effect is to be felt in adverse in decades to come. Two phase attempts are in the anvil for us. Reduce the methane and carbon monoxide contribution and plan to mitigate the adverse effect on health and production of livestock.

The platform provided by the NRCE ,Hisar during XXII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology(ISVIB), nicknamed as VIBCON 2015 is opt to debate the current scenario in terms Immunology and Biotechnology . With this basic theme ISVIB has provoked the intelligence of the galaxy of scientists assembled, to come up with presentations ,critical discussions and lobby debates. Our scale of preparedness is poor to combat microbial mutations in warming temperatures. The productivity is also at risk at the enhanced temperatures. The threat appears to be more than opportunities as of today but I am sure that the talented scientific community will aggressively thwart by close cooperation,integration and superior coordination. The Nation builders and the present Governments expect short and long term benefits in relation to the quantum of grants provided by them to the benefit of the ultimate target group, here our farming community. The expectations should be fulfilled by our scientific creativity. The challenges should be won with championship. This is my message to the enthusiastic young scientists. While thanking organisers for providing excellent platform,

I wish the conclave a great success. Best.

(PALANISWAMI K.S.)

DR. BALDEV RAJ GULATI
Principal Scientist
ICAR-National Research Centre on Equine
Organizing Secretary, VIBCON-2015

Message

National Research Centre on Equines (NRCE), Hisar is a unique organization working in the frontier areas of immunology, biotechnology and infectious diseases of equines. It has also established repository of animal pathogens i.e. National Veterinary Type Culture Facility at its Campus. Recently, NRCE has been conferred with Sardar Patel Outstanding ICAR Institution Award 2014 for its contributions. I express my gratitude to the Indian Society for Veterinary Immunology and Biotechnology for imposing faith on me for organizing XXII Annual Convention (VIBCON-2015) of ISVIB & National Symposium on "Immunomics and Proteogenomics in Livestock Health & Productivity" at NRCE from 17-19 December 2015.

With the technological advances in genomics and proteomics, scientists have been able to infer inter-relationships between genes and/or proteins to better understand how the immune system functions and how it is regulated. The current genomic technologies, like microarrays, T-cell and B-cells-epitope mapping, have been aiding in the development of newer tools and diagnostics & vaccines for infectious diseases affecting livestock health and productivity. This Convention will provide an excellent platform for deliberations on applications of principles of genomics, immunomics, systems biology, engineering, etc, for ameliorating livestock health and productivity.

The overwhelming response from scientific colleagues, researchers, students from all over India for participation has been driving force for the Organizing Committee. I extend my heartfelt thanks to all the delegates for joining in the Convention in the harsh winter climate of Hisar.

The guidance and encouragement from the office bearers of the ISVIB, specifically, Dr R K Singh (President) and Dr K S Palaniswami (Vice-President) and Dr A Thangavelu (Secretary) has been source of inspiration for me to take this onerous responsibility with ease. I express my gratitude to Dr B N Tripathi, Director NRCE & Chairman Local Organizing Committee for taking keen interest in every activity of Convention and providing all support for the event. Without the untiring support from team of wonderful colleagues like Dr Sanjay Barua (Co-organizing Secretary), Dr S C Yadav, Dr Rajender Kumar, Dr Nitin Virmani, Dr Balvinder Manuja, Dr Sanjay Kumar, Dr Rajesh Vaid, Dr S K Khurana, it was not possible to organize the event of this scale in a short span of time.

The financial assistance from the Indian Council of Agricultural Research, National Bank for Agriculture & Rural Development, Department of Biotechnology (Govt of India) is duly acknowledged and industry is duly acknowledged.

(BALDEV R. GULATI)

NRCE at A Glance

Since the dawn of Indian civilization equines find an important place in day to day life in context of our history. There might be debates about the first domestication of horse in India but they have been mentioned in the oldest scriptures of Hinduism- "Vedas" and have been the main source of transport for men and materials in past. In the present era, mechanization has decreased the utility of animal power; yet, equines have great relevance, especially in the hilly and difficult terrains of the country, where other means of transport are inaccessible. The increase of 43.34 % in mule population (0.19 m) in the recent livestock censes is a food for thought for planners and policy makers to focus more on mule production in country. At present, equine population in India is 1.14 m, which includes horses and ponies (55%), mules (17%) and donkeys (28%). Major population of these equids provide livelihood to the landless, small and marginal farmers and other sections of unprivileged rural societies.

In order to improve the health, performance and production potential of equines in India, the Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on November 26, 1985 at Hisar (Haryana) as its main campus. It has state-of-the art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. Equine Production Campus (EPC), a sub-campus of NRCE was established in 1989 at Bikaner in Rajasthan to undertake research on equine production, genetics and breeding, reproduction, physiology and nutrition. The research activities are supported by centralized services like animal and agriculture farms, experimental animal facility, BSL-III facility, ARIS cell, ATIC, library and Info-equine museum. The Centre has well maintained herd of Marwari, Zanskari and Manipuri horses and indigenous and exotic donkeys at Equine Production Campus, Bikaner.

Since its inception, institute's efforts have been focused on infectious diseases confronting equines, surveillance and monitoring of equine diseases, development of diagnostics and vaccines and improvement of equine health and production which has led to its recognition at national and international levels. The vision of the Centre is to enhance the utilization of equines in agricultural and transport through development programmes in order to elevate socio-economic status of under-privileged. Veterinary Type Culture Collection (VTCC) established in the year 2005 at Hisar for collection and preservation of microbes of animal origin and veterinary importance, is an integral part of the Centre.

Mandate of NRCE

- To undertake research on health and production management in equines;
- To act as a national referral facility for diagnosis of equine diseases and
- To provide advisory and consultancy services.

Objectives

- Generation of demand-driven technologies for equine health & production management.
- Capacity building for competitive equine power utilization in agricultural operations to serve the under-privileged in changing environment & socio-economic scenario.

Major Issues

- Achieving freedom from dreaded equine diseases through development of modern diagnostics & vaccines.
- Transfer of technology for superior mule & true-to-breed indigenous horse production in their home tracts.
- Artificial insemination and embryo transfer technology with an aim to establish embryo bank of Marwari/Kathiawari horses to enhance export.
- Enhancing performance of working equids especially in arid, semi-arid & mountainous regions.
- Income generation through market intelligence activities.

Thrust Areas

- Refinement of diagnostic tests and associative assays, kits and reagents
- Preparedness for developing newer diagnostic methods and preventive and control measures against infectious diseases of equines
- Understanding pathogen evolution through mutation or interaction of exotic genetic material, early warning against emerging/re-emerging diseases

- Emergency preparedness in terms of early diagnosis of disease, forewarning, and taking strategic control measures for the diseases with emphasis on clinical proteomics, whole genome sequencing in disease diagnosis, pathogen characterization and nanonized molecule(s) - targeted drug/vaccine delivery
- Use of bioinformatics and modern biotechnology tools in designing vaccines, drugs and stem-cell therapeutic approach for control of important equine diseases
- Development of national policy on disease control, prevention and management
- Conducting epidemiological investigations especially in widely distributed working equine populations with a statistically sound population sampling design so as to formulate disease forecast and control measures
- Establishment of equine sanctuary and in situ conservation of indigenous breeds of horses and donkeys by way of perfecting artificial insemination (AI) and embryo transfer technology (ETT)
- Indigenous breed conservation approaches and initiate immediate action plans with state government's/ NGO/SAUs and, agencies/ department approved by Government of India
- Initiate research work on equine welfare issues
- Generation of database and validation of ITKs in equine production and utilization
- Genetic improvement of mules, donkeys and ponies used for draught purposes
- Enhancing nutritional quality of indigenous feed/fodder for formulation of ration for equids
- Training of personnel including veterinarians and livestock assistants, educating equine breeders and farmers for adopting scientific equine practices
- Explorative research for value addition of equine products
- Equine work physiology of horses, ponies, donkeys, and mules
- Evaluating endurance potential of Marwari horses for equestrian events like Thoroughbred horses
- Evaluate donkey gut physiology and microbiome
- Assess potential of horse milk and donkey milk as of cosmetic value, sports drink for athletes, and therapeutic drink for ailing and recovering human patients for their rejuvenation
- Establishing equine sports medicine with special emphasis on creating infrastructure for studies on body scanning/mapping for kinetics of racing, athleticogenomics
- Elucidation of complete behavioural responses of equines under various physiological states

MAJOR ACHIEVEMENTS

Diagnostics for equine diseases

The Centre has been recognized as National Referral Centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture (Government of India). The Centre has developed and refined diagnostics against various equine diseases including immunodiagnostics and molecular diagnostics such as:

Equine Herpes virus 1 (EHV 1): A highly sensitive and specific neutralizing monoclonal antibody-based diagnostic kit, namely, Equiherpes B-ELISA was developed by the Centre for diagnosis of EHV-1 antibodies. This kit tests serum samples using single dilution, thus, making it very economical. Presently the kit is under the process of commercialization.

Equine Herpes virus 4 (EHV 4): A type-specific ELISA using EHV 1/4 recombinant glycoprotein G has been developed for differentiation of EHV 1 and EHV 4 infections. A multiplex PCR targeting glycoprotein C and G genes has also been developed for differentiation of EHV 1 and EHV 4 and is routinely used in the laboratory.

Equine Rotavirus: A sandwich enzyme-linked immunosorbent assay (s-ELISA) was developed employing a monoclonal antibody (mAb) raised against VP6 of rotavirus, for detection of equine rotavirus (ERV) from stool samples. An RT-PCR using VP6 gene primers was also developed and its results were compared with the s-ELISA. The RT-PCR was found to be equally sensitive as s-ELISA.

Equine influenza virus (EIV): EIV is routinely diagnosed by haemagglutination inhibition (HI) assay. RT-PCR for equine influenza diagnosis and typing has also been developed. Furthermore, real-time RT-PCR based assay targeting M & NP genes have also been developed for diagnosis of EIV.

Theileria equi: For serodiagnosis of T. equi, a recombinant

antigen based-ELISA has been developed using a truncated gene segment of a merozoite surface protein, EMA-2.

Trypanosomosis: An indirect ELISA has been standardized using whole cell lysate antigen of Trypanosoma evansi. RoTat 1.2 gene-specific PCR has also been standardized for sensitive detection of surra.

Japanese encephalitis virus (JEV): Serum neutralization test (SNT) and haemagglutination inhibition (HI) assay have been standardized for diagnosis of JE. Monoclonal antibodies against JEV have also been raised and are under trial for development of mAb-based capture ELISA.

Equine infectious anemia: Coggins test for EIA is routinely being used at the Centre. A recombinant protein from a synthetic gene of 26 kDa expressed in E. coli was evaluated for use in AGID/indirect ELISA in a pilot study for sero-diagnosis of EIA.

Equine viral arteritis: Virus neutralization is routinely used for serodiagnosis of EVA.

Small Animal models for understanding pathology and disease mechanisms

a) Equine influenza: ICAR-NRCE has developed a novel BALB/c mouse model for studying pathology and pathogenesis of equine influenza virus. The model will help in understanding disease mechanisms, host-pathogen interaction while simultaneously working for screening of vaccine candidates for their protective efficacy and immune response.

b) Equine Herpes Virus 1: ICAR-NRCE has standardized the BALB/c mouse model for EHV-1 for respiratory infection and abortion studies. The abortion model has been widely utilized in pathology laboratory of NRCE for protective efficacy of inactivated EHV-1 vaccine. The respiratory model has been used for immune prophylactic studies of recombinant proteins of EHV-1.

Vaccines and Immuno-biologicals developed by NRCE

a) EHV 1 vaccine: An equine herpes virus-1 (EHV 1) killed vaccine, namely, "EquiherpAbort" incorporating indigenous strain (Hisar-90-7) of EHV 1 has been developed by the Centre. This killed vaccine has already undergone field trials in mares. The vaccine with a three dose schedule induced good immune response in pregnant mares. The vaccine generates protective immune response, which is comparable to that of commercially imported Pneumabort 'K' vaccine in pregnant mares and is providing encouraging results.

b) Updated Equine influenza vaccine: Previously, the Centre had developed equine influenza vaccine (EI) using indigenous isolate (A/equi-2/Ludhiana/87). During 2008-09, India experienced another outbreak of equine influenza. An antigenically and genetically divergent EIV strain was isolated, which was significantly different from the previous (1987) isolate. Thus, the vaccine has been updated in 2010 incorporating epidemiologically relevant isolate {A/eq/Katra-Jammu 06/08 (H3N8)} responsible for EI outbreaks during 2008-09. The updated vaccine is safe and efficacious as evident by the protective immune response generated by the vaccine in field trials in equines.

c) Salmonella Abortus equi vaccine : Improved bacterin and outer membrane protein-based vaccines have been developed for Salmonella Abortus equi.

d) Monoclonal antibodies: Monoclonal antibodies have been developed for diagnosis and characterization of equine herpes virus 1, equine rotavirus, equine influenza and Japanese encephalitis viruses.

e) Kits for disease diagnosis: HERP kit & Equiherpes B-ELISA kit (For EHV 1 diagnosis), recombinant protein based ELISA for the diagnosis of Theileria equi & COFEB kit for diagnosis of Theileria equi have been developed by the Centre.

Surveillance and monitoring of equine diseases in India

ICAR-NRCE is involved in nation-wide monitoring and sero-surveillance of important equine infectious diseases, with a view to manage, control and eradicate diseases. Important achievements of the Centre in disease surveillance are:

- Information generated by institute about the status of African horse sickness (AHS) in the country helped in declaring India free of AHS in 2006 by Office International des Epizooties (OIE).
- Outbreaks of glanders in equines have been detected since 2006-07 and control measures are being adopted for preventing its further spread.

Molecular characterization of equine pathogens

Equine influenza virus (EIV): HA genes of EIV isolates from 2008 outbreak (A/eq/Jammu-Katra/08, A/eq/Mysore/08 and A/eq/Ahmedabad/09) were cloned and sequenced. Phylogenetic analysis established that 2008 EI outbreak in India was due to eq/2 (H3N8) subtype and that Indian isolates were identical to the Clade 2 of American lineage of H3N8 subtype. Also, the genetic analysis and selection pressure of matrix (M) gene of the Indian isolates

from 2008-09 outbreaks were studied and it was found that M1 and M2 proteins shared 98.41% and 99.54% homology with other Clade 2 viruses of Asian origin for M1 and M2 amino acid (aa) sequences, respectively. Phylogenetic analysis revealed clustering of Indian and Chinese isolates in a separate cluster designated as "Asian clade" for M gene. All the eight gene set (HA, NA, NP, NS, M, PA, PB1 & PB2) of EI isolates from 2008-09 outbreak have been cloned, sequenced and analyzed.

Equine rotavirus (ERV): Sequencing of VP7 gene of ERV isolates indicated circulation of G10, G3 and G6 serotypes in India. Sequencing of outer surface proteins (VP4 and VP7) of equine rotaviruses for their genotyping and molecular epidemiology was done.

Japanese encephalitis virus (JEV): Sequence analysis of E-gene of JEV isolated from an equine indicates genotype 3 was responsible for causing the disease in equine and that the equine JEV isolate clustered with Vellore group among isolates responsible for JEV in humans in India.

In vitro culture of Trypanosoma evansi: The Centre succeeded in in-vitro cultivation of bloodstream forms of T. evansi in artificial media by using specially formulated cell culture medium supplemented with 20% adult horse serum.

Biological resource Bank

NRCE has a strong biological resource base having numerous pathogens, recombinant clones, reference sera, equine sera, monoclonal antibody secreting hybridomas, etc.

- Pathogenic isolates (viruses, bacteria and parasites) of equine origin available with NRCE include EHV 1, EHV 4, equine rotavirus, equine influenza, Japanese encephalitis virus, West Nile virus, Rhodococcus equi, Streptococcus equi, S. zooepidemicus, S. Equisimilis, Burkholderia mallei, Salmonella Abortusequi, Enterobacter aerogenes, E. coli, Staphylococcus aureus and Trypanosoma evansi.
- ICAR-NRCE has a number of hybridomas secreting monoclonal antibodies against equine herpes virus 1, equine influenza, equine rotavirus, Japanese encephalitis virus, West Nile virus and Trypanosoma evansi.
- ICAR-NRCE has a repository of more than 15,000 equine serum samples collected from different geographical locations in its Equine Serum Bank.
- ICAR-NRCE has a collection of recombinant plasmid clones with recombinant genes of pathogens including equine influenza virus, equine rotavirus, EHV 1, EHV 4, EIA, JEV, EIAV, R. equi, Burkholderia mallei, Trypanosoma evansi and Theileria equi.

Indigenous breed characterization

- Phenotypic characterization of Indigenous horse and pony breeds

Populations of the six equine breeds registered by the Indian National Bureau of Animal Genetic Resources have drastically decreased due to indiscriminate breeding and their low utilization. These breeds namely Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri, were characterized phenotypically on the basis of their biometric indices and coat colour. Significant differences among different biometric indices were observed due to breed as well as sex.

On the basis of their heights at wither, Kathiawari and Marwari breeds were grouped under "horse", while Zanskari, Manipuri, Bhutia and Spiti fell under "pony" breeds.

In Marwari and Kathiawari breeds, both stallions and mares were found to rotate their ears at an angle of 180° making the ear tips meet in the centre, which is a typical characteristic of the two breeds.

- **Genotypic characterization of Indian equine breeds**

Genetic diversity analysis, population structure and relationship among six Indian horse (Kathiawari, Marwari) and pony breeds (Manipuri, Spiti, Zanskari and Bhutia), along with English Thoroughbred horses as an out group was carried out which indicated high genetic diversity in all Indian breeds except Spiti ponies with maximum genetic differentiation between Spiti and Thoroughbred (0.1729), followed by Spiti and Kathiawari (0.1725), while Zanskari and Manipuri were the least differentiated (0.0379). Individual assignment indicated admixture in all the breeds except Thoroughbred horses.

Establishment of Nucleus Herd

- Exotic Donkeys: Jennies and jacks of European breed (Poitu), imported from France & UK, are being maintained at EPC, Bikaner for the improvement of indigenous donkeys and production of superior mules.

- Marwari Horses: In effort to conserve the true to breed equids, the Centre has also established a nucleus herd of Marwari horse at EPC, Bikaner.
- Indigenous Donkey: The Centre has initiated the establishment of nucleus heard of small grey and large white donkeys found in India for conservation and improvement of donkeys.
- Equine sanctuary at EPC, Bikaner: ICAR-NRCE has initiated an in-vivo conservation programme in the form of developing an equine sanctuary at EPC, Bikaner. Under this 12 Zanskari ponies (8 mares & 4 stallions) were brought from Zanskar valley, Kargil, Ladakh, Jammu & Kashmir in November, 2009. In 2014, a total of 11 Manipuri ponies (7 mares & 4 stallions) were brought from Imphal, Manipur.

Improvement in production potential of equines

- Semen cryopreservation and artificial insemination (AI): In order to conserve the germplasm of indigenous equine breeds, the technique for cryopreservation of semen of Marwari stallions and donkeys have been standardized. The technique of artificial insemination using frozen semen for production of superior quality Marwari horses, superior mules and donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology.
- Pregnancy diagnosis: Pregmare - An eCG based sandwich ELISA kit has been developed for detection of pregnancy between days 30 to 150 of gestation in mare. The kit is cost effective, horse specific and animal friendly. Also pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares.

Utilization of animal energy with enhanced system efficiency

- Single animal drawn matching plough, seed drill (two furrow) and harness were designed and developed for donkeys and mules for performing various agricultural operations. Animal energy potential was utilized successfully in agricultural operations namely ploughing and sowing for different work hours without any adverse effect on the animals.
- Similarly, mules used in different ploughing experiments indicated that these can also be used efficiently in agricultural operation as all resumed to normal physiological conditions by the next morning.

Sustainable utilization of mule power for chaffing operation

The mules were successfully used for chaff cutting operation to reduce women drudgery. Average output capacity of chopped bajra straw in rotary mode chaff cutter was 660 kg/hour. Deployment of mules for operating a chaff cutter in rotary mode of operation is a viable option for sustainable utilization of equine power during idle hours.

Utilization of equine dung for preparation of vermicompost

The Centre was facing the problem of equine dung disposal as it cannot be utilized directly as manure in fields. It does not decompose properly due to low moisture content and poor water absorption. To overcome the problem of dung disposal, vermicompost is being prepared using equine dung in readymade vermibeds successfully and it is being applied in agricultural fields, lawns and plants.

Patents

- A highly sensitive kit for detection of antibodies against Theileria equi in serum of equids. Application No. 2763/DEL/2012, dated 06.09.2012.
- Nano-drug delivery for quinapyramine sulphate. Application, No.2560/DEL/2011, dated 06.09.2011. (NRCE, Hisar and GJUS &T, Hisar).
- Polynucleotide sequence, processes, composition and methods thereof- Application No. 1575/CHE/2010 and PCT/IB 2011/052475 (IISc Bangalore and NRCE, Hisar).
- A recombinant haemagglutinin domain-containing protein for the detection and diagnosis of glanders and method of preparation thereof. Application No.1328/DEL/2010 dated 08.06.2010. (DRDE Gwalior and NRCE, Hisar).

Services

ICAR-NRCE provides following services to the farmers and equine breeders:

- The Centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, state animal husbandry departments, police and army horses.

- Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- NRCE is providing health certification for movement of equines within and outside the country. This facility has helped in promotion of export of horses.
- Assessment and transfer of technology using the latest know-how of information technology is also given due importance to extend the technologies to the end-users.
- Extension activities: To receive feedback from the equine owners, various activities like health camp, awareness and farmers meets are organized on regular basis in different areas of the country.

Veterinary Type Culture Collection

Veterinary Type Culture Collection was established at NRCE by ICAR in 2005 as a national repository of animal microbes including dairy and rumen microbes with the aims of:

- Exploration and collection of microorganisms of animal origin/significance/relevance
- Central storage of animal microbes from existing culture collection centres, institutions and universities
- Characterization, documentation and digitization of microbial database of cultures of animal microbes
- Development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes
- Conservation (both short-term and long-term) and utilization of microorganisms

This microbial resource centre focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other microbial resources for research in Veterinary and life sciences.

Mandate

- To act as a national repository of micro-organisms including recombinant cultures and plasmids
- Identification, characterization and documentation of animal microbes
- Conservation, maintenance, surveillance and utilization for R & D
- Human Resource Development (HRD)

Milestone Achievements

- Several vaccine strains from livestock and poultry have been added to VTCC.
- Complete genome sequencing of two isolates of Classical swine fever virus was carried out.
- A total of 19 bacteriophage isolates against a variety of bacteria such as Aeromonas hydrophila, Ecsherichia coli, Staphylococcus spp., Bacillus spp., Pseudomonas spp. were isolated and accessioned in VTCC repository.
- Strengthening of repository with Veterinary microbes during the year:-
  - Bacteria accessioned : 227
  - Virus accessioned : 21
  - Bacteriophage : 19
  - Recombinant clones accessioned : 140
  - Genomic DNA accessioned : 47

So far, a total of 2546 cultures/clones have been deposited in VTCC after authentication, and conventional and molecular characterization including GC-FAME and sequence analysis of 16S rRNA & other genes. These microbes are being contributed by 19 network units including veterinary (7), rumen (8), and dairy (4) network units, and other ICAR institutes and State Agricultural and Veterinary Universities. These cultures/clones include veterinary pathogens including viruses, bacteria, bacteriophages, clones, rumen microbes comprising anaerobic bacteria and fungi, and dairy microbes. VTCC repository includes bacterial isolates represented by more than 900 isolates belonging to more than 50 genera. Viral isolates viz. camelpox virus, buffalopox virus, goatpox virus, orf bovine herpes virus 1, equine herpes virus 1 & 4, equine influenza virus, bovine rotavirus, human rotavirus, Japanese encephalitis virus, RD virus, BTV, NDV, CSF, PPRV, Avian virus are represented in VTCC repository.

Major Landmarks

1985	NRCE established at Hisar with Prof. P. K. Uppal joining as Founder Director
1987	Outbreak of Equine Influenza in Northern India
1989	Sub Campus of NRCE established at Bikaner for research on production in equines
1989	Occurrence of Equine Infectious Anaemia in India
1990	Exotic donkey germplasm with Poitu blood introduced from France
1991	Artificial insemination (AI) initiated in equines using fresh extended liquid semen
1991	Early pregnancy diagnosis (15 days post insemination) using ultrasonography
1995	Ciq-ELISA developed for detection of circulating immune complexes in EIA-infected horses
1995	Development of field-oriented immune-stick ELISA kit for detection of EHV-1 latent infection in Throughbred horses
1995	Cryopreservation of Jack semen and technology of AI perfected using frozen semen with 40% conception rate
1996	Establishment of a nucleus herd of Marwari horses at Bikaner campus
1996	Crystal structure of mare milk lactoferrin deduced by crystallography
1996	New carpet fabric developed by blending of donkey and sheep hair (Assheep)
1997	Equine Influenza vaccine using indigenous isolate (A/Equi-2/Ludhiana/87) released
2001	Patent for complement fixation test based diagnostic (COFEB)
2003	An Indian patent granted to a diagnostic kit for forecasting EHV
2005	Mab-based sELISA for detection of animal rotaviruses
2005	Establishment of Veterinary Type Culture Centre, at NRCE, Hisar
2006	Collection and cryopreservation of stallion semen at farmer's door using mobile laboratory
2006	World Organization for Animal Health declared India free of African horse sickness
2006	Outbreaks of Glanders in equines
2008	Re-emergence of Equine Influenza after 1987
2008	Equine Herpes Virus-1 diagnosis kit released
2008	ELISA based pregnancy diagnosis kit (Pregmare kit) for pregnancy diagnosis in mares released
2009	Development of Equine Herpes Virus-1 vaccine
2009	A nucleus herd of Zanskari ponies established at Bikaner
2009	First loboratory confirmed Camelpox zoonosis in the world
2009	Japanese Encephalitis Virus isolated from equines in India
2009	Re-emergence of Glanders in Chhattisgarh
2009	Updation of Equine Influenza vaccine
2009	First isolation of *Bordetella bronchiseptica* from horse, *Staphlococcus hyicus* from pig, *Corynebacterium pseudotuberculosis* and *Corynebacterium bovis* from horse & Methicillin- resistant Coagulase Negative *Staphylococcus sciuri* from goats
2010	Equine sanctuary for conservation of indigenous breeds of horses and indigenous donkeys initiated
2010	A new clade designated as 'Asian Clade' of Equine Influenza Virus reported

2010	Award of OIE twining project on Equine Poroplasmosis between NRCPD, Japan and NRCE, India
2010	EIA-positive mule detected in Haldwani: Re-emergance of EIA after 1998
2010	Phenotypic characterization of all six indigenous equine breeds
2010	Re-emergence of glanders in Himachal Pradesh and Uttar Pradesh
2010	Standardization of AI using semen of Poitu donkeys & Marwari horses
2010	Zanskari stallion semen cryopreserved
2010	Started toll-free helpline no. 1800-180-1233 for advisory services to equine owners at NRCE Hisar
2011	First laboratory confirmed report on BPXV causing disease in Buffalo, human and cow in same time and space
2011	Whole genome sequencing of Indian strain of Japanese Encephalitis virus
2011	Whole genome sequencing of *Pasteurella multocida* B : 2 strain
2011	First isolation of *Trueperella pyogenes* from buffalo, *Enterococcus asini* from horse & *Exiguobacterium* spp. from pig and *Brevibacterium* spp. and *Brevibacillus* spp.from Equine
2011	Indigenous donkeys (Small grey & Large white) inducted in Equine Sanctuary at EPC, NRCE, Hisar
2012	MOU with NRDC for commercialization of technologies generated by NRCE
2012	OIE twinning proposals for Equine Influenza and Glanders with Animal Health Trust, UK and Friedrich Loeffler Institute, Germany initiated
2012	Re-emergence of Equine Infectious Anaemia in Thoroughbred Polo horse in Haryana
2012	Started toll-free helpline no. 1800-180-6225 for advisory services to equine owners at EPC Bikaner
2012	Isolation of *Rhodococcus equi* from double-humped camel of Leh & Ladakh
2012	Development of recombinant protein -based ELISA kits for Glanders and Equine Piroplasmosis
2012	Development of EIA virus p26 synthetic protein -based ELISA for diagnosis of Equine Infectious Anaemia
2012	Whole genome sequencing of *Bordetella bronchiseptica, Pasteurella multocida, Actinobacillus equuli, Salmonella gallinarum and EHV-1*
2012	Single donkey/mule use ploughs and double donkey/mule use ploughs developed
2012	Work-Rest-Cycle established for indigenous donkeys/mules for ploughing/sowing
2012	Technique for Vermi-composting using equine dung developed
2013	Microbial Containment Laboratory (BSL-3 facility), Phase 1 of VTCC Laboratory Complex, ATIC and Info-Equine Museum at NRCE dedicated to nation inaugurated by Dr S. Ayyappan, Secretary DARE and DG ICAR
2013	Foundation stone of BSL-3 Facility of VTCC laid by Dr S. Ayyappan, Secretary DARE and DG ICAR
2013	First isolation of a *Nocardia otitidiscaviarum* from equine granulomatous pneumonia case and *Moraxella (Branhamella) ovis* from ovine keratoconjunctivitis in sheep
2014	First isolation of *Mannheimia varigena* from pneumonia in buffalo.
2014	Monoclonal raised against *T. evansi* for development of diagnostics.
2014	Recombinant protein based ELISA for diagnosis of *Burkholderia mallei*.
2014	Recombinant heat shock protein (HSP70) based ELISA for diagnosis of *Trypanosoma evansi* infection.
2015	Two technologies viz. Equiherpabort vaccine (EHV1 vaccine) and *Theileria equi* antibody detection kit released by Hon'ble Minister of Agriculture on 18 February 2015 at Annual General Body Meeting of ICAR
2015	Whole genome sequencing of classical swine fever virus completed
2015	"Sardar Patel Outstanding ICAR Institution Award-2014" conferred to ICAR-National Research Centre on Equines

XXII ANNUAL CONVENTION

INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY

(VIBCON-2015)

and National Symposium on

Immunomics and Proteogenomics in Livestock Health & Productivity

DAY 1: DECEMBER 17, 2015 (THURSDAY)

Time	Programme	Venue
9:00 AM – 10:00 AM	Registration	NRCE Reception
10:00 AM – 11:30 AM	Inaugural Session	NRCE Auditorium
11:30 AM – 12.00 Noon	High Tea	NRCE Lawns
12:00 Noon- 1.00 PM	S1: Dr. P. Richard Masillamony Oration and other ISVIB Awards	NRCE Auditorium
12:30 PM – 04:30 PM	POSTER SESSION 1: Genomics and Proteomics in Diagnosis and characterisation (PG)	NRCE Lawns
1:00 PM – 2:00 PM	Lunch	NRCE Lawns
2:00 PM – 4:00 PM	S2: Role of Immunomics in Livestock and Human Health (Plenary Session)	NRCE Auditorium
4:00PM – 4:30 PM	Tea Break	Lobby Auditorium
4:30 PM – 6:30 PM	S3: Advances in livestock production and reproduction technologies for improving livestock productivity	NRCE Auditorium
6:30 PM – 7:00 PM	General Body Meeting	NRCE Auditorium
7:30 PM – 9:30 PM	Get Together and Dinner	Surya Greens, Hisar

DAY 2: DECEMBER 18, 2015 (FRIDAY)

Time	Programme	Venue
9:00 AM – 11:0 AM	S4: Recent Developments in Emerging Infectious Animal Diseases	NRCE Auditorium
9:00 AM – 11:00 AM	S5: Immunomic advances in development of vaccines and therapeutics	CIRB Auditorium
11:00 AM – 11:30 AM	Tea	Lobby NRCE Auditorium
11:30 AM-1:00 PM	S6: Young Scientist Award Session	CIRB Auditorium
11:30 AM-1:00 PM	S7: Mid-Career Award Session	NRCE Auditorium
1:00 PM – 2:00 PM	Lunch	NRCE Lawns

Time	Programme	Venue
2:00 PM – 4:00 PM	S8: Novel Technologies and Informatics for Improving Livestock Health and Productivity	NRCE Auditorium
2:00 PM – 4:00 PM	S9: Advances in Diagnosis and Epidemiology of Livestock Diseases	CIRB Auditorium
4:00 PM – 4:30 PM	Tea Break	Lobby Auditorium
4:30 PM – 6:30 PM	S10: Applications of genomics and proteomics in diagnosis and control of animal and aquatic diseases	NRCE Auditorium
4:30 PM – 6:30 PM	S11: Application of Genomics and Proteomics in Characterization of Veterinary Pathogens	CIRB Auditorium
6:30 PM – 7:30 PM	ISVIB Executive Council Meeting	NRCE Auditorium
7:30 PM – 9:30 PM	Dinner	NRCE Lawns

DAY 3: DECEMBER 19, 2015 (SATURDAY)

Time	Programme	Venue
9:30 AM – 11:30 AM	S12: Genomic and Proteomic Advances for Improvement of Animal Productivity	NRCE Auditorium
11:30AM-12:00 Noon	Tea	Lobby NRCE Auditorium
12:00 Noon -1:00 PM	Valedictory Function	NRCE Auditorium
1:00 PM – 2:00 PM	Lunch	NRCE Lawns
2:00 PM – 4:30 PM	Site Seeing Tour to Agroha	NRCE Auditorium
4:30 PM – 5:00 PM	Tea Break	NRCE Lawns
7:00 PM – 8:30 PM	Dinner & Family Get Together	NRCE Lawns

Session 1: Dr. P. Richard Masillamony Oration Award and other ISVIB Awards

Session Time: 17 December 2015 (12:00 to 1:00 PM)

Venue: NRCE Auditorium

Sr. No.	Author	Title	Nature
OL-1	Dr J M Kataria, Director, Central Avian Research Institute, Izatnagar	Roadmap for strategical diagnosis and control of avian viral diseases in India	Oration Lecture

Session 2: Plenary Session (PS)

Role of Immunomics in Livestock and Human Health

Session Time: 17 December 2015 (2:00 PM to 4:00 PM)

Venue: NRCE Auditorium

Sr. No.	Author	Title	Nature
PS-1	Dr D T Mourya, Director, NIV, Pune	Emerging and re-emerging zoonotic infections	Key Note
PS-2	Dr R K Singh, Director, IVRI, Izatnagar	Immunomics and its potential applications in developing novel diagnostics, vaccines and therapeutics	Key Note
PS-3	Dr Shailendra K Saxena, CCMB, Hyderabad	Molecular mechanism of early innate immune response during Japanese encephalitis virus infection	Lead Paper
PS-4	Dr B N Tripathi, Director, NRCE, Hisar	Equine infectious diseases	Lead Paper

Session 3: Reproductive Technologies (RT)

Advances in livestock production and reproduction technologies for improving livestock productivity

Session Time: 17 December 2015 (4:30 PM to 6:30 PM)

Venue: NRCE Auditorium

Sr. No.	Author	Title	Nature
RT-1	Dr Abhijit Mitra, Director, NRC on Mithun, Jharnapani, Nagaland	Sperm mediated gene transfer as an alternate to production of transgenic animals	Lead Lecture
RT-2	Dr G Taru Sharma, Head Physiology and Climatology Division, IVRI, Izatnagar	Molecular portraits of buffalo oocytes and blastocysts as a consequence of the transition from maternal to embryonic control of gene expression	Lead Lecture
RT-3	Dr S Majumdar, National Institute of Immunology, New Delhi	Buffalo beta casein promoter driven production of therapeutic protein in the milk	Lead Lecture
RT-4	Dr Prem Singh Yadav, Central Institute for Research on Buffaloes, Hisar	Fetal adnexa tissues: Ideal source of mesenchymal stem cells	Invited
RT-5	Dr Sujoy K Dhara, Senior Scientist, Indian Veterinary Research Institute, Izatnagar	ELOVL6 expression may regulate expression of ELOVL2, 4 and 5 in porcine mesenchymal stem cells	Oral
RT-6	Dr Naresh L Selokar, ICAR-Central Institute for Research on Buffalo, Hisar	Somatic cells cryobanking: Future hope for multiplication and conservation of quality buffalo (Bubalus Bubalis)	Oral
RT-7	Dr A Palaniswami, Madras Veterinary College, Chennai	Characterization of stem cell like cells from pre implantation goat (Capra hircus) embryos	Oral

DAY 2: 18 DECEMBER 2015

Session 4: Emerging Infectious Diseases (ID)

Recent Developments in Emerging Infectious Animal Diseases

Session Time: 18 December 2015 (9:00 AM to 11:00 AM)

Venue: NRCE Auditorium

Sr. No.	Author	Title	Nature
ID-1	Dr. Dilip Kumar Sarma, Director, ICAR-National Research Centre on Pig, Rani- Guwahati	Classical swine fever (CSF): genomic and immunomic of CSF virus	Lead Paper
ID-2	Dr G Saikumar, Division of Pathology, Indian Veterinary Research Institute, Bareilly	Japanease encephalitis among Indian pigs and an update on genetic relationship between viruses circulating in countrsy	Invited
ID-3	Dr Minakshi Prasad, College of Veterinary Sciences, LUVAS, Hisar	Bluetongue in India: A journey from virus to vaccine	Invited
ID-4	Dr Sushila Maan, Deptt. of Animal Biotechnology, College of Veterinary Sciences, LUVAS, Hisar	Gaps in Orbivirus Research	Invited
ID-5	Dr H C Gera, Ex Nodal officer, NVBDCP Chandigarh	Vector Bionomics, Prevention and control of Vector Borne Diseases in Chandigarh	Invited
ID-6	P. Manesh Kumar, Ph.D.,Research Scholar, Madras Veterinary College, Vepery, Chennai	Expressional profiling of virus specific genes in avian cells infected with different pathotypes of New castle disease virus	Oral
ID-7	Dr Manoharan S, Professor, Vaccine Research Centre-Bacterial Vaccines, Centre for Animal Health Studies, TANUVAS, Chennai	Cloning and expression of major glycoprotein genes of Duck plague virus towards the development of field based diagnostics	Oral
ID-8	Dr Riyesh T, Scientist, ICAR-NRC on Equines, Hisar	Sequence analysis of host-range genes of Swinepox virus isolated from India	Oral
ID-9	Dr M Priyanka/Dr K G Tirumurugaan, Translational Research Platform for Veterinary Biologicals, 2nd floor, Central University Laboratory, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai-51	Isothermal detection of Peste des petits ruminants virus genomes from clinically infected small ruminants using uracil DNA glycosylase to avoid carry over contamination	Oral
ID-10	Dr Himanshu Sharma, Ph.D Scholar, Lala Lajpat Rai University of Veterinary & Animal Sciences, HSR	Development of in vitro Model for Persistent Equine Herpesvirus-1 Infection	Oral
ID-11	Dr. Richa Sood, Senior Scientist, National Institute of High Security Animal Diseases, Anandnagar, Bhopal	Emergence of naturally transmitted sheep associated malignant catarrhal fever in diverse ruminant species in India	Oral

Session 5 : Vaccines and Therapeutics (VT)

Immunomic advances in development of vaccines and therapeutics

Session Time: 18 December 2015 (9:00 AM to 11:00 AM)

Venue: CIRB Auditorium

Sr. No.	Author	Title	Nature
VT-1	Dr Gaya Prasad, Former ADG (AH), ICAR, New Delhi	Decoding immunologic Memory for Designing better Vaccines	Lead Paper
VT-2	Dr N K Kakker, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar	Success of Foot-and-Mouth disease control programme in Haryana	Invited
VT-3	Dr S. K Srivastva, Indian Veterinary Research Institute, Izatnagar	Leptospirosis Vaccines: Present status and future prospects	Invited
VT-4	Dr Praveen Malik, Ministry of Agriculture & Farmers Welfare, Government of India, Baghpat	Harmonization of Quality of Veterinary Biologicals in India	Invited
VT-5	Dr Shishir Kumar Gupta, Molecular Biology Lab, Division of Animal Biotechnology, Indian Veterinary Research Institute, Bareilly	Potential of oncolytic virotherapy for the treatment of animal cancers	Invited
VT-6	Dr. A. Thangavelu, Professor, Department of Veterinary Microbiology, Madras Veterinary College, Chennai	Evaluation of a DNA vaccine expressing peste des petits ruminants virus haemagglutinin	Oral
VT-7	Dr Yanglem Pushpa, Chingamakha Yanglem Leikai, PO/PS: Singjamei, Manipur	Some immunological studies on a new vaccine derived from bacteriophage lysate of Pasteurella multocida in mice	Oral
VT-8	Dr. Elaiyaraja. G., PhD Research Scholar, IVRI, Izatnagar, Bareilly	Nanoemulsion and rHSP 60 adjuvanted Foot and Mouth Disease antigen vaccine induced immune response in Guinea pigs	Oral
VT-9	Dr Sanjay Barua, Principal Scientist, ICAR-National Research Centre on Equines, Sirsa Road, Hisar	Screening of kinase and phosphatase inhibitors for their antiviral efficacy against buffalopoxvirus	Oral
VT-10	Dr Sumit Mahajan, Scientist, ICAR- IVRI, Izzatnagar	Nitrosative Stress Indices in Demyelinating Neuropathies in Dogs	Oral

Session 6: Young Scientist Award (YS)

Young Scientist Award Session

Session Time: 18 December 2015 (11:30 AM to 1:00 PM)

Venue: CIRB Auditorium

Sr. No.	Author	Title
YS-1	Dr Subas Chandra Jena, Ph.D Scholar (Animal Biotechnology), Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar	Identification of promoter region of dog survivin gene and its exploitation for development of gene constructs for selective apoptosis in dog cancer cells

Sr. No.	Author	Title
YS-2	Dr Rajanikant Sharma, PhD student, Division of veterinary Microbiology, SKAUST-Jammu	Molecular Characterization of *E. coli* virulence factors from calves
YS-3	Dr Anjali Somal, Division of Physiology and Climatology, ICAR-Indian Veterinary Research Institute, Izatnagar	Comparative profiling of fetal adnexa derived caprine stem cells
YS-4	Dr Vinay Joshi, Assistant Scientist, Department of Animal Biotechnology, College of Veterinary Sciences, Lala Lajapat Rai University of Veterinary & Animal Sciences Hisar	Development of Cell Penetrating Peptides for cellular delivery of peptide nucleic acid (PNA), a model study for nanodelivery and therapeutic interventions
YS-5	Dr Shishir Kumar Gupta, Division of Animal Biotechnology, Indian Veterinary Research Institute, Bareilly	Poly (I:C) enhances anti-tumor and antimetastatic activities of canine parvovirus NS1 protein
YS-6	Dr Aman Kamboj, PhD Scholar, Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar	Construction of infectious cDNA clone derived from a classical swine fever virus field isolate in BAC vector using in vitro overlap extension PCR and recombination
YS-7	Dr Adarsh Mishra, PhD Scholar, Department of Veterinary Microbiology, Madras Veterinary College, TANUVAS, Vepery, Chennai	Genotyping of single nucleotide polymorphism in goat poxvirus field isolates through a a novel p32 gene based tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) assay
YS-8	Dr Suswetha Das Mitra, Research Associate, NIVEDI, Bangalore	Staphylococcus aureus ST-2219t6877 and ST-2182t267 intramammary inoculation in a mouse model reveals modulation of transcriptome landscape with alternative Splice variants
YS-9	Dr Mamta Pandey, School of Animal Biotechnology, GADVASU, Ludhiana	Erythroblastic Leukemia Viral Oncogene Homolog 2 (ErbB2) as a diagnostic biomarker of canine mammary carcinomas

Session 7: Mid Career Award (MC)

Mid-Career Award Session

Session Time: 18 December 2015 (11:30 AM to 1:00 PM)

Venue: NRCE Auditorium

Sr. No.	Author	Title
MC-1	Dr Vikas D Dighe, National Institute for Research in Reproductive Health, Parel, Mumbai	Identification and Characterization of Brain and Testis Specific Homing Peptides using Phage Display Library
MC-2	Dr B C Bera, Scientist, ICAR-National Research Centre on Equines, Hisar	ORFeome of equine influenza virus: state-of-the-art clone resource for functional genomics
MC-3	Dr Taruna Anand, Scientist VTCC, National Research Centre, Hisar	Emergence and spread of antibiotic resistance genes in environmental bacteriophage
MC-4	Dr Yashpal S Malik, Principal Scientist, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar	Whole genome analysis of bovine rotavirus strain carrying novel genomic constellation with evidence of multispecies reassortment

Sr. No.	Author	Title
MC-5	Dr Varij Nayan, Scientist, ICAR-CIRB, Hisar	In silico identification of a novel peptide epitope and generation of antibody for sensing buffalo Luteinizing Hormone
MC-6	Dr Sonika Ahlawat, Scientist, National Bureau of Animal Genetic Resources, Karnal, India	Extraordinary diversity in the zinc finger domain of the speciation gene PRDM9 but multiple disruptive mutations in its paralog PRDM7 in ruminants
MC-7	Dr Rajeshwari Shome, Principal Scientist, ICAR-NIVEDI, Bengaluru	Indigenous Indirect ELISA kit for Diagnosis of Brucellosis in sheep and goat
MC-8	Dr S. K. Maurya, Department of Veterinary Physiology & Biochemistry, COVS & AH, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad	Polymorphism of prolactin gene and its association with egg production performance in Kadaknath hens
MC-9	Dr K.G. Tirumurugaan, Programme Head (Diagnostics), TRPVB, DCAHS, TANUVAS, Chennai	Understanding the Circulating Indigenous Strains and Innate Resistance Potential of Indigenous Livestock-To Enable Better Control Strategies

Session 8: Novel Technologies and Informatics (NI)

Novel Technologies and Informatics for Improving Livestock Health and Productivity

Session Time: 18 December 2015 (2:00 PM to 4:00 PM)

Venue: NRCE Auditorium

Sr. No.	Author	Title	Nature
NI-1	Dr B P Mishra, Joint Director, Research, IVRI, Izatnagar	Application of transcriptomics in understanding host-pathogen interaction	Lead paper
NI-2	Dr Praveen Singh, I/C CIF Bioengineering, Division of Vet Biotechnology, IVRI, Izatnagar	Point of Care Nano-diagnostics for Pathogens and Parasites	Invited
NI-3	Dr Arvind Kumar, Dept. Veterinary Microbiology, LLR University of Veterinary & Animal Sciences, Hisar	Information technology enabled spatial epidemiology based approach for control and eradication of Hemorrhagic septicemia	Invited
NI-4	Dr Ajit Singh, Department of Veterinary Microbiology, Prof., LLR University of Veterinary and Animal Sciences, Hisar	Potential applications of single- domain antibodies ('Nanobodies') produced by phage display technology	Invited
NI-5	Dr G.V.P.P.S. Ravi Kumar, Senior Scientist, Animal Biotechnology, Indian Veterinary Reseach Institute, Izatnagar	Transcriptome analysis: Deciphering the Interactome	Invited
NI-6	Dr Subhash Verma, Department of Veterinary Microbiology, DGCN -COVAS, CSK-HPKV, Palampur	Nano-immunobiologicals:associated interference with standard cytotoxicity tests	Invited

Sr. No.	Author	Title	Nature
NI-7	Dr Sonika Ahlawat, Dr Sonika Ahlawat, Scientist, National Bureau of Animal Genetic Resources, Karnal	Identification of novel splice variants of meiotic recombinase DMC1 gene in ruminants	Oral
NI-8	Dr Poonam Jayant Singh, National Bureau of Fish Genetic Resources, Lucknow	Patinformatics for Technological Competence in the Area of Proteo-Genomics for improving Livestock Health and Productivity	Oral
NI-9	Dr Pandukar CY/Ramakrishanan MA, Indian Veterinary Research Institute, Mukteswar	Differentiation of capripoxviruses at species level by a newly developed PCR assay	Oral

Session 9: Diagnosis & Epidemiology (DE)

Advances in Diagnosis and Epidemiology of Livestock Diseases

Session Time: 18 December 2015 (2:00 PM to 4:00 PM)

Venue: CIRB Auditorium

Sr. No.	Author	Title	Nature
DE-1	Dr L K Gupta, Director, IgYImmunologix, Hyderabad	IgY antibodies - Potentials for research diagnostics and human nutraceuticals	Lead
DE-2	Dr P P Goswami, Division of Veterinary Biotechnology, IVRI, Izatnagar	Opportunities for Improved Serodiagnosis of Paratuberculosis in animals	Invited
DE-3	Dr Satish Kumar, Emeritus Scientist, ICAR-IVRI, Izatnagar, Bareilly-243122, U.P., INDIA	Biomolecules infused nanomaterials in making rapid affordable diagnosis	
DE-4	Dr Rashmi Singh, Associate Professor, College of Veterinary Sciences & Animal Husbandry, DUVASU, Mathura	3AB3 recombinant protein based DIVA ELISA for screening of anti-non structural protein antibodies against FMD virus in Uttar Pradesh	Oral
DE-5	Dr. K. Sripad, Deputy Director, Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore	A study on comparison of various diagnostic techniques for diagnosis of Rabies in and around Bangalore	Oral
DE-6	Dr Himani Dhanze, Scientist, Division of Veterinary Public Health, IVRI, Izatnagar	Comparative evaluation of serological assays for diagnosis of Japanese encephalitis in swine	Oral
DE-7	Dr K Senthil Kumar, Zoonoses Research Laboratory, Centre for Animal Health Studies, TANUVAS, Chennai	Seroprevalence studies on animal leptospirosis in South Andaman	Oral
DE-8	Dr Aman Kumar, Asstt. Res. Officer, College of Veterinary Sciences, LUVAS Hisar	An outbreak of bovine popular stomatitis virus infection in buffalo calves	Oral

Sr. No.	Author	Title	Nature
DE-9	Dr K Anbu Kumar, Asstt Prof, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai	Porcine Circovirus Type 2 predisposes swine to infection with classical swine fever virus	Oral
DE-10	Dr Naveen Kumar, Senior Scientist, Veterinary Type Culture Collection, ICAR-National Research Centre on Equines, Hisar	Peste des petits ruminants virus and foot-and -mouth disease virus coinfection in goats: Long-term *in vitro* co-persistence of two acute pathogenic viruses	Oral
DE-11	Dr Yashpal S Malik, Principal Scientist, ICAR-IVRI, Izatnagar	Frequency distribution of picobirnavirus in diarrheic and non-diarrheic bovine calves	Oral
DE-12	Dr Parimal Roy, Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai	An outbreak of PPR in goats at Nilagiris hills of Tamil Nadu	Oral
DE-13	Dr Hari Mohan, Assistant Prof., Center for Medical Biotechnology, M.D. University, Rohtak-124001, Haryana, India	Genotypic linkages of VP6 gene of human rotavirus isolates circulating in pediatric patients with acute gastroenteritis in Haryana indicated close proximity of antigenic epitopes with bovine strains	Oral

Session 10: Diagnosis & Control (DC)

Applications of genomics and proteomics in diagnosis and control of animal and aquatic diseases

Session Time: 18 December 2015 (4:30 PM to 6:30 PM)

Venue: NRCE Auditorium

Sr. No.	Author	Title	Nature
DC-1	Dr Rajesh Vaid, Principal Scientist, ICAR-NRC on Equines, Hisar	Exploration of cultural bacterial biodiversity and discovery of unusual animal pathogens	Invited
DC-2	Dr. S.S. Mishra, Principal Scientist & Head, Central Institute of Freshwater Aquaculture (ICAR), Bhubaneswar, Odisha	Advances in genomics and immuno diagnostics in fish disease diagnosis	Invited
DC-3	Dr Rajender Kumar, National Fellow, ICAR-National Research Centre on Equines, Sirsa Road, Hisar	Recent advances in diagnosis of trypan-osomosis in animals using immunological and molecular approaches	Invited
DC-4	Dr Alka Tomer, Immunology Section, Indian Veterinary Research Institute, Izatnagar	Mixed infection of avian leukosis virus (ALV) and Marek's disease virus (MDV) among dead commercial chickens	Oral
DC-5	Dr Sanjoy Das, Kakdwip Research Centre of CIBA, Buddhapur, West Bengal	Bacteriological study of the wound of juvenile Hilsa (Tenualosa ilisha, Hamilton)	Oral
DC-6	Dr S Dey, ICAR-Indian Veterinary Research Institute, Izzatnagar	Myelin basic protein in cerebrospinal fluid: a diagnostic marker for neuroinflammation	Oral

Sr. No.	Author	Title	Nature
DC-7	Dr. V. Balamurugan, Senior Scientist, ICAR- NIVEDI, Yelahanka, Bengaluru	A novel two-tube based Multiplex PCR assay for the detection and differentiation of pathogenic leptospira with specifically identification of five pathogenic serogroup	Oral
DC-8	Dr K. Manimaran, Assistant Prof., Tamil Nadu Veterinary and Animal Sciences University, Chennai	Detection of Avian Mycoplasmosis by Conventional and Molecular Techniques in Poultry in Tamil Nadu state	Oral
DC-9	Dr Padmashree BS/Dr Rajeswari Shome, Principal Scientist, NIVEDI, Bangalore	Lab-to- Land technology: A pen-side diagnostic tool for brucellosis infection in multiple livestock species	Oral
DC-10	Dr B Vasanthi / Dr K.G. Tirumurugaan, Programme Head, (Diagnostics), TRPVB, DCAHS, TANUVAS, Chennai	Viability PCR to Detect the Most-Probable-Number of Probiotic Bacteria from Commercial Preparations	Oral
DC-11	Richa Salwan/Aneesh Thakur, DGCN COVAS, CSKHPKV, Palampur	Pasteurella multocida infection modulate inflammatory and immunological responses in mice by altering the expression of virulent genes	Oral
DC-12	Dr K.Senthil Kumar, Centre for Animal Health Studies, TANUVAS, Chennai	Isolation and molecular characterization of leptospira sp strain isolated from canine by PCR and 16s rRNA gene sequence analysis	Oral
DC-13	Dr Rajesh Vaid, Principal Scientist, ICAR-NRC on Equines, Hisar	Isolation and identification of an antimicrobial resistant persistent cell culture contaminant as Achromobacter spp	Oral

Session 11: Characterization of Pathogens (CP)

Application of Genomics and Proteomics in Characterization of Veterinary Pathogens

Session Time: 18 December 2015 (4:30 PM to 6:30 PM)

Venue: CIRB Auditorium

Sr. No.	Author	Title	Nature
CP-1	Dr Nitin Virmani, Principal Scientist, ICAR-NRC on Equines, Hisar	Equine influenza virus : An insight into evolution and interspecies transmission	Lead Paper
CP-2	Dr Sanjay Kumar, Principal Scientist, ICAR-NRC on Equines, Hisar	Equine piroplasmosis-an insight into interacting molecules and novel drug targets	Lead Paper
CP-3	Dr Chakradhar Tosh, Principal Scientist and OIEExpert, ICAR-National Institute of High Security Animal Diseases, Anand Nagar, Bhopal	Evolution of avian influenza viruses: Indian and global context	Invited
CP-4	Dr Yashpal S Malik, Principal Scientist, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar	Porcine enteric rotavirus infections: Etiology and genotypic distribution with impact assessment	Invited

Sr. No.	Author	Title	Nature
CP-5	Dr Amit Kumar Pandey, Assistant Professor, Translational Health Science and Technology Institute, Faridabad	Mycobacterium tuberculosis on steroids	Invited
CP-6	Dr. Shakil Ahmad Wani, Sher-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar	Changing pattern of bovine group A rotavirus in Kashmir, India	Oral
CP-7	Dr Chakradhar Tosh, Principal Scientist and OIE Expert, ICAR-National Institute of High Security Animal Diseases, Anand Nagar, Bhopal	Multiple introductions of a reassortant H5N1 avian influenza virus of clade 2.3.2.1c with PB2 gene of H9N2 subtype into Indian poultry	Oral
CP-8	Dr. Niraj Kumar Singh, Assistant Scientist, School of Animal Biotechnology, GADVASU, Ludhiana	Heterologous expression of exposed outer domain of 86 kDa outer membrane protein (OPR86) of Pseudomonas aeruginosa	Oral
CP-9	Dr Balvinder Kumar, ICAR-National Research Centre on Equines, Sirsa Road, Hisar	Prediction of functional changes in equine Mx protein and association with susceptibility vis-a-vis resistance against Equine Influenza	Oral
CP-10	Dr Shalini Sharma, Assistant Prof., Lala Lajpat Rai University of Veterinary and Animal Science, Hisar	Cytomegalovirus infection enhances the immune response to influenza	Oral
CP-11	Dr Sankar Muthu, IVRI, Mukteswar campus, Nainital IVRI, Izatnagar	Quantitative real-time PCR (qPCR) based diagnosis of benzimidazole resistance and comparison of various tests for its efficacy	Oral

DAY 3: 19 DECEMBER 2015

Session 12: Animal Productivity (AP)

Genomic and Proteomic Advances for Improvement of Animal Productivity

Session Time: 19 December 2015 (9:30 AM to 11:30 AM)

Venue: CIRB Auditorium

Venue: NRCE

Sr. No.	Author	Title	Nature
AP-1	Dr Naveen Kumar, VTCC, NRCE, Hisar	Host-targeting antiviral agents	Invited
AP-2	Dr Mamta Chauhan, Senior Scientist, ICAR-NRC on Equines, Hisar	Scope of genomic tools in enhancing animal breeding potential	Invited
AP-3	Dr Shalini Sharma, Assistant Prof., Lala Lajpat Rai University of Veterinary and Animal Science, Hisar	The two faces of heterologous immunity: protection or immunopathology	Invited

Sr. No.	Author	Title	Nature
AP-4	Dr T R Talluri, Scientist, ICAR-NRC on Equines, Bikaner, Rajasthan	Cytoplasmic injection of murine zygotes with Sleeping Beauty transposon plasmids and mini-circles results in the efficient generation of germline transgenic mice.	Oral
AP-5	Dr Papori Sharma, PhD Scholar, Animal Genetics and Breeding, LUVAS, Hisar	Effect of sodium butyrate (histone deacetylase inhibitor) on donor cell physiology, those are used for production of handmade cloned buffalo (Bubalus bubalis) embryos	Oral
AP-6	Dr Ratan K Choudhary, Assistant Prof., Guru Angad Dev Veterinary and Animal Science University, Ludhiana	Immunolicalization of MSI1 and FNDC3B in water buffalo mammary glands	Oral
AP-7	Dr Anuradha Bhardwaj, Scientist, ICAR-National Research Centre on Equines, Sirsa Road, Hisar	Molecular Docking studies of equine Chorionic Gonadotropin through insilico approach	Oral
AP-8	Dr Balvinder/Dr Anju Manuja, Senior Scientist, ICAR-National Research Centre on Equines, Sirsa road, Hisar	Hepatic gene expression profiling of rats reveals key enzymes involved in quinapyramine sulfate metabolism	Oral
AP-9	Dr Vijay Kumar, NRC on Equines, Bikaner	Seasonal variation in estrous cycle characteristics of Marwari mares in two different seasons as monitored by behavioral cues and ultrasonography	Oral
AP-10	Dr Deepak Kumar/Sandeep Gera, COVS LUVAS, Hisar	Hematological Profile of Hardhenu Strain of Cattle in Comparison to Sahiwal and Hariana Breed.	Oral
AP-11	Dr Yash Pal, Principal Scientist, ICAR-NRC on Equines, Hisar	Seminal plasma protein profile in indigenous jacks	Oral
AP-12	Dr Mamta Chauhan, Senior Scientist, ICAR-NRC on Equines, Hisar	Importance of genomic tools for parentage testing in equines	Oral
AP-13	Dr Harisankar Singha, ICAR-National Research Centre on Equines, Hisar	Expression of recombinant equine interleukin (IL) -4 and interleukin (IL) -10 and its effect on cytokine production by equine peripheral blood leukocytes	Oral
AP-14	Dr Pallvi/ Dr R S Sethi, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab	Expression of TLR4, TLR9, TNF-α and IL-1β in lungs following exposures to poultry barn air	Oral
AP-15	Dr. Subodh Kumar, Principal Scientist, ICAR - Indian Veterinary Research Institute, Izatnagar	Identification of polymorphism in TLR4 genes and their association with occurrence of Paratuberculosis in cattle	Oral

POSTER SESSION 1

Genomics and Proteomics in Diagnosis and characterisation (PG)

17 DECEMBER 2015 12:30 PM-04:30 PM

Sr No.	Name	Address	Title
PG-1	Dr A S Selvaramesh/ Dr Pushpendra Kumar	IVRI, Izatnagar	Cloning and characterization of partial chicken Mx1 gene
PG-2	Dr N Nagpal / Dr Pushpendra Kumar	Division of Animal Genetics, IVRI, Izatnagar	Single Strand Conformation Polymorphism (SSCP) in Caprine mLYS (Intron-III) gene and its effect on serum lysozyme activity and SCC
PG-3	Dr Kush Shrivastava/ Dr Pushpendra Kumar	IVRI, Izatnagar	MHC Class II DRB gene polymorphism in Rohilkhandi goats
PG-4	Dr Vikas Kumar Singh	PhD Scholar, IVRI, Izatnagar	Peripheral blood mononuclear cells of brucella infected goats showed more expression of TLR 4 and INF γ.
PG-5	Dr Mehtab S Parmar	ICAR-IVRI, Izatnagar	Expression and secretory profile of homologous fetal fibroblasts and Wharton's jelly feeder layers used for propagation of buffalo embryonic stem cells
PG-6	Dr Kirthika P	PhD student, College of Vety. Sciences & AH, Central Agricultural University, Mizoram	Dynamics of mitogen stimulated cytokine gene expression in peripheral blood mononuclear cells of indigenous and exotic breeds of pigs
PG-7	Ms Swasti Kakker	M.Sc. Scholar, Birla Institute of Technology and Science, Pilani, Rajasthan	Genomics and proteomics in genetic testing of animals
PG-8	Dr IRFAN AHMAD MIR/ Dr Sunil Maherchandani	SRF, College of Veterinary and Animal Science, RAJUVAS, Bikaner	Characterization of Salmonella enterica Using PCR based Genotyping techniques
PG-9	Dr Rahul Yana	PhD Scholar, Rajasthan University of Veterinary and Animal Science, Bikaner	Detection of coagulase gene polymorphism in Staphylococcus aureus isolated from cattle and buffalo mastitic milk
PG-10	Dr. Dibyajyoti Hazarika	Department of Veterinary Microbiology, GADVASU, Ludhiana	Detection of bovine brucellosis by bruce ladder and Hinic real time PCR
PG-11	G Ravikumar / S Senthil Kumar	Assistant Professor, Centre for Animal Health Studies, Chennai	Molecular detection of an outbreak of anthrax in ovine in Tamil Nadu
PG-12	Dr Pawan Kumar	Assistant Professor, Department of Animal Biotechnology LUVAS, Hisar	Intragenic variations among Indian isolates of Bluetongue serotype 9 demonstrate Genomic Diversity
PG-13	Dr Bhuvana Priya	PhD Scholar, Division of Bacteriology, IVRI, Izatnagar	Microarray analysis of host and bacterial gene expression in Pasteurella multocida infected mice model

Sr No.	Name	Address	Title
PG-14	Dr Shishir K Gupta/ Dr. Aditya Prasad Sahoo	Scientist, Molecular Biology Lab, Division of Veterinary Biotechnology, IVRI, Izatnagar, Bareilly	Canine parvovirus NS1 protein induced apoptosis in HeLa cells causes accumulation of reactive oxygen species and follows intrinsic pathway of apoptosis
PG-15	Dr. P. Raja	Ph.D Scholar, Madras Veterinary College, Chennai	Rapid detection of infectious bursal disease in chicken by reverse-transcription loop mediated isothermal amplification
PG-16	M Kumar/C Tosh	Principal Scientist and OIE Expert, ICAR-National Institute of High Security Animal Diseases, Bhopal	Isolation and characterization of an Indian H6N2 avian influenza virus
PG-17	Dr Paviter Kaur	Assistant Professor, Deptt. of Veterinary Microbiology, GADVASU, Ludhiana	Genus specific Real-time PCR using IS711 specific primers and Taqman probe chemistry for identification of Brucella
PG-18	Dr Paviter Kaur	Assistant Professor, Deptt. of Veterinary Microbiology, GADVASU, Ludhiana	Multiplex PCR assay for detection of different bacterial pathogens associated with reproductive disorders in cattle and buffaloes
PG-19	Dr Shahnaj Parbin Ahmed	College of Veterinary Science, Assam Agricultural University, Assam	Detection and Genotypic Characterization of Rotavirus from Bovine Calves
PG-20	Dr Mahavir Singh	PhD Scholar, College of Veterinary Sciences, LUVAS, Hisar	Relative expression of proinflammatory cytokines in milk somatic cells of subclinical mastitis affected buffaloes
PG-21	N. Chacko/ S.B. Shivachandra	Senior Scientist, ICAR-NIVEDI, Ramagondanahalli, Bengaluru	Oligomerization of recombinant non-structural protein 3 N-terminus (NS3Nt) of bluetongue virus by Coiled coil motif (CCM)
PG-22	Dr Vinay Kumar	Assistant Scientist, College of Veterinary Sciences, LUVAS, Hisar	Development of Real Time PCR for Diagnosis of Brucella Sp.
PG-23	Dr Mukesh Bhatt, Dr Sankar Muthu	IVRI, Mukteswar campus, Nainital	Cloning and expression of protease inhibitor, cystatin of Haemonchus contortus and its expression kinetics during infection
PG-24	Dr Prabhat Kumar	ICAR-National Research Centre on Equines (NRCE), Hisar	Isolation and characterization of porcine adenovirus (PAdv) for possible use as a vector in vaccine delivery
PG-25	Dr Kapil Gupta, Dhirendra Meena	Department of Veterinary Microbiology and Biotechnology, Rajasthan University of Veterinary and Animal Science, Bikaner	Genetic confirmation of Escherichia coli obtained from different sample of water in nearby places at Bikaner

Sr No.	Name	Address	Title
PG-26	Dr Irfan Ahmad Mir, Dr Sunil Maherchandani	COVAS, RAJUVAS, Bikaner	Phenotypic and Genotypic Investigation of Antibiotic Resistance determinants in Salmonella enterica isolated from Poultry
PG-27	Dr Himanshu Sharma	Ph.D Scholar, Deptt. of Veterinary Microbiology, Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana, India	Prevalence of Latent equine herpesvirus infection among horses in India
PG-28	Dr Dheeraj Pal	PhD Scholar, Gene Expression Laboratory, IVRI, Izatnagar, Bareilly, Uttar Pradesh-243122, India	Study of gene expression encoding 16 and 18.5kDa Proteins from Mycobacterium avium Subspecies paratuberculosis (MAP)
PG-29	Dr. Shaheen Farooq	Subject Matter Specialist (Animal Science), Krishi Vigyan Kendra, Kupwara, J&K	Prevalence, isolation and strain variation of Fusobacterium necrophorum from ovine footrot in Kashmir, India
PG-30	Dr. Amit Ranjan Sahu	Ph. D. Scholar, ICAR-IVRI, Bareilly	Analysis of the goat PBMCs transcriptome in response to infection with virulent Peste-des-petits Ruminants (PPR) Izatnagar/94 isolate virus
PG-31	Dr Anuj Chauhan	Scientist, IVRI, Izatnagar	Semi quantitative estimation of differential expression of interferon-gamma gene in cattle
PG-32	Dr Jonathan Lalsiamthara/ Dr Pallab Chaudhuri	IVRI, Izatnagar	Brucella abortus S19Δper mutant is moderately attenuated and mounted protective immunity to mice
PG-33	Dr Minakshi Prasad	Professor, Department of Animal Biotechnology LUVAS, Hisar	Detection of novel avian rotaviruses in poultry birds and zoonotic importance
PG-34	Dr. Manoranjan Rout	Scientist, Project Directorate on Foot and Mouth Disease, Mukteswar	Demonstration of foot-and-mouth disease virus infection specific nonstructural protein-antibodies in a vaccinated herd comprising cattle, buffalo and goats in India
PG-35	Dr Poonam Kumari/ Dr Yashpal S Malik	Principal Scientist, ICAR-IVRI, Izatnagar	Epidemiology of group A rotavirus in bovine population of northern states of India discloses its high prevalence
PG-36	Dr Ritesh Kumar	Research Fellow, Dept. of Bio & Nanotechnology, GJUS&T, Hisar	Synthesis and Evaluation of Gold nanoparticles for applications in Animal disease diagnosis
PG-37	Dr Suman Bishnoi	MVSc Scholar, Department of Veterinary Medicine, LUVAS, Hisar	Canine parvovirus in Dogs: molecular detection and haematological alterations

Sr No.	Name	Address	Title
PG-38	Dr Nimita VC/ Susweta/BR Shome	National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru	Methicillin-Resistant Staphylococci in Livestock–A Duplex PCR Assay for its Rapid Detection
PG-39	Dr N. N Mohanty/ S.B. Shivachandra	Senior Scientist, ICAR-National Institute of Veterinary Epidemio-ogy and Disease Informatics, Bengaluru	Gene cloning, expression and purification of recombinant OmpLA protein of Pasteurella multocida B:2
PG-40	Dr Prasanta Kumar Mishra	PhD Scholar, Division of Biochemistry, IVRI, Izzatnagar	Cloning and Sequencing of Thioredoxin Reductase (trxB) Gene of Salmonella enterica serovar Typhimurium Isolated from Poultry
PG-41	Dr Shikha Saxena	Senior Research Fellow, Division of Veterinary Biotechnology, IVRI, Izatnagar	Intracellular Delivery of Histidine and Arginine Rich Cell Penetrating Peptides into different cell lines

POSTER SESSION 2

Proteomic and Immunological Tools in Diagnosis and Characterisation (PP)

18 DECEMBER 2015 10:00 AM-02:00 PM

Sr No.	Name	Address	Title
PP-1	Dr. Prasenjit Dhar	Assistant Professor, Deptt. of Veterinary Microbiology, DGCN COVAS, CSKHPKV, Palampur	Use of Dot-ELISA for detection of FAV-4 propagated in different cell cultures
PP-2	Dr Swati Dahiya	Assistant Scientist, Deptt. of Veterinary Microbiology, LUVAS, Hisar	FOOT-AND-MOUTH DISEASE INCIDENCES IN HARYANA DURING 2013-2015
PP-3	Dr. Somesh Banerjee	Senior Research Fellow, CIRB, Hisar	Single domain antibody selected from phage display library of Indian desert camel (Camelus dromedaries L.) neutralizes LPS of E. coli O153 in chicken embryo model
PP-4	Dr Anuj/Sushila Maan	Department of Animal Biotechnology, College of Veterinary Sciences, LUVAS Hisar	A comprehensive study on seroprevalence of bluetongue virus in Haryana state of India
PP-5	Dr Yogesh Kumar/ Rashmi Singh	MVSc Scholar, DUVASU, Mathura	Comparison of serological response to FMD virus antigens in FMD-hemorrhagic septicemia- black quarter combined vaccine and FMD vaccine
PP-6	Dr Aditya Baruah	M.V.Sc Scholar, College of Veterinary Science, AAU, Guwahati	Sero-prevalance of JEV anti-bodies in different domestic animals in the state of Assam

Sr No.	Name	Address	Title
PP-7	Dr Ajay Pratap Singh/ Rashmi Singh	Assistant Professor, College of Veterinary Sciences & Animal Husbandry, DUVASU, Mathura	Seromonitoring in bovines vaccinated against foot and mouth disease in six model villages of Mathura District, U.P.
PP-8	Dr Pankaj Kumar	Assistant Prof., Deptt. of Veterinary Microbiology and Biotechnology, CVAS, RAJUVAS, Bikaner	Production and Characterization of monoclonal antibody against Pasteurella multocida
PP-9	Dr Basanti Brar/ Dr B R Gulati	ICAR- NRC on Equines, Hisar	Surveillance of Japanese Encephalitis Infection in Animal Population of North-East India
PP-10	Dr Shailja Katoch	Ph.D. Scholar, Department of Veterinary Microbiology, DGCN COVAS, CSK HPKV, Palampur	Comparison of Humoral response in calves following intranasal challenge with P. multocida B:2 and subcutaneous vaccination with formalin killed alum adjuvant vaccine
PP-11	Dr Namrata Singh/ Dr Neelima Patel	College of Veterinary Science and A.H. Hisar (Haryana)	Seroprevalence study of cattle brucellosis using Rose Bengal plate test and milk ring test
PP-12	Dr K Triveni/ Rajeswari Shome	National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru	Fluorescence Polarization Assay: A DIVA test for the diagnosis of brucellosis
PP-13	Dr Ameya Gupte	MVSc Scholar, Department of Veterinary Microbiology, LUVAS, Hisar	Development of peptide ELISA for serodiagnosis of Equine Herpesvirus 1
PP-14	Dr. Uiase Bin Farooq	Assistant Professor, School of Veterinary Medicine, Hawassa, Ethiopia	Faliure of Passive Transfer (FPT) of Immunity in Foals
PP-15	Dr Susweta Das Mitra	Research Associate, National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru	Streptococcus uberis Induce Inflammatory Responses in a Mouse Intramammary Infection Model in a strain directed manner
PP-16	Dr Lalrengpuii Sailo	PhD Scholar, Indian Veterinary Research Institute, Izatnagar	Disease resistance in livestock: challenges and opportunities
PP-17	Dr Rajni Chaudhary	PhD scholar, Indian Veterinary Research Institute, Bareilly, India	Neonatal Fc receptor of IgG (FcRn) and their role in Failure of Passive Transfer (FPT) of Immunity
PP-18	Dr Ramesh Dedar	Scientist, ICAR-NRC on Equines, Bikaner, Rajasthan	Serum Status of Trace Elements in Indigenous Donkeys from two donkey fairs
PP-19	Dr Sanjay K Ravi	Scientist, ICAR-NRC on Equines, Bikaner, Rajasthan	Freezability of Kathiawari horse semen under field conditions
PP-20	Dr. Arpita Padhy	Assistant Prof., Arawali Veterianry College, Sikar, Raj.	Detection of biofilm formation of canine persistent wound pathogens by Congo Red Agar Method.

Sr No.	Name	Address	Title
PP-21	Dr Harish DR/ Dr. Aditya Prasad Sahoo	Scientist, Indian Veterinary Research Institute, Izatnagar, Bareilly	Identification of mammary tumor homing peptides by in vivo biopanning using phage display peptide library
PP-22	Dr Krishan Dutta Rawat	Research Associate, VTCC, NRC on Equines, Hisar	Study of chemokines of CXC family expressed during granuloma formation in mycobacterial infection in guinea pig
PP-23	Dr Heena Sharma	PhD Scholar, Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar	Evaluation of raw chicken sausages incorporated with blend of clove and holy basil essential oils during refrigeration storage
PP-24	Dr Archana Sarangi LUVAS	MVSc Scholar, Department of Veterinary Physiology, LUVAS, Hisar	Intervention strategies against Salmonellas
PP-25	Dr Neelam Yadav	MVSc Student, LUVAS, Hisar	Haematological alteration and antibiogram profile of E. coli isolates in dogs with gastroenteritis
PP-26	Dr Babita/ Archana Sarangi	MVSc Scholar, Deptt. of Veterinary Physiology, LUVAS, Hisar	Principles of viral disease management
PP-27	Dr Geetika Verma/ Dr R S Sethi*	Professor, School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana	Genotoxicity evaluation of acute and chronic doses of ethion
PP-28	Dr Dhirendra Meena	Department of Veterinary Microbiology and Biotechnology Rajasthan University of Veterinary and Animal Science, Bikaner	Potential risk of brucellosis on animal and human beings
PP-29	Dr Dhirendra Meena	Department of Veterinary Microbiology and Biotechnology Rajasthan University of Veterinary and Animal Science, Bikaner	Studies on brucellosis in animals in Bikaner district and its public health significance
PP-30	Dr Rahul Yadav	PhD Scholar, Rajasthan University of Veterinary and Animal Science, Bikaner	Detection of antibiotic resistance patterns of Escherichia coli isolates among healthy and diseased camel
PP-31	Dr P Nathawat/ Dr Rahul Yadav	PhD Scholar, Rajasthan University of Veterinary and Animal Science, Bikaner	Antibiogram studies of Klebsiella oxytoca from nasal discharge of respiratory tract infection in Dromedary camel
PP-32	Dr Neha Gaur	Rajasthan University of Veterinary & Animal Sciences, Bikaner	Recent advances in equine reproduction management
PP-33	Dr Y Vashi/ Dr Soumen Naskar	Scientist, ICAR-National Research Centre on Pig, Guwahati	Influence of seasonal variation on production in pigs: Physiogenomic perspectives

Sr No.	Name	Address	Title
PP-34	Dr S. K. Maurya	Department of Veterinary Physiology & Biochemistry, College of Veterinary Science & AH, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad	Relationship of seminal plasma protein with semen characteristics of Murrah buffalo bulls
PP-35	Dr P Bala	Scientist, ICAR-NRC on Equines, Bikaner, Rajasthan	Identification of acetogens, methanotrophs and methanogens in the equine hindgut
PP-36	Dr Suman	MVSc Student, Department of Veterinary Medicine, LUVAS, Hisar	Antibiogram pattern against major pathogens responsible for diarrhoea in murrah buffaloes
PP-37	Dr. Raj Narayan Trivedi	PhD Scholar, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar	Methionine sulphoxide reductase (MsrA) prevents neutrophill mediated killing of salmonella typhimurium
PP-38	Dr Surbhi/ Dr Nirmal Sangwan	MVSc Scholar, Deptt. of Veterinary Physiology nad Biochemistry, LUVAS, Hisar	Isolation and identification of platelet aggregating inhibitors from salivary glands of Hyalomma anatolicum anatolicum ticks
PP-39	Dr Praveen Kumar/ Dr Rajendra Yadav	Assistant Professor, College of Veterinary Sciences, LUVAS, Hisar	Antibiogram of bacterial isolates from clinical cases of BRD complex in buffaloes
PP-40	Dr Neelesh Sindhu	Assistant Prof., Deptt. of Veterinary Medicine,LUVAS,Hisar	An outbreak of organophosphate (Ethion 50%) in buffaloes
PP-41	Dr. Rajesh Singathia	Assistant Prof., Veterinary Microbiology, Veterinary University Training and Research Centre, Churu	Cell mediated immune response against Newcastle disease virus in chickens
PP-42	Dr Shafiya Imtiza Rafiqi	PhD Scholar, IVRI, Izatnagar	Nanovaccinology: Dawn of Biomemetic Vaccine Carriers in Parasitology
PG-43	Dr Anita Dalal	PhD Scholar, LLR University of Veterinary and Animal Sciences, Hisar	B2L and IL-2 gene sequence based characterization of Orf viruses from North-India

Indian Society for Veterinary Immunology and Biotechnology
XXII Annual Convention and National Symposium on
"Immunomics and Proteogenomics in Livestock Health & Productivity"
DECEMBER 17-19, 2015

Roadmap for strategical diagnosis and control of avian viral diseases in India

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Background

Poultry production in India has emerged as a rapid growing sector among agricultural and livestock sector. Indian poultry industry has made a significant impact in economic, nutritional and socio-cultural aspects including livelihoods of poor rural households. Ever since its inception and is presently emerging as sun rise sector with a growth rate of 10-15% and overall turnover of Rs 70,000/- crore. Despite of frequent ingress with various infectious diseases including avian influenza, which is a severe setback for the industry, India has sustained globally as 3rd and 5thposition for egg and meat production, respectively. Emerging and re-emerging poultry diseases in the country are posing significant challenges to the poultry industry. In the recent past, this sector has faced frequent onslaught of newer poultry diseases like bird flu (Avian Influenza) leading to enormous losses to the poultry sector not only in India but globally. In addition to this, other existing diseases viz. infectious bronchitis, infectious bursal disease, Ranikhet disease, Marek's disease and fowl pox have emerged in the recent past in more virulent form. Therefore, scientific interventions are urgently needed to curb the menace of such emerging poultry diseases in the Country as well as effective control measures against already existing major poultry diseases.

Existing situation in India

The scenario of diseases has been changing frequently with a vast impact on poultry industry. Respiratory disease complex has emerged as a greatest challenge with multiple etiological pathogens. The existence of Infectious laryngotracheitis (ILT) in India was dated back to 1964, phylogenetic analysis in India revealed a close relatedness to vaccine strains. Infectious bronchitis also affected several Indian flocks with its ubiquitous existence. Mass, 793B (4/91) and THA280252 are the important pathogenic strains that are commonly reported in the country. GHV Marek's disease, a lymphoproliferative disease of chickens is a great concern to poultry industry. The annual loss in India accounts to Rs. 4 crores and the incidence is reportedly higher in dust prone and mining areas. The virus is shifting towards a more pathogenic trend with a striking emergence from mild to very virulent (vv) and very virulent plus (vv+). This developed a new clinical picture characterized by more than 90% morbidity and mortality and absence of classical signs, the lymphomas and peripheral neuritis while showing acute rashes. Recent epidemiological reports from India indicated high prevelance rate of 86% (serological) and 73.3% (molecular) for chicken infectious

anaemia (CIA), vertically transmitted immunosuppressive disease of chicken. Phylogenetic analysis indicated a possibility of contaminated vaccines or infected breeders. Infectious bursal disease is found in Indian poultry flocks and continues to be as economic hardship for farmers. Contagious nature of virus coupled with geographically limited antigenic drift is always challenging since it is not possible to produce vaccine for every new strain encountered. In addition, at time broiler farmers are skipping the vaccination protocol to raise their economic statue. Non-viral pathogenic agents Mycoplasma and *E.coli* are posing significant threats to the industry in form of secondary infections with frequent occurrence.

On other side of coin, poultry production is lagging behind with continuous hits with avian influenza in the form of LPAI and HPAI. Ever since the inception of first outbreak, India accounts for a loss of Rs. 2432.08 lakhs from culling of 72.46 lakh live birds. With the LPAI as silent infections, HPAI is evolving as endemic disease affecting exports and economic situation of the country.

Diagnosis and control

Employment of successful disease control strategies is mainly possible with the accurate and in-time diagnosis which is possible only through accurate, easy, simpler, on farm and cost effective tools. Lateral flow assay (LFA) offers promising value as pen side test for rapid diagnosis of disease. LFA's and biosensors based diagnosis are already in place for avian influenza. Molecularly imprinted nanoparticles- the artificial antibodies for detecting circulating viruses in field, RNA binding stretches (aptameres) for detection of MDV-1, 2 & HVT-3 in poultry house dust in a robust and sensitive manner, loop mediated isothermal amplification (LAMP) at a single temperature, proteomics and oligonucleotide micro-arrays for differentiating multiple strains of salmonella, Microsphere -based suspension array technology and Nucleic acid based amplification for detection of avian influenza species. Rapid multiplex PCR provides pin point solution for rapid identification and differentiation of respiratory disease complex.

Following are the suggested line of action for the effective control of emerging poultry diseases in the country

- Characterization of pathogenicity of new virus strains in time.

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Role of Immunomics in Livestock and Human Health Emerging and re-emerging Zoonotic infections

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It has been observed that, over the last 30 years, new infectious agents and diseases affecting humans are emerging rapidly. About seventy per cent emerging and reemerging infections are zoonotic and some recent examples are severe acute respiratory syndrome (SARS), avian influenza, Crimean Congo Hemorrhagic fever (CCHF) and Nipah virus (NiV). Beside from causing human mortality, these infections have had devastating effects on the populations, economies and livelihood systems.

The key to detect and control the emerging zoonoses infections needs coordinated action on the part of animal and human health sectors. In particular, it is crucial to detect and control early any emerging and re-emerging zoonoses at the animal source to prevent it from infecting human population. Thus it is essential to establish good collaborations between animal and human health sector to ensure synergistic actions, make rational use of available resources, improve efficiency and avoid duplication of work. India reported outbreaks of highly Pathogenic Avian Influenza (HPAI) H5N1 viruses and more than 90 explosive outbreaks of H5N1 viruses have been reported from 15 states in poultry from 2006 to 2015. One of the best ways to intercede a possible pandemic is through systematic surveillance. Growing concerns about AI and its impact on agriculture and human health have highlighted the need to understand the role of wildlife in maintaining and spreading the virus. AI surveillance in wild birds may be useful for risk assessments in poultry, humans, pigs, and other animals. Simultaneously, country need to be prepared to deal with emerging diseases like Ebola and Marburg which has long persistence of virus in human body fluid and severe outcome. In India Nipah and CCHF also pose big threat due to lack of vaccine/treatment and a organized public health infrastructure.

World has witnessed the rise of avian influenza, SARS, MERS Corona Virus, Ebola and CCHF, Nipah and Marburg etc as a result of mutations, habitats destruction and increased connectivity among populations. About 37 per cent of emerging and re-emerging pathogens are viruses and prions followed by protozoa (25%). Emerging diseases causing 26 per cent of annual deaths worldwide has also witnessed many outbreaks in India,

Immunomics and its potential applications in developing novel diagnostics, vaccines and therapeutics

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"Immunomics" is the linking immunology with genomic techniques and has lead to great revolution in understanding various concepts and dynamics of immunology. It is the study of all types of antigens of an organism, presented either to T or B cell, and the ways to identify these with perspectives for designing and developing new generation novel diagnostics, vaccines and therapeutic targets. It helps in avoiding time and labor consuming conventional methods of identifying immune stimulating epitopes of an antigen. Origin of immunomics includes cDNA microarrays to investigate immune response. Various techniques of studying Immunomics include Immuomic microarrays, Lymphochip, T- and B- cell epitope mapping tools, flow cytometry, ELISPOT, deeper sequence mining and other advanced methods. Immunomics pave ways towards understanding immune cell activation and differentiation, generation and dynamics of immune responses to several pathogens, and immune regulatory networks. Immunomics approach can be exploited to identify biological markers for various disease conditions viz., autoimmune diseases, HIV, diabetes and others. Linking immunology with computational methods has revolutionized the field of immunology research. Modern advances in the areas of immunology, genomics, biotechnology, molecular biology, genetic engineering and proteogenomics have presented newer and effective diagnostics, vaccines and therapeutics for safeguarding the health of humans and their companion animals. Most of the research works regarding to Immunomics has been conducted on animal models pertaining to human diseases such as autoimmune diseases, infectious diseases and cancers, and its potential applications against animal diseases are still awaited to be utilized. The present paper is an overview describing immunomics and its potential applications in designing novel diagnostics, vaccines and treatment regimens to counter various disease conditions with a perspective vision for its exploitation in the field of animal and veterinary sciences for protecting health of livestock and poultry health.

Immunomics in developing novel diagnostics

Accurate and early diagnosis of any disease holds significance towards appropriate follow up of any treatment and preventive measures. For this diagnostic tests should be strengthened to detect many disease conditions simultaneously. Immunomics not only helps in diagnosis of any disease once it has occurred but also predicting it long before its occurrence, thus foretelling of future diseases seems to be possible. Staging of any

disease condition and its prognosis in an individual patient can be known by developing a suitable database. Immunomics approach can be utilized in predicting autoimmune disease by analyzing individual's antibody repertoire via microarray anlaysis as in mouse for type 1 diabetes. Identification of autoantigens or potential pathogenetic antigen by tissue microarrays (TMA) and proteomics can provide better ways to predict pathophysiology of autoimmune conditions such as Rheumatoid arthritis. Autoantibody analysis could diagnose occurrence of neurodegenerative diseases (Alzheimer's and Parkinson's disease). Microarray analysis of autoantibodies can predict and diagnose systemic lupus in mice. Occurrence of various malignant conditions can also be determined as are associated with presence of autoantibodies viz. p53, L-myc, glycosylated annexins I and II or HER2-neu, termed as "cancer immunomics". Recently, circulating antibody to FOXP3 has been evaluated to diagnose and monitor the course of cervical cancer. Tumor associated antigens (TAA) can also be investigated by mini-arrays to diagnose ovarian cancer.

Immunomics can also help in diagnosis of infectious diseases by identifying antibody repertoire generated due to invasion of a particular pathogen and characterizing most important antibody type generated which can be employed in sero-diagnostic tests. High density peptide microarray for identifying antibody specificities has been developed for Chagas disease caused by Trypanosoma cruzi, thus facilitating disease diagnosis and vaccine designing for combating this disease. B-cell linear epitopes of T. cruzi responsible for clinical disease have been predicted using Bepipred progam which can be used for sero-diagnosis and serotyping using ELISA. Identification of highly immunoreactive antigens of malaria parasite Plasmodium vivax by using sera has paved way for development of better diagnostics based on antibody surveillance. Immunoproteomics approach has also been extended to evaluate the presence of certain antigenic markers of particular disease such as schistosomiasis. Thus by combining peptide microarray with bioinformatic peptides, various diagnostic epitopes can be analysed which could help in designing effective diagnostic test with higher specificity and sensitivity.

Immunomics and vaccine designing

Modern approach for vaccine designing includes developing vaccine antigen from most significant epitope on sequence based advances which generates protective immune response rather than microbiological approach. Newer directions for vaccine development include Reverse vaccinology and genome based approaches. Immunomic approach is based not only upon in silico prediction algorithm but also on the use of biological samples from humans and animals with specific immunity to the particular pathogen. Computational approaches have also been utilized to design such epitope for developing vaccines for many pathogens with long lasting immunity. Exploiting immunoinformatics, peptide based antigens consisting of epitopes for both T and B lymphocytes can be easily identified and used in designing novel and effective vaccines with help of conformational epitope server (CEP) and 3D-Epitope-Explorer (3DEX) softwares. Protein microarrays can be used to predict immune reactive protective epitopes for antibody, while epitope prediction algorithm and high throughput cellular assays (multi-parameter flow cytometric assay) for including T cell epitopes in vaccines. Future trend in designing potent vaccine antigen / epitope could be a fruitful combination of multiple "omic", reverse vaccinology, various computational and immunological techniques.

Cell mediated immunity protects visceral leishmaniasis or kala azar, utilizing algorithms through bioinformatics approach to predict epitopes to be used in vaccines producing protective immune response could pave way for designing an effective vaccine to counter this disease. It involves combination of genomic database, evolutionary relationships, 3D structure of proteins, protein domains etc. to evaluate interaction between epitope and immune cell receptors. Immunoinformatics can also be employed to design vaccines for Middle East Respiratory Syndrome corona virus (MERS-CoV), Chagas disease, Dengu fever and others. Similarly, for Schistosomiasis, immunomic approach involving identification of a surface protein epitope through microarray has been employed to provide protective immunity. Sequencing of Plasmodium flaciparum genome has helped in determining protective epitope via protein microarray for antibodies and antigen, epitope based prediction algorithms, which could pave way for malaria vaccine development. Using protective epitopes information for Hepatitis C virus by in silico analyses of its certain nonstructural proteins can certainly provide an effective vaccine for this important disease.

Immunomics and developing novel therapeutics

Immunomics has lead to the development of protein based drugs such as recombinant

Molecular mechanism of early innate immune response during Japanese encephalitis virus infection

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Japanese encephalitis virus (JEV, an arthropod-borne flavivirus) infection remains one of the major causes of encephalitis with significant mortality among children throughout the temperate and tropical zones of Asia and part of Australia, including India. Approximately 3 billion people and 60% of the world's population live in endemic region and about 50,000 cases with 10,000 deaths were notified annually from a wide geographic range. Following an infective mosquito bite, the initial viral replication may occur in local regional lymph nodes. Viral invasion of the central nervous system (CNS) probably occurs via the blood. Leucopenia is a frequent feature of most of the viral infections, while JEV infection is characterized by polymorphonuclear leucocytosis with variable effects on different components of the peripheral blood leucocytes. An early influx of macrophages followed by neutrophils at the site of injury in different human organs and in experimental animals has been reported; yet the mechanism of recruitment of these cells is uncertain. Our observations have revealed that JEV induces splenic macrophages secrete a highly potent chemotactic factor (peptide) of low molecular weight (~10 kDa), which causes chemotaxis of neutrophils and is named as macrophage derived factor (MDF). Previously it was thought that MDF is a pathogenesis related protein. However, we showed that MDF plays a key role in early anti-JEV host defense. MDF attracts neutrophils and has a variety of

Equine infectious diseases in India: Current status and future research

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ICAR-National Research Centre on Equines (ICAR-NRCE) plays an important role as a technical leader in the development of equine sector by initiating efficient R&D for equine health and production. It is the only institute responsible for researches on equine production, health, management and husbandry in the country. The activities include addressing researchable issues in disease surveillance and monitoring, diagnosis, prevention, production including reproduction, nutrition, feeds and fodder, developing value-added products and all aspects of management and husbandry as well as policy and planning, enhancing awareness among stakeholders about equine husbandry practices. The institute got recognition in national and international arena through its technological interventions in the field of diagnostics, vaccines and concerted efforts in the direction of conservation of indigenous equines.

The NRCE has initiated work in the frontier areas of technologies such as reverse genetics approach for equine influenza vaccine, bacterial artificial chromosomes for the viral diseases, molecular approaches to crack the riddle of latency due to herpes viruses, preparedness for the exotic diseases through development of diagnostics using synthetic peptide technology, development of recombinant antigens and monoclonal antibody based diagnostics for Trypanosoma evansi and Theileria equi parasites, expression of recombinant equine cytokines and use of nano-technological approaches for therapeutics and diagnostics. Sequencing of ORF68 and ORF30 genes was done for geno-grouping of various EHV1 isolates and identification of neuropathogenic strains, respectively. Further PCR/qPCR assays were developed for detection of EHV1 latency in equines. The Centre was able to develop a mouse model for equine influenza, which has shown great potential for future research on host pathogen interaction and screening of vaccine candidates.

All six recognized breeds (Marwari, Kathiawari, Spiti, Zanskari, Manipuri and Bhutia) have been phenotypically and genotypically characterized using microsatellite techniques. The institute is actively engaged in in-situ and ex-situ conservation and propagation of important breeds of equines by perfecting AI and semen cryopreservation technology that has resulted in the increase of horse population by 2.3% and mule population by 43% over last five years (Livestock Census 2012). Our studies on utilization of donkeys' and mules' energy in agricultural operations for sustainable livelihood have become a baseline data for policy makers.The Centre has also ventured into the area of equine nutrition through

studies on requirement of ration for various stocks and how that can be met through the locally available feed and fodder. For complete characterization of donkey breeds, NRCE has taken a major step towards phenotypic and genotypic characterization of the locally available gene pool in various geographical conditions of the country.

Equine Infiuenza

Equine influenza (EI) is a highly contagious respiratory viral disease of equines caused by RNA virus - Influenza virus A- belonging to family Orthomyxoviridae. The EI disease is caused by two subtypes viz. H7N7 and H3N8. The outbreaks due to H7N7 are limited as the isolation of the virus has not been reported for more than three decades. The virus of subtype H3N8 spreads very rapidly in the susceptible population and is known to cause severe clinical disease in equines, characterized by pyrexia, dyspnoea, dry hacking cough and serous nasal discharge that becomes mucopurulent due to secondary bacterial infections. India faced an epizootic of equine influenza in 2008-09 after a gap of two decades. The outbreak started in last week of June 2008 (confirmed on July 7, 2008), from Jammu and Kashmir (Katra), northern state of India and affected 14 states. Phylogenetic analysis of HA gene established that 2008 EI outbreak in India was due to H3N8 subtype belonging to Clade 2 of Florida sublineage. All the genes of Indian isolates were cloned and sequenced. Matrix gene revealed a separate clustering of EIV of Asian origin and was distinguished as Asian Clade.

EIV is routinely diagnosed by RT-PCR, virus isolation and haemagglutination inhibition (HI) assay. RT-PCR for equine influenza typing has also been developed. Furthermore, real-time RT-PCR based assay targeting M gene has also been developed for diagnosis of EIV. Additionally monoclonal antibody based sandwich ELISA has been developed and is currently under the process of validation. NRCE has updated its previous inactivated EI vaccine through strain substitution. The updated vaccine is safe and efficacious as evident by the protective immune response generated by the vaccine in field trials in equines. The centre has developed a novel BALB/c mouse model for studying pathology and pathogenesis of equine influenza virus. The model will help in understanding disease mechanisms, host-pathogen interaction while simultaneously working for screening of vaccine candidates for their protective efficacy and immune response. NRCE is currently having an OIE twinning project on equine influenza with Animal Health Trust, UK with an aim of capacity building so that the Centre can act as an OIE referral centre for the region. NRCE has also initiated an inter-institutional project on development of recombinant EIV using reverse genetics technology.

Equine herpes virus infection

Equine rhinopneumonitis is a collective term for EHV-1 and EHV-4 infection. Both EHV-1 and EHV-4 are endemic in India. While EHV-4 causes respiratory disease only, EHV-1 can cause a range of clinical signs including, respiratory disease in young horses, late-term abortion in pregnant mares, neonatal foal mortality and equid herpesvirus myeloencephalopathy (EHM) resulting in paresis/paralysis.

During past one decade, the global incidence of abortion and rhinonpneumonitis is on decline, while that of EHM is on the rise, primarily due to emergence of neuropathogenic

EHV1 strains and also due to widespread vaccination practices. EHV1 strains causing EHM show single nucleotide polymorphism (Ato G) at position 2254 in the EHV1 DNApolymerase gene (encoded by ORF30). EHV1 strains possessing guanine (G2254) at this site are considered to have neuropathogenic potential. In recent years, incidence of neurological illness with hind leg paralysis has been frequently reported by equine practitioners in India. The involvement of neuropathogenic strain of EHV1 in these cases is speculated. Based on sequencing of ORF30 genes and SNP real-time PCR results, we established that neuropathogenic strains are circulating in India. Sequence analysis revealed that 2 out of total of 24 isolates contained the neuropathogenic marker (D752/G2254).

The ability of equid herpesviruses to establish life-long latent infection in lymphoid and neural tissues with periodic reactivation and shedding is central to the maintenance of these viruses in horse populations. Over 50% of horses become latently infected after infection with EHV1 and EHV4. In a study to determine the presence of latency in lymph nodes and trigeminal ganglion of horses at post-mortem, it was observed that 37-74% horses in India were latently infected with EHV1. During latency, expression of viral genes is highly restricted, with expression of few or no viral proteins. The establishment of latent infection is highly coordinated process regulated by inter-play of viral, host and environmental factors. Samples of trigeminal ganglion and bronchial lymph nodes were collected from PM of 19 horses and peripheral blood lymphocytes (n = 25) of mares with previous history of EHV1 associated abortion and recovery from acute infection were tested for latency. On testing of clinical samples for latency, 20 (31.7%) and 28 (44.4%) samples were found to be latently infected by RT-PCR and real-time RT-PCR, respectively.

The Centre has also initiated work on eukaryotic expression of glycoproteins of the virus. However, understanding the disease pathogenetic mechanism and host-Pathogen interaction is an ever increasing area of active research for devising the control measures. Most recently, the Centre has been successful in generating infectious clone of genome of a field isolate of EHV1 in the form of Bacterial Artificial Chromosome (BAC).

Equine infectious anemia (EIA)

Equine infectious anemia (EIA) - a retroviral disease caused by equine infectious anemia virus (EIAV) - is a chronic, debilitating disease of horses, mules, and donkeys. EIAV infection has been reported worldwide and is recognized as pathogen of significant economic importance to horse industry. This disease falls under regulatory control program in many countries including India.

Serological evidence of EIAV infection has been reported in many countries around the world and the disease is most prevalent in Europe and Americas. In Asia, disease is less prevalent and had not been reported for many years. In India, the first case of EIA was detected in Karnataka in 1987; thereafter intense sero-surveillance throughout the country diagnosed 228 positive cases in the following years. However, recent emergence of EIAV infection in Asian continent like, Japan, Mongolia, China, Thailand, Uzbekistan, Philippines, and Malaysia during 2008-12 and detection of EIAV infection in 2010 and 2012 in India after a long gap underlines the importance of continued surveillance to monitor the disease prevalence and identifying the genetic variation among field strains of EIAV.

Currently, internationally accepted test for diagnosis of EIA is the agar gel immune-diffusion test (AGID). The ELISA has been reported to be more sensitive than AGID in detecting acute cases of EIA and helpful in disease eradication programme. However, commercial EIA ELISA kit is expensive and is not affordable by poor equine owners in Asian countries. NRCE has also optimized a recombinant rp26 antigen based ELISA for detection of Equine infectious anaemia virus antibodies in equine population and till date we have tested more than 8000 serum samples with the newly developed ELISA. The relative sensitivity and specificity of the newly developed ELISA were 100% and 98.6%, respectively.

Equine glanders

Glanders is a fatal infectious and notifiable disease of equines caused by Gram-negative bacteria, Burkholderia mallei. The natural hosts for B. mallei are horses, donkeys and mules. The disease in equines is characterized by chronic suppurative lesions of skin and mucous membrane, pneumonia and septicemia. Human may become infected through direct contact with organism and prolonged contact with infected animals. An extremely high rate of mortality can occur in untreated humans.

Glanders has been very much prevalent throughout India in early 1900s and major incidence of glanders is believed to occur before the implementation of control programme through the 'Glanders and Farcy Act 1899' (Act No. XIII of 1899). Sudden re-emergence of glanders in India was detected in 2006 after a gap of eight years. From 2006 to 2015 (August), a total of 39641 equines were surveyed for glanders from nine glanders affected states including Andhra Pradesh (n=8406), Chhattisgarh (n=930), Haryana (n=3624), Himachal Pradesh (n=2030), Jammu & Kashmir (n=2305), Maharashtra (n=11519), Punjab (n=4206), Uttar Pradesh (n=3385) and Uttarakhan (n=3236). Out of these, 227 equines were found positive for glanders and majority of the cases were detected from Uttar Pradesh (n= 115), followed by Maharshtra (n=23), Uttrakhand (n=22), Jammu & Kashmir (n=17), Himachal Pradesh and Andhra Pradesh (n=16), Chattisgarh (n=14), Punjab (n=3) and Haryana (n=1).

The Complement Fixation test (CFT) is an OIE-prescribed sero-diagnostic method for glanders for international trade. However, considering its questionable sensitivity and specificity with mule and donkey serum, the need for the development of a supplementary confirmatory test with a high negative likelihood ratio for international trade is essentially required. Very recently, ICAR-NRCE, Hisar has produced three recombinant B. mallei proteins, namely A, H, and TssB, and evaluated their diagnostic potential in the indirect ELISA format. Repeatability and reproducibility of the assays have been performed in six different laboratories and had comparable results. The diagnostic specificity and sensitivity of the assay were found to be 99.6% and 99.8%, respectively. Molecular epidemiological studies on B. mallei isolates was carried out by multilocus sequence typing (MLST) and VNTR typing. Whole genome sequencing (WGS) of one isolate is recently completed and sequence analysis is being done. NRCE is also running an OIE Twinning program on Glanders for capacity development and to get OIE referral laboratory status for this region.

Equine trypanosomsosis

Trypanosoma evansi is an extracellular protozoan parasite, multiplies in blood and body fluids, and causes a wasting disease in animals commonly known as Surra. It affects a wide range of domestic and wild animals viz. camels, horses, donkeys, mules, cattle, buffaloes,

dogs, pigs, sheep , goats, cats tigers, and elephants.The recombinant antigens (HSP-70, Flagellar genes) based ELISA were standardized for detection of antibodies against T. evansi infection in equines. Further, a monoclonal antibody based ELISAwas optimized for detection of antigens bound to immune-complexes in serum of experimentally infected donkeys with T. evansi. The assay could detect antigen successfully in serum samples 14th d.p.i. onwards.

For sensitive and specific diagnosis of T. evansi infection in animals, PCR assays were developed targeting multi copy genes viz., TBR1/2, ITS-1, ISG, ESAG genes. The LAMP-PCR assays were optimized using primers targeting RoTat 1.2 VSG and 18S genes. For quantitative estimation of infection in equines, a quantitative PCR (qPCR) assay was developed using primers targeting the internal transcribed spacer 1 (ITS-1) region of rRNA

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Sperm-mediated gene transfer (SMGT) - a potential tool for making transgenic livestock

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Transgenic animals have become valuable tools for both research and applied purposes. Over the recent years, a variety of transgenic approaches has been assessed and developed in the pursuit of a more efficient and easier method. Among them, sperm-mediated gene transfer (SMGT), which exploits the ability of sperm cells to bind, internalize and transport the exogenous DNA into an oocyte during the process of fertilization, is considered as a promising alternative (Lavitrano et al, 1989). The method of SMGT appears to be a simple, efficient and applicable to a variety of species that uses spermatozoa for its propagation. The DNA binding efficiency, however, varies widely ranging from 0.3 to 78% among the sperms of different species (Canovas et al., 2010, García-Vázquez et al., 2011 and Zhao et al., 2012). The success of SMGT is influenced by several factors including the donors of spermatozoa, incubation media, size and type of the exogenous DNA, and the kind of assisted reproductive technique used.

In order to improve the DNA uptake efficiency of sperm cells, several strategies have been employed which include DNA-liposome complexes (Lai et al., 2001), electroporation (Gagne et al., 1991), virus-mediated transfection (Takehashi et al., 2007), linker (receptor) based method (Wu and Wu, 1987), combination of restriction enzyme-mediated integration (REMI) with SMGT (Kroll and Amaya, 1996), as well as combination of intracytoplasmic sperm injection (ICSI) with sperm/DNA interaction (Perry et al., 1999). Amongst them, electroporation-aided SMGT is considered as a cheaper and more efficient method. Using electroporation-aided SMGT, transgenic offsprings are successfully produced in finfish and shellfish (Tsai 2000), and also in Rohu fish (Thayanithy et al. 2004). Recently we report, for the first time, a protocol for electroporation aided SMGT in goat (Pramod et al, 2015). Our study demonstrated that under the optimized condition, electroporation can result in maximum DNA uptake by the caprine sperm cells with minimum adverse effect on their vital parameters including fertilizing ability. We also produced transgenic fluorescent embryos using the transfected sperms.

Traditional SMGT experiments are potentially characterized by lack of reproducibility. In vivo gene transfer to introduce the transgene into testicular (sperm) stem cells namely testis-mediated gene transfer (TMGT) could be an alternative approach (Dhup and Majumdar, 2008) to solve this problem. This technique would, in principle, remove the need to collect, manipulate or transfer eggs, thus providing a major streamlining of germline

transgenesis. This in vivo technology introduces foreign DNA directly into testis by injection. In order to increase the efficiency, several strategies including virus-mediated as well as non-viral physical and chemical methods have been employed for TMGT (Dhup and Majumdar, 2008; Sehgal et al., 2011). Owing to a higher efficiency, virus-aided TMGT offers an attractive proposition, but it is constrained by biohazard risk and possible harmful effects such as uncontrolled infection or inflammation. Amongst the non-viral methods, both lipofection and electroporation aided TMGT are considered as an easier and safer method (Umemoto et al., 2005).

The available literature indicates that electroporation method of TMGT has immense potential to produce transgenic laboratory animals (Dhup and Majumdar, 2008). However, till now there is no report of TGMT in large animal species. We developed a successful method of transgenesis in goat by gene transfer into testicular cells using electroporation. We demonstrated the expression of the transgene in the spermatogonial stem cells (SSCs), sperms as well as in the embryos (Pramod, 2014). Finally, one of the natural matings of the bucks, with transfected testis, resulted in the birth of one female goat carrying the transgene.

In conclusion, SMGT appears to be simple, efficient, and relatively inexpensive methods in modifying animals and the genome of animals. However, its underlying molecular basis is generally neglected and the inconsistencies concerning the reproducibility associated with this method remain unsolved. In spite of it is having a well-accepted and established method, SMGT is still subject to development and new approaches are being developed to make the technique even more widespread and reliable. Given the wide availability of livestock semen, SMGT can be considered the method of choice for the production of genetically modified farm animals and represents the most powerful tool available today for medical research purposes. As the TMGT method has high success rate in the production of transgenic laboratory animals, we can try this technique in farm animals using optimized conditions.

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Molecular portraits of buffalo oocytes and blastocysts as a consequence of the transition from maternal to embryonic control of gene expression

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One of the major problems of infertility in livestock species is mainly due to the poor oocyte competence which could be due to the insufficient storage of maternal transcripts. Oocyte competence in turn affects the early embryogenesis which itself is dependent on a tightly controlled and well orchestrated program of gene expression. Oocyte attains the complement of cytoplasmic organelles and accumulates mRNAs and proteins to get enabled for the fertilization and to progress through the first cleavage until zygotic genome activation. The transcriptional activity decreases as the oocyte reaches maximal size, during the follicular growth. At later stages oocyte depends on stored RNAs for normal function during maturation, fertilization and early embryonic development. Therefore, it is logical to get the molecular portraits of oocytes and blastocysts specifically during the transition from maternal to embryonic control and characterization of specific transcripts, to help augmenting fertility.

The ability of an oocyte to develop into a viable embryo depends on the accumulation of specific information and molecules such as RNA, protein or imprinted genes during oogenesis. The poor developmental competence of in vitro matured oocytes has been proposed due to failure of the timely onset of embryonic genome activation resulting from incomplete cytoplasmic maturation of these oocytes. The development programme becomes dependent on new transcripts derived from activation of the embryonic genome. High developmental competence is dependent on normal follicular and oocyte growth, maturation and expression patterns. Studies on oocyte gene expression have revealed the specific molecular markers to characterize successful oocyte maturation. A good number of transcripts have been identified as potential predictors of oocyte competence both in cattle and buffaloes. Studies have also been conducted on oocyte competence marker genes in surrounding cumulus cells. As these transcripts are good indicators of the oocyte

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Buffalo beta casein promoter driven production of therapeutic protein in the milk

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In the era of high throughput sequencing technology driven genomics, information about genes and its products (proteins) in remediation of diseases are being piled up. The information, unless translated into products, is useless These proteins may be used for replenishment in patients (man or animal) lacking them. For this replenishment, these proteins need to be produced in large amount as biotherapeutics. In vitro production of therapeutic proteins by microbes or cultured cells is a common choice for production with several drawbacks like tedious recovery process, improper folding and modification of expressed proteins, etc. Transgenic animals stand a very good chance for production and isolation of therapeutic proteins. Mammary Gland being exocrine in nature produce large amount of milk at the time of lactation and hence can be used for targeted expression of desired proteins. Methods of transgenesis in large animals is very cumbersome, time consuming and costly affair. Promoters, along with other regulatory elements of genes coding for milk proteins, have been cloned from few species for directing the expression of desired proteins in the milk of farm animals. However, buffaloes, which are the second largest source of milk production in the world, have remained unexplored for such use. Since mammary epithelial cell-specific -casein is the most abundantly expressed protein found in buffalo milk, we have isolated the promoter region and the transcriptional regulatory element along with exon 1, Intron 1 and partial exon 2 of the ß-casein gene from the genome of the Indian river buffalo (Bubalus bubalis) and have characterized the same (GenBank accession no. KF612339). Mammary epithelial cells of buffalo and human (MCF7) expressed Enhanced green fluorescent protein (EGFP) upon transfection with the

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Fetal adnexa tissues: Ideal source of mesenchymal stem cells

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Among the many cell types used in regenerative medicine, the fetal adnexa tissues such as amnion, chorion, amniotic fluid and umbilical cord derived cells have attracted attention of human and animal science scientists and clinicians (Pappa and Anagnou 2009; Cremonesi et al. 2011; Yadav et al. 2012a). The adnexal tissue cells preserve some primitive characteristics of embryonic layers (Cremonesi et al. 2011) and represent ideal alternative source of stem cells for regenerative medicine due to being easily accessible in large quantity, non-invasive isolation procedure, multi lineage differentiation and presenting no ethical reservation like embryonic stem cells since they get routinely discarded as biomedical waste (Pappa and Anagnou 2009; Yadav et al. 2012a). These stem cells are easily accessible in larger quantities, and can be used for various applications includes assisted reproduction, regenerative medicine or production of induced pluripotent stem cells. In this article, we have discussed our experiences on stem cells derived from fetal adnexa tissues in buffalo.

Amniotic fluid (AF) is being a safe and reliable source of stem cells. The AF stem cells for tissue regeneration offers advantages over the use of embryonic or adult stem cells, viz., i) AF represents a convenient and non-contested source for obtaining stem cells, ii) isolating them is relatively simpler and rapid, iii) no feeder layers are required for their culturing, iv) they display no spontaneous differentiation in culture, v) their stem cell phenotype is not affected by long-term culture. We have isolated, cultured and characterized the amniotic fluid-derived cells from pregnant water buffalo uterus (Yadav et al., 2011). These undifferentiated AF cells expanded without feeder layer over a period of 100 days upto passages 20 and the positive for expression of alkaline phosphatase, and expression of Oct-4/Nanog/ Sox-2 were detected by RT-PCR. The cells exhibited uniform morphology and normal chromosome number. This study noted that bubaline AF cells could be cultured and maintained in vitro for a prolonged period and offers a potential source of multipotent cells for applications like therapeutic assisted reproduction in farm animals (Yadav et al., 2011).

The placenta is comprised of three layers namely amnion, chorion, and decidua. Amnion and chorion are derived from the epiblast and trophoblast cells of embryo proper respectively, whereas decidua is derived from the cells of uterine wall. We have successfully cultured the amniotic epithelial and mesenchymal stem cells from both gravid pregnant uterus (50-70 days) (Man et al., 2012) and term placenta (Gosh et al., 2015a,b). These cells have positive expression of pluripotency markers (OCT-4/SOX-2/NANOG), mesenchymal

stem cell markers (TERT, CD29, CD44, CD105) and negative for haematopoietic marker (CD34) genes at different passages. In addition, these cells were also positive for alkaline phosphatase staining. These cells have potential to differentiated into adipogenic, chondrogenic and osteogenic lineages of cells in vitro (Gosh et al., 2015a,b)

The umbilical cord blood (UCB) is normally discarded and can be easily collected at the time of delivery. Collection can be accomplished by venipuncture of the umbilical vein of the placenta still in utero, or after the expulsion of the placenta itself. Advantages of UCB stem cells are their high proliferative capacity, low risk of viral contamination response to alloantigen, their availability and donor safety. We have reported that bubaline cord matrix cells could be cultured for more than 100 days in continuous cells culture which expressed Oct-4, Nanog and Sox (Singh et al., 2013).

Fibroblasts cells are the most ubiquitous in complex organisms and play an important role in repair and healing of damaged organs or tissues. We have reported that the pluripotency genes expressed by ESCs are also expressed by fetal fibroblasts which survived up to passage 47. These cells have adipogenic and osteogenic differentiation ability in vitro (Yadav et al., 2012b). This indicated that the fetal somatic explants contains a subpopulation of stem cells, which can be induced to display features of lineage uncommitted stem cells, hence these might serve as a new source of easily accessible stem cells.

Conclusion

The use of mesenchymal stem cells derived from fetal adnexa tissues in veterinary medicine is of great promise and is likely to show rapid uptake as with adipose and bone marrow-derived stem cells. These types of stem cells have a great potential for augmenting assisted reproduction and treatment of several degenerative diseases in farm animals, those don't have effective therapy. For instance, bone fracture and damage of cartilages, tendons and ligaments. We have described here the features and potential therapeutic properties of buffalo mesenchymal stem cells of fetal adnexa tissues, and are expected to be studied in more depth and then implemented at the clinical level following international guidelines.

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ELOVL6 expression may regulate expression of ELOVL 2, 4 and 5 in porcine mesenchymal stem cells

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Mesenchymal Stem Cells (MSCs) are tri-potential cells that can give rise to bone, fat, and cartilage cells. MSCs are popularly used in in vitroadipogenic differentiation studies. During lipogenesis, elongation of long chain (or very long chain) fatty acids is catalyzed in a cascade of reactions by elongase enzymes in microsomes. Elongase 6 encoded by ELOVL6 gene initiates the elongation process. Fatty acids produced by elongase 6 are used as substrates for subsequent enzymatic catalysis by other elongases. Given this, we test the hypothesis that expression level of ELOVL6 gene would also regulate expression of other elongasesnamely, ELOVL2, ELOVL4 and ELOVL5 in MSCs. Porcine bone marrow MSCswere isolated, cultured, and characterized. MScs were transfected with short interfering RNA (siRNA) against ELOVL6 gene. Expression levels of ELOVL2, ELOVL4, ELOVL5 and ELOVL6 genes were quantified by real-time qPCR in wild type and siRNA transfected MSCs. Transfection of siRNA against ELOVL6 (at 10 nM concentration) caused down-regulation by ~36% of the gene ($p<0.05$). Down-regulation of ELOVL6 gene resulted in significant lower expression of ELOVL2 ($p<0.05$), ELOVL4 ($p<0.001$) and ELOVL5 ($p<0.05$)

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Somatic cells cryobanking: Future hope for multiplication and conservation of quality buffalo (Bubalus Bubalis)

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Assisted reproductive technologies like artificial insemination, in vitro fertilization and embryo transfer are vital components of breeding programmes for multiplication and conservation of high value livestock. Apart from gametes and embryos, somatic cells are also play a key role in reproduction as they can be used for cloned animal production, materials for genomic/proteomic studies and iPSc cell generation. Somatic cells are easily and harmlessly collected during biopsies and necropsies from high value animals. Therefore, through their collection and cryopreservation, we can preserve the possible genetic value of both male and female population for future applications. Considering this in mind, we thought of isolation, culture and cryopreservation of somatic cells from quality male and female buffaloes. In the present study, we have selected 10 elite Murrah/NiliRavi buffaloes (male and female) for isolation of somatic cells, these buffaloes are of farm both the institute farm and champions in different fairs. Tissue piece from ventral side of tail from each animal was mechanically chopped into small pieces and cultured in DMEM medium. Cells were come out after 5-7 days of explant culture and exhibited spindle shape morphology and were positive for vimentin expression indicated their fibroblast origin. These cells were sub passage more than 15 times indicated that culture conditions were efficient for these cells to proliferate continuously in vitro. For cryopreservation by slow freezing, aliquots of cells (approximately 1x105 cells/ml) at early passages (passage 2-3) in DMEM/F12 containing 10% dimethyl sulphoxide (DMSO) and 20% FBS were subjected to slow freezing in cryovials at the rate of 1.0°C/ min to -80°C after which the cryovials were stored in liquid nitrogen. Whenever required, the cells were thawed, and washed once with the culture medium before being replated. Out of 10 cell lines cryopreserved, one male cell line was used for production of cloned embryos using handmade cloning. Rates of embryo development (cleavage and blastocysts) were recorded on day 7 of in vitro culture and percent development of each stage was determined. We found that cleavage (89.30± 2.1)

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Characterization of stem cell like cells from pre-implantation goat (Capra hircus) embryos

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Establishment of stable immortal ES cell lines using embryo as a source of isolation in domesticated farm animals, in particular for Goat, which are closer to human than mouse has not been reported. This information could contribute to the improvement of agricultural biotechnology, genetic modifications, developmental biology and regenerative medicine in humans, biotechnology and agriculture. Therefore, the discovery of effective protocols to derive and maintain ES cells and the induction of somatic cells from ES cells in goat is of importance.

ES cells in their undifferentiated state are characterized by distinct morphology and expression of specific markers is an important criterion for pluripotent or undifferentiated state of cells. The expression of these markers is found to be exclusive to a particular species. In vitro derived embryos were subjected to enzymatic and mechanical method to derive the blastomeres.

Blastomeres were cultured in knockout DMEM medium on mitotically inactivated goat fetal fibroblast feeder layer at 37ºC until the primary outgrowth of ES-like cells were observed. The ES cell like cells were found positive for SOX-2, OCT4 and NANOG

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Classical swine fever (CSF): genomic and immunomic of CSF virus

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Classical swine fever (CSF) is a highly contagious viral disease of domestic and wild pigs caused by CSF virus (CSFV) of the genus Pestivirus and family Flaviviridae. CSFV is a small RNA virus that is closely related to bovine viral diarrhoea virus (BVDV) and border disease virus, which also cause infection in pigs. CSF is enzootic in pig herds in India, and outbreaks of the disease continue to occur. Among various infectious diseases affecting the growth of piggery, CSF has emerged as the major obstacle for the development of profitable pig farming in India. Several recent studies reported occurrence of CSF among pig herds in Assam and other parts of India. Accurate and timely diagnosis is important to prevent the spread of CSF and to minimize the economic losses. Genetic typing has proved as a useful means of tracking the spread of CSFV and is generally considered superior to antigenic methods. Three regions of CSFV genome have been widely used to determine their ability to discriminate between CSFV strains and for genetic grouping of the viruses. For sequence comparison, 150 nucleotides of the 5 / non-translated region (5 / NTR), 190 nucleotides of the E2 envelope glycoprotein gene and 409 nucleotides of the NS5B polymerase gene have been used. This widely followed genetic grouping scheme divides CSFV in to three genogroups (1-3) with three to four subgroups in each genogroup. CSFV subgenogroup 1:1 has been reported in majority of the field isolates in India, however there are recent reports of the other sub genogroups like 2:1, 2:2 from India. Detailed epitope characterization of the E2 and Erns of CSFV is found to be important for understanding of interactions between the

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Japanese encephalitis among Indian pigs and an update on genetic relationship between viruses circulating in the country

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Japanese encephalitis (JE) is an arthropod-borne zoonotic viral disease caused by Japanese encephalitis virus (JEV) that belongs to the Flavivirus genus in the Flaviviridae family. In India, JE was first reported in 1955 from Vellore, in Tamil Nadu. JE which was confined to south India spread to Burdwan and Bankura districts of West Bengal in 1973 and by 1978 it was recorded in 21 States of India. During 1978 to 2009, a dramatic increase in the number of JE cases was recorded in Gorakhpur district of Uttar Pradesh.

JEV infection in pregnant pigs can result in reproductive problem characterized by abortion, stillbirths and mummified fetuses. In India, JEV has been recovered from 19 different mosquito species. There is only one report of JEV isolation from a sentinel pig in India, but there is no information on the genotypes involved and its genetic relationship with viruses detected in humans.

In a recent study conducted at ICAR_IVRI, Izatnagar, investigation of reproductive failure among pigs in an organized farm led to the isolation of JEV from stillborn piglets with neuropathological lesions. The isolated virus showed neurovirulent properties in experimentally infected mice. It was closely related to JEV GIII associated with the 2005 outbreak in India and certain old isolates from Japan and Indonesia. Phylogenetic analysis of Indian JEV from 1956 to 2014 categorized the GIII viruses into different clades and their spatial distribution which was discernible in the previous century seems to be blurring.

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Bluetongue in India: A journey from virus to vaccine

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Bluetongue (BT) is an economically important viral disease around the globe. In small ruminants especially in sheep and goat causing morbidity and mortality whereas in large ruminants maintains carrier status consequently affecting Indian sheep and dairy industries. BT is a Culicoides borne infectious disease, the carrier status of the virus in domestic and wild ruminants and circulation in the environment plays an important role making the disease endemic in many parts of India. The results of studies carried out under AINP-BT, an ICAR funded project in last 15 years based on samples collection, virus isolation, antibody detection and molecular characterisation of the virus has revealed a true picture of the disease in the country. Presence of BT antibodies in goats, cattle and deer was regularly observed without manifestation of clinical disease. Till date BTV 1, BTV 2, BTV9, BTV10, BTV16, BTV21 and BTV23 have been isolated from sheep during BT outbreaks.

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Gaps in Orbivirus Research

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The 'Virology Discussion Group' recently had a wide ranging and useful discussion concerning recent research developments, new opportunities and current challenges for orbivirus research, the need for greater international collaboration, and the possibility of a collaborative global network of researchers / organisations to combat these important trans-boundary diseases.

Basic virology includes studies of the interactions among the orbiviruses, their vertebrate hosts and arthropod vector species. It also explores how these viruses replicate and make use of the interactions between their hosts and vectors at the molecular level, to spread and cause disease in new individuals and populations.

A better understanding of the basic virology of bluetongue and the other orbiviruses, will help to underpin all the topics of discussion including Vaccines, Pathology, Immunology, Entomology and Epidemiology. The virology data generated will also help us to model current and future disease outbreaks, and to understand the global risks we face, from BTV, EHDV, AHSV, EEV, and any of the 23 other orbivirus species, or additional novel

Vector Bionomics, Prevention and control of vector borne diseases in Chandigarh

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Malaria, Dengue, Chikungunya and J.E. are among other vector borne diseases continue to be of major public health importance in India. Undoubtedly the prevention & control of these diseases are closely linked to ecological, geographical and Environmental factors. The bionomics of vectors plays an important role in deciding the scientific strategy for prevention and control of Vector Borne Diseases to a great extent. The habit and habitat of vectors, the transmission months, flight range, longevity, biting time, presence of human and animal blood and survival of vectors in extreme conditions are equally important to institute effective vector control measures in an area.

Ecology is changing fast. Urbanisation, rapid transport involving frequent movements of the people from one place to the other has added another factor to bring changes in the bionomics of vectors. Global warming and change of weather conditions due to deforestation and other factors has led to affect the ecology and the behaviour of the vectors. This needs intelligent planning to introduce newer techniques to institute the effective control measures in an appropriate manner.

In Union Territory Chandigarh Vector control measures were applied keeping the bionomics of the vectors in view. Environmental manipulation, anti-larval and anti- adult control measures with massive behaviour change and communication programme

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Expressional pro› ling of virus speci› c genes in avian cells infected with different pathotypes of Newcastle disease virus

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Expression of structural and non-structural genes of Newcastle disease virus (NDV) encoding the viral protein plays a pivotal role in the replication of the virus and also associated with the infectivity and pathogenicity of the virus in avian hosts. Regardless of the extensive research carried out on the characterization of different pathotypes of NDV based on virulence, little is known about the expression of the virus specific genes of the different pathotypes during the course time of infection in different avian cells. Hence this study was performed to investigate the in vitro transcriptional profiling of the virus specific genes in different avian cells infected with different NDV pathotypes.

Relative quantification of all the virus specific genes of NDV strains, LaSota [lentogenic], R2B [mesogenic] and 2K3 [local velogenic isolate] in Chicken embryo fibroblast (CEF), Native chicken embryo fibroblast (NCEF), Japanese quail embryo fibroblast (JQEF) and QT-35 (Quail tracheal cell line) cells infected with m.o.i. of 0.01 were investigated in vitro at 24, 36, 48, 60, 72, 84 and 96 hours post infection (hpi) using gene specific, designed SYBR green qPCR primers. Virus copy number was determined by absolute quantification based on the standard curve obtained by qPCR technique. In NDV-LaSota infected avian cells, expression of the genes was high at 72, 84 and 96 hpi, whereas in NDV-R2B infected cells expression was high at 60, 72 and 84 hpi. However, in case of NDV-2K3 infected cells, expression was high at 36 and 48 hpi. Over all, the expression of virus genes was high in CEF cells followed by NCEF, JQEF and QT-35 cells through the course time of infection.

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Cloning and expression of major glycoprotein genes of Duck plague virus towards the development of › eld based diagnostics

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Duck viral enteritis (DVE), or duck plague (DP), is an acute, contagious herpesvirus infection of ducks, geese, and swans of all ages and species. The disease has been responsible for significant economic losses in domestic and wild waterfowl as a result of mortality, condemnations and decreased egg production. Several studies indicate that survivors of DVE may become carriers of the virus for up to 4 years. This disease is difficult to be monitored and controlled, because DEV establishes an asymptomatic carrier state in waterfowl that is detectable only during the intermittent shedding period of the virus. Development of rapid and field based diagnostics is the need of the hour for early diagnosis and culling of the infected birds. To develop such diagnostics, envelop proteins are targeted. In this study, the major envelop protein genes viz. glycoprotein C glycoprotein D, glycoprotein E and glycoprotein G were cloned and expressed in prokaryotic system to use the recombinant protein in the diagnostic assays. Initially all the envelop genes were cloned in pGEMT vector and then subcloned into pET28a vector for expression in prokaryotic system. Confirmation of the recombinant protein was done by western blotting method using duck plague virus positive serum raised in ducks. Optimization of the diagnostic assay and the interpretation of the results would be discussed in the

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Sequence analysis of host-range genes of Swinepox virus isolated from India

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Swinepox virus (SWPV), the only member of the genus Suipoxvirus is the etiological agent of swinepox, a disease of pigs characterized by fever, dullness and acute appearance of round cutaneous pock-like lesions all over the body. In comparison to Orthopoxvirus and Parapoxvirus infections which are frequently reported from India, there are very few recorded incidence of swinepox and is usually associated with poor sanitation and/or intensive breeding with open-herd management. Genome analysis of the SWPV indicates that this virus is most closely related to Lumpy skin disease virus (LSDV) and is genetically very distant from commonly occurring pox viruses in the country. Although, SWPV is host-specific (infects only pigs), in context of species jumping potential shown by related poxviruses viz., Buffalopox virus and Camelpox virus recently in the country, the sequence analysis of some important host-range genes genes viz., Ankyrin-repeat protein (ANK), Kelch-like protein (KLP), Extracellular enveloped virus protein (EEV) of this virus was carried out to ascertain its sequence identity/changes with reference SWPV as well as with other reported poxviruses. Sequence analysis of host-range genes revealed that SWPV isolated from India is closely related (97-98%) to SWPV reference isolates from U.S.A. The aa identity of the SWPV with other poxviruses (other than SWPV isolates) ranged from

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Isothermal detection of Peste des petits ruminants virus genomes from clinically infected small ruminants using uracil DNA glycosylase to avoid carry over contamination

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Peste des petits ruminants (PPR) is an acute, highly contagious disease of small ruminants (mainly goats and sheep) caused by the Peste des Petits Ruminants virus (PPRV). During an outbreak of PPR, rapid diagnosis will be useful for managemental decisions such as isolation of infected animals, vaccination of susceptible flocks etc. Conventional diagnostic methods such as virus isolation in cell culture are time-consuming. Whereas the genome detection methods such as RT- PCR and real-time RT-PCR are rapid but are expensive and require sophisticated instruments. In this study we have developed a Loop Mediated Isothermal Amplification (LAMP) assay for detection of PPRV in clinical samples. Our assay format uses the dUTP/ Uracil-DNA glycosylase strategy to avoid the laboratory cross contamination due to free circulating templates which is of major concern in laboratories using LAMP for routine diagnosis. This assay can be performed in a water bath and the results can be rapidly interpreted using a simple visual detection. Of forty sheep/ goat nasal swab samples collected from PPRV outbreaks, 22 were found to be

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Development of in vitro model for persistent Equine Herpesvirus-1 infection

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Equid herpesvirus 1 (EHV1) is an important viral pathogen of equines which causes significant losses to the equine industry. Organism is primarily responsible for rhinopneumonitis, abortion, neonatal foal mortality and encephalomyelitis. As with other herpesviruses, EHV1 establishes lifelong persistent infection in recovered horses following EHV1 infection. Our knowledge of EHV1 persistent infection is based on the various experiments made in living animals (mice, rabbits, horses etc.). Such animal experimentation has certain limitation in terms of costs, homogeny, ethical issues etc.; so there is need to develop in vitro system for study of EHV1 persistent infection. In the present study, persistent infection was induced in a human lymphoblastoid cell line (LCL-2) and Marmoset EBV- transfected B-cell Line (B-95) at a multiplicity of infection (MOI) of 0.3. Persistently infected cells were maintained and observed for 120 days. Samples of culture were taken at regular time interval to monitor virus production. Presence of virus in both the cells was confirmed by gB-based nested PCR and real-time PCR, immunoflorescence, infection of RK13 cells. Both the cells showed production of virus as early as 3rd day and remain infected till 120 days, as detected by nested and real-time PCR. In the infected cells, virus was localized intra-cellular in the nucleus, as seen by immunoflorescence at different intervals post-inection. The RK13 cells were co-cultured with EHV1-infected B-95 and LCL-2 cells at different intervals post-infecton. The RK13 cell monolayers were infected by co-cultivation, showing characteristic cytopathic effects (CPE), with virus titers of 4.1 and 2.9 TCID50/ml in B-95 and LCL-2, respectively. The persistently infected cells also produced excretory virion particles, as confirmed by infection of RK-31 with cell-free supernatant,

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Emergence of naturally transmitted sheep associated malignant catarrhal fever in diverse ruminant species in India

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Malignant Catarrhal fever (MCF) is a fatal herpesvirus infection, with a short and dramatic clinical course characterized primarily by high fever, severe depression, swollen lymph nodes, salivation, diarrhoea, dermatitis, neurological disorders and ocular lesions often leading to blindness. A series of clinical cases of sheep-associated malignant catarrhal fever (SA-MCF) have been recorded in susceptible species Karnataka, Tamil Nadu and Mizoram within a span of 2 years. The first case was recorded in a captive bison in the year 2013 with typical symptoms of SA-MCF which was later confirmed by OIE approved laboratory test. Subsequently, fatal clinical cases of SA-MCF were identified in cattle with symptoms of diarrhoea, respiratory distress, conjunctivitis, nasal discharges. Laboratory diagnosis from the samples of two ailing animals confirmed the detection of ovine herpesvirus-2 (OvHV-2) genome in the peripheral blood samples of cattle. Recently 4 buffalo samples from in and around the villages of Namakkal with typical symptoms of MCF were diagnosed with the disease. The sheep blood samples collected subsequently from the neighbouring areas also showed presence of OvHV-2 genome indicating a nidus of infection in the state. Very recently one of the cattle samples submitted from Mizoram was diagnosed for MCF and ailing pig samples also submitted from the same place were diagnosed as positive indicating the far and wide spread of the disease. The laboratory test

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Decoding immunologic memory for designing better vaccines

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Immunological memory has been an exciting field of immunology for over five decades and has attracted several outstanding immunology scholars to study and understand complex mechanism of immunological memory generation and its maintenance for short term as well as long term. One of the major questions related to generation of immunological memory has been why certain antigens induce long term memory where as only short term memory is generated in response to some antigens. Clear answer to this question has huge implication in development of more effective vaccines which could produce long lasting immunity.

It is now fairly well understood that any exposure of mammalian host with antigen either naturally in the form of infectious agent or deliberate inoculation, the host immune response is induced which could be either humoral or cellular or both depending upon the nature of the antigen. During the process of induction of primary immune response B and T memory cells are also generated which are capable of mounting rapid and more vigorous immune response in the face of encounter with the same pathogen/antigen in future. However, we still do not have clear idea about how B and T memory cells are generated and what are the genes that are responsible and how the short term and long term memory is maintained in the host. Recent studies suggest that primary immune response can also generate memory natural killer (NK) cells. Driven by new tools and technologies such as

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Success of Foot-and-Mouth disease control programme in Haryana

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Govt. of India launched FMD Control Programme (FMD-CP) during 2003-04 initially in 54 districts of country including eight districts of Haryana (Bhiwani, Fatehabad, Hisar, Jhajjar, Jind, Rohtak, Sirsa, and Sonepat). The animals in the remaining districts of Haryana were also vaccinated under ASCAD. The Regional Research Centre on FMD, Hisar, a unit of ICAR-Project Directorate on FMD, Mukteswar participated actively in the implementation of FMD-CP by providing logistic support in the form of epidemiological surveillance and sero-monitoring work in all the 21 districts of Haryana.

A total of 239 FMD outbreaks were recorded in Haryana over a period of fifteen years between January 2000 and December 2014. Of these, 204 FMD outbreaks were recorded before (2000 to 2003) and only 35 after (2004 to 2014) the launch of FMD-CP. Maximum outbreaks (111) were recorded in 2003 which reduced to 15 in 2004; three in 2005 and 2010; only one each in 2006, 2007, 2009 and 2011; two in 2008, four each in 2012 & 2014 and none in 2013 and 2015 (upto Sept. 2015). Further, the sero-monitoring data generated by analyzing the serum samples from FMD vaccinated animals (pre- and post-vaccination) by high throughput single dilution liquid phase blocking ELISA (sdLPBE) demonstrated that

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Leptospirosis vaccines: Present status and future prospects

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Leptospirosis has in recent years emerged as one of the priority diseases in the country as it is responsible for substantial economic losses to the farmers as a result of various reproductive disorders it causes in animals such as abortions, still birth and repeat breeding including mastitis and agalactia. Research on the development of a suitable vaccine for control of the disease has continued since last 50 years with mixed findings. Presently we have inactivated whole cell vaccines which comprise of one or preferably several Leptospira serovars with or without adjuvant to provide a broad range of immunity. Selection of serovars in preparing these vaccines depends on the prevalence of the serovars in the target area or animal population. Repeated vaccinations are required to generate protective immunity in animals which may not last for more than 6 months. A few such multivalent killed commercial vaccines are currently in use in countries such as USA, Australia, New Zealand and U.K. Due to short lived immunity generated by these vaccines, development of live vaccines was attempted using highly prevalent serovars, Pomona and Icterohaemorrhagiae. Though these vaccines generated higher level of immunity in animals, however, the problem of maintenance of the vaccine strains and reversion to the virulent form have been the constraints in their wider acceptability. Subunit vaccines prepared from surface proteins separated from outer cell membrane have been a much accepted option. A few protein sub units targeted for such studies are 39 kDa, Omp L1, Lip41, LipL32 and Lig proteins. Efforts on the expression of some of these proteins for

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Harmonization of Quality of Veterinary Biologicals in India

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Livestock contribute over one-fourth to the agricultural gross domestic product. Though the livestock sector has been growing faster than crop sector, it has remained under-invested and neglected. Nevertheless, there is immense potential of growth in this sector, however, achieving growth rate of 5-6% would require addressing challenge of frequent occurrence of some deadly infectious diseases besides nutrition and breeding. In order to fight the challenge of infectious diseases through various strategies of treatment, containment and control, the most important is to accomplish the basic goal of prevention. The uncomplicated and most cost-effective way of achieving this is by way of immunization with acceptable benefit/risk consideration. These veterinary vaccines have a major impact not only on animal productivity but also on human health through increasing safe food supplies and preventing animal-to-human transmission of infectious diseases.

In India, many State-owned Veterinary Biologicals Production units and companies in private sector are engaged in production of veterinary biologicals. However, majority of them does not observe Good Manufacturing Practice (GMP) and quality control for veterinary biologicals was almost non-existent. The organization and function of veterinary vaccine production and quality control is governed by the statutes of the Drug and Cosmetic Act 1945, alongside a series of schedules based upon Indian pharmacopoeia. Quality control of veterinary vaccine generally involves safety, potency and sterility. It also includes some other less common tests intended specifically for individual vaccines. There is lack of uniformity in the provisions of Act and IP monographs, which warrants their review. These should be revised for harmony across different States and sectors on the lines of OIE and with the international standards. It is mandatory that for successful productivity programmes, vaccine production units in the States are updated for creating

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Potential of oncolytic virotherapy for the treatment of animal cancers

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Oncolytic viruses are a group of viruses that infect and kill cancer cells preferentially to normal tissues. The idea for using these viruses as a cancer therapy is not a new one and is based on the observation of tumor regression in the face of natural viral infection. Virus therapy has been limited in the past, however, because of the limited capacity for generating high titers of viruses to be used clinically. Furthermore, advances in molecular engineering has allowed for manipulation of the viral genome to both make them safer by deleting viral genes involved in pathogenesis and by insertion of novel transgenes to enhance antitumor activity. As a result, several viruses have now been tested in clinical trials with varying degrees of success. ONYX-015, an adenovirus, has been tested in randomized trials and its cousin, H101, has been licensed in China. Meanwhile, reovirus, and herpes simplex virus (HSV) are in late phase testing. Various other viruses including measles virus (MV) and vaccinia virus (VV) have completed phase I testing with promising results.

Initially, it was thought that direct lysis of cancer cells by a virus would be the primary mechanism for the anticancer effects of a virus therapy, hence the term "oncolytic virus therapy". Although virus-induced cancer cell death does occur, there is a large amount of evidence that indicates the immune response to the virus is a key component of an effective anticancer virus therapy. The ideal oncolytic virus should destroy tumor cells throughout the body and induce antitumor immunity without damaging healthy cells. Indeed, safety is a key concern with oncolytic viruses, particularly when used in immunosuppressed patients. Safety may be enhanced by using nonpathogenic, genetically stable virotherapeutics derived from wild-type viruses that do not cause severe disease and lack the ability to integrate into the patients' DNA.

Tumor speci› city of oncolytic viruses

Increasing the tumor specificity of a virotherapeutics also can enhance its safety. Interestingly, some of the common cellular pathway alterations found in neoplasms allows viruses to productively and selectively infect tumor cells. Several tumor cells have mutations in key components of intracellular signaling pathways which block apoptosis and/or promote proliferation, making them susceptible to viral infection. Other tumor cells lack adequate antiviral interferon responses that prevent virus replication in noncancerous cells. Some viruses are inherently tumor-selective or can be genetically altered to improve tumor specificity. For example, in many oncolytic adenoviruses, the viral E1a gene is

disrupted and/or the E1b-55K gene is deleted so that the viruses can only replicate in cells with defective retinoblastoma or p53 pathways, respectively. Also, several oncolytic vaccinia viruses (VACVs) have the viral thymidine kinase gene deleted, which limits virus replication to actively dividing cells. Additionally, oncolytic viruses may be designed to contain tumor-specific promoter elements that only permit replication of the virus in tumor cells.This can be achieved by the insertion of a tumor-specific promoter driving the expression of a critical gene. Others viruses either possess naturally [e.g., CoxsackievirusA21 and measles virus (MV)] or have been designed to have specific tropism based on the expression of cell surface receptors unique to cancer cells.

Important points to remember

1. Viruses can be engineered to selectively infect and/or replicate in cancer cells.
2. Selective infection uses redirection of viral binding away from normal and towards normal cells.
3. Selective replication can be targeted towards cells with overactive RAS or defective interferon signaling, cells with defects in the p16/RB tumour-suppressor pathway, or replication use tumour-selective promoters to drive expression of viral genes.
4. Clinical trials have shown that oncolytic viruses - that are derived from adenoviruses such as herpes simplex virus and Newcastle disease virus - are well tolerated without notable side effects.
5. The main goals of oncolytic viral research are to increase tumour selectivity by modifying the viral genome, to combine oncolytic viral therapy with standard radiation and chemotherapy, and to 'arm' the viruses with suicide cDNAs and/or cytokine cDNAs for multimodal treatment.
6. Improving our understanding of the mechanisms of viral oncolysis and the immune responses that viruses induce, as well as improving the ability to image these viruses in vivo, should make this therapeutic approach safer and more effective.

The immune response to virus therapy

Oncolytic virotherapy has the potential benefit of altering the tumor microenvironment enough to break existing tumor immunotolerance. Failure of the immune system to recognize tumor cells may be due to a paucity of stimulated immune cells infiltrating the tumor and/ormasking of tumor antigens. Aberrant cytokine patterns in the tumor microenvironment may severely limit an antitumor immune response. Additionally, regulatory T-cells and myeloid-derived suppressor cells may be present in increased numbers in cancer patients. These immunologic hurdles can be overcome by oncolytic viral therapy if infected tumor cells express viral antigens that trigger a fully functional cellular immune response and alter cytokine production to promote infiltration of immune cells. Ideally, when the cellular immune response lyses infected tumor cells and presents viral antigens to cells of the immune system, hidden tumor antigens that the immune system can respond to will also be exposed. This would lead to clearance of the virus by the

immune system and concurrent amplification of an antitumor immune response.

Challenges of Oncolytic Virotherapy

Oncolytic viruses can rapidly replicate in and spread through2D cell cultures that are derived from a variety of differenttumor types. However, there are several factors that couldhamper the efficient spread of oncolytic viruses within a solidtumor mass. Physical barriers such as necrotic areaswithin the infected tumor, normal stroma cells and extracellular matrix, or the presence of the basal membrane, could limitthe distribution and infection of the diffuse virus. Viral replication has underlined the importance of diffuse

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Evaluation of a DNA vaccine expressing peste des petits ruminants virus haemagglutinin

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A DNA vaccine against peste des petits ruminants (PPR) was developed by cloning Haemagglutinin (H) gene of peste des petits ruminants virus in pEGFP-C1 expression vector. Vaccination trials were carried out in PPR seronegative goats with three groups. viz. DNA vaccine group, live vaccine group and control group. In the DNA vaccine group the animals were injected with 300µg of plasmid intramuscularly with boosters on 7th and 14th day. The animals in the live vaccine group were inoculated with 1 ml of live attenuated PPR vaccine subcutaneously. The animals were challenged with 103 SID50 Poondi virulent virus on 28th day. The animals were bled on days 0, 14, 21 and 28 post vaccination and 14 days post challenge and antibody titres assessed by competitive ELISA and neutralization tests. In DNA vaccine group the mean per cent inhibition values were 28.75±0.62, 84.6±2.53, 91.275±1.45, 93.3±1.13 and 92.1±1.28 on day 0, day 14,day 21,day 28 post vaccination and 14 days post challenge respectively when tested in c ELISA. The animals in the live vaccine group showed mean per cent inhibition values of 29.5±2.50, 93.29±1.06, 96.6±0.60, 97.5±0.50 and 96.8±0.40 on day 0, day 14, day 21, day 28 post vaccination and 14 days post challenge respectively. Mean neutralization titres were 3.75±0.25 log2, 4.75±0.25 log2, 4.75±0.25 log2 and 4.75±0.25 log2 on day 14,day 21,day 28 post vaccination and 14 days post challenge respectively for the DNA vaccine group. In the live vaccine group mean neutralization titers

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Some immunological studies on a few vaccine derived from bacteriophage lysate of Pasteurella multocida in mice

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The present study was undertaken to evaluate the humoral immune response in mice induced by a novel phage lysate vaccine against Hemorrhagic Septicemia developed from Pasteurella multocida grown under iron-restricted conditions and lysed by a bacteriophage. Two groups of mice were immunized with lysate vaccine (LV) and conventional HS alum precipitated vaccine (CV) and blood was collected at various days post - immunization (DPI). The total serum protein concentration in lysate vaccinated mice (4.675±0.223) was significantly higher ($p<0.05$) than the conventional vaccinated mice (4.100±0.282) at 150 DPI. The serum globulin levels at 90 DPI and 180 DPI in LV mice (1.330±0.071 and 0.650±0.100) were significantly ($p<0.01$) higher than the CV mice (0.850±0.084 and 0.366±0.098). The serum IgG levels at 150 DPI and 180 DPI in LV (0.564±0.188 and 0.485±0121) mice were significantly higher ($p<0.01$) than the CV mice

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Nanoemulsion and rHSP 60 adjuvanted Foot and Mouth Disease antigen vaccine induced immune response in Guinea pigs

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Foot and Mouth Disease (FMD) is a highly contagious, acute vesicular viral disease of cloven hoofed animals caused by FMD virus (FMDV). Vaccination is the most promising way in control of FMD. In this connection, the inactivated vaccine adjuvanted with Oil or Gel based vaccine is currently being used at the field level. However, the Conventional vaccines fail to induce prolonged humoral immunity. Accordingly, we developed and characterized a nanoemulsion (NE) in which FMDV O/IND/R2/75 antigens were incorporated to achieve effect immunity in vivo. For effective antigen presentation to immune system, it was adjuvanted with recombinant heat shock protein 60 (rHsp60) @ 10 µg/dose. Experimental guinea pigs were divided into six groups and the novel vaccine or its component(s) was administered either intradermal (n=12/group) or intramuscular (n=12/group) route. Sera were collected at three intervals post-vaccination for the assay of serum neutralizing antibody, total IgG, IgG1 and IgG2 for humoral immune response analysis. Lymphocyte transformation test (LTT) was performed to assess the cell mediated immune response (CMI). Further, protection studies were conducted following homologous FMD virus challenge in guinea pigs. The results indicated that the NE showed thermodynamic and

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Screening of kinase and phosphatase inhibitors for their antiviral ef› cacy against buffalopoxvirus

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High mutation rate in viral genome allows the virus to become resistant to antiviral drugs and preexisting immunity. The rise in incidence of drug resistance has prompted a shift to newer strategies for the development of antiviral drugs. Compared to the high rate of mutations in the virus genome, genetic variability of the host is quite low and hence host targeting agents are considered to impose a higher genetic barrier to generation of resistant viruses. Therefore, a potential approach for development of novel antiviral therapeutics is to target host factors essential for viral replication. Targeting host factors might be an attractive prospect as it could make an impact on multiple virus genotypes (strain/serotype) and provide a broad spectrum inhibition against different families of viruses that might use the same cellular pathway(s) for replication. This novel approach has led to the development of some promising compounds for treatment of HCV and HIV. Poxviruses are double-stranded DNA viruses that belong to the family Poxviridae and have a wide host-range which includes both vertebrates and invertebrates. The disease is characterized by fever, formation of localized or general pox-like lesion and mortality that depends on specific virus involved. Though the vaccine against few of the proxviruses is

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Nitrosative stress indices in demyelinating neuropathies in dogs

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Nitric oxide (NO) an unconventional neurotransmitter plays potential role in pathophysiology of neuronal disease. Canine distemper virus can cause demyelinating neuropathy but little is known about role of nitric oxide n Nitric Oxide Synthatase (nNOS). We report here the status of NO and nNOS in CD induced neuropathies in dogs. The study was conducted on clinical cases of canine demyelinating neuropathies (DMN) presented to Referral Veterinary Polyclinic (RVP), Indian Veterinary Research Institute (IVRI), Izatnagar, from 2013-14. Fifteen apparently healthy dogs maintained at Division of Nutrition, IVRI, served as healthy control. Out of 3934 clinical cases, 139 dogs (3.53%) cases were positive for DMN; and among these DMN cases 94 cases were positive for canine distemper (CD) specific IgM and IgG. There was significant (p?0.001) increase in the concentration of Myelin Basic protein (MBP) both in plasma and Cerebrospinal fluid (CSF) of dogs suffering from DMN and CD when compared to healthy control group. Similarly significant (P<0.05) increase in the plasma and CSF level of NO and activity nNOS, in dogs suffering from DMN

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Identi› cation of promoter region of dog survivin gene and its exploitation for development of gene constructs for selective apoptosis in dog cancer cells

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Canine mammary tumours (CMTs) are amongst the most common neoplasms of female dogs with thrice higher mortality rates than human breast cancer. Specific expression of apoptotic genes in cancer cells utilizing tumour specific promoters is a promising strategy forcancer therapy. Survivin, a member of the IAP family of proteins, is expressed in 50-70% of dog cancers but not in normal differentiated adult tissues. Thus survivin promoter can be utilized for cancer gene therapy, but no attempts have yet been made to characterize promoter of survivin gene in dogs. In this study, promoter region of survivin gene was identified and further exploited for development of gene constructs for selective expression of apoptotic genes in cancer cells.To characterize survivin promoter, 5' UTR region of dog survivin gene, retrieved from the Ensembl Genome Browser, was analysedin silico for prediction of probable promoter regions (n=8), which were amplified and cloned directionally in pGL4.17 luciferase reporter vector. Further the luciferase gene was replaced with RFP gene to obtain constructs expressing RFP under the identified promoter regions. The reporter gene constructs were transfected in cancerous cells (established cancer cell lines) as well as non-cancerous dog cells obtained upon primary culture of different tissues of a still born pup.The 1945 bp promoter region with maximal expression of reporter gene was identified using dual-glo luciferase assays as well as RFP reporter assays. The identified promoter region gave specific expression of the reporter genes in dog, human,

Molecular Characterization of E. coli virulence factors from calves

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Calves are most susceptible to Esherichia coli infections in the first few weeks of birth. In the present study, isolation, serogrouping, molecular characterization and antibiotic resistance of E. coli isolated from faecal samples of 150 calves (80 diarrheic and 70 non-diarrheic) less than 3 month of age was undertaken. A total of 250 E. coli isolates (163 diarrheic) were recovered which belonged to 18 different serogroups. Among various serogroups O2 and O6 were most prevalent in non-diarrheic whereas O9 is most prevalent among diarrheic ones. In multiplex PCR for f5, f41, f17, stx1, stx2, eae, sta and lt, 28 (11.2%) isolates were found positive for atleast one virulence gene. The study revealed that f5, f41, sta and lt gene carrying strains were common in calves less than 1 week with severe diarrhea. These 28 E. coli isolates were divided into different virotypes on the basis of different virulence genes present, like shiga-toxin producing E. coli (12 isolates),

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Comparative pro› ling of fetal adnexa derived caprine stem cells

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Objective of the present study was to evaluate the comparative profile of caprine MSCs derived from fetal adnexa, {amniotic fluid (AF), amniotic membrane (AM), umbilical cord matrix (UCM) and umbilical cord blood (UCB)}. Three months old gravid caprine uteri were collected from local abattoir and used for the derivation of all the fetal adnexa derives stem cells. All the four types of stem cells of adnexa, were expanded in vitro; the cells were characterized for their growth kinetics, specific surface antigen and pluripotency marker localization as well as molecular expression, and, and mesenchymal lineage differentiation during their in vitro expansion at 3rd passage using the standard protocols of our laboratory.

Morphology of the proliferating cells showed fibroblast like appearance, whereas, the population doubling time (DT) was significantly different amongst these cells. Cell proliferation and doubling was noted to be faster for UCM-MSCs and UCB-MSCs compared to AF-MSCs and AM-MSCs. Although all the four lineages expressed pluripotent and mesenchymal stem cell surface markers, such as Oct-4, SOX-2, NANOG, KLF, cMYC, FOXD3, CD73, CD90 and CD105. Expression of the hematopoietic stem cell marker i.e. CD34 was absent in all fetal adnexa derived cells. The isolated cells of the four cell lineages at P3 showed multipotent capacity and got differentiated in vitro into adipocyte, osteocyte, and chondrocyte, as demonstrated by specific stains. These results were also confirmed at protein level using flow cytometric analysis and immune-cytochemical localization.

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Development of cell penetrating peptides for cellular delivery of peptide nucleic acid (PNA), a model study for nanodelivery and therapeutic interventions

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Peptide nucleic acid (PNA) is being used as molecular probes for the diagnosis of infectious diseases. The PNA probes execute special characters like charge neutrality and resistance to nucleases and proteases. These characters make PNA a candidate molecule for antisense therapy. However due to charge neutrality, polycationinc lipids cannot be used for cellular delivery of PNA limiting their therapeutic applications. In present study we discuss development of peptide based nano-delivery mechanism for non covalent cellular delivery of PNA. The peptides form small nanoparticles on interactions with PNA ensuring their cellular entry. The peptides showed efficient PNA delivery up to 97% as observed in

Poly (I:C) enhances anti-tumor and anti-metastatic activities of canine parvovirus NS1 protein

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Some viral genes induce apoptosis selectively in the tumor cells without harming the normal cells. The NS1 protein of parvoviruses is one of such agents. The NS1 protein of canine parvovirus (CPV2.NS1) is also cytotoxic to malignant cells. In the present study, we evaluated the oncolytic activity of CPV2.NS1 in mammary mouse tumor model. Further, we also evaluated its combination with poly (I:C) with an aim to determine if the combination can induce a potent anti-tumor immune response which may further potentiate the anti-tumor activity of CPV2.NS1. The results suggested that Poly (I:C) when given along with CPV2.NS1, not only inhibited the tumor growth significantly, but also augmented the immune response against the tumor antigen(s) as indicated by the increase in blood CD4+,

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Construction of infectious cDNA clone derived from a classical swine fever virus › eld isolate in BAC vector using in vitro overlap extension PCR and recombination

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Classical swine fever (CSF) is a highly contagious, fatal disease of pigs and wild boars prevalent worldwide. It is caused by the CSF virus (CSFV), a pestivirus belonging to the family Flaviviridae. The disease is enzootic in most of the pig producing states and particularly in the North Eastern states of India. It is highly desirable to develop a live modified vaccine against classical swine fever, derived from a field isolate belonging to the genotype prevalent in India. The present study was aimed to establish an infectious cDNA clone for developing reverse genetics system for CSFV. To develop reverse genetics system of RNA viruses, cloning of full-length viral genome is required which is often challenging due to many steps involved. In this study, we report cloning of cDNA from an Indian field isolate (CSFV/IVRI/VB-131) of CSFV using in vitro overlap extension PCR and recombination, which drastically reduced the number of cloning steps. The genome of CSFV was amplified in six overlapping cDNA fragments and linked together by overlap extension PCR using thermostable DNA polymerase, and cloned in a bacterial artificial chromosome (BAC) vector using in vitro recombination method to generate full-length cDNA clone. The full-length CSFV cDNA clone was found stable in Stellar and DH10B E. coli

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Genotyping of single nucleotide polymorphism in goat poxvirus › eld isolates through a novel p32 gene based tetra-primer ampli› cation refractory mutation system-polymerase chain reaction (ARMS-PCR) assay

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Goatpox disease is one of the most widespread Capripoxvirus diseases affecting small ruminants. It exhibits moderate to even severe pathological consequences in the endemic areas. Existing techniques for characterizing and genotyping of these goat poxviruses are limited to some sophisticated techniques like high thoroughput sequencing technologies and restriction fragment length polymorphism techniques. Single nucleotide polymorphisms (SNPs) are most often associated with some pathological implications. To screen out the presence of such mutations is extremely sought to know the nature of the disease outbreak. Furthermore, the allele specific distributions of the virus are to be known for effective epidemiological strategies. Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) is a simple, rapid and inexpensive

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Staphylococcus aureus ST-2219t6877 and ST-2182t267 intramammary inoculation in a mouse model reveals modulation of transcriptome landscape with alternative splice variants

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Epidemiological investigations of mastitis causing pathogens gleaned across the globe shows Staphylococcus aureus is the leading pathogen with diverse genetic lineages circulating in the dairy cattle population causing mastitis. Using an established intramammary infection mouse model, we found the endemic clones in the province causing mastitis showed specific alteration of epigenetic modulators (histone modifications and microRNA) resulting in deviating immune response. Histopathological analyses showed 24h post inoculation a critical time point influencing the fate of infection. To further gain a deeper insight of the the modulation of transcriptome landscape, we expanded the studyhere using high throughput, paired end RNAsequencing analysis of the mouse mammary gland infected with three strains of S. aureus possessing specific genotype (ST-2182t267;n = 2 &; ST-2219t6877n=1), virulence and enterotoxin traits.

RNAsequencing of 24h post inoculated mammary gland tissues infected with SA1 (ST-2219t6877),SA2 and SA3 (ST-2182t267)over all detected 20756 transcripts. Expression of ~1001 transcripts was >2 FPKM of transcripts per million mapped fragments and ~ 37% of multi exonic genes were alternatively spliced. Gene ontology (GO) and pathway analysis revealed that majority of the significant genes with altered expression clustered into inflammatory response, cell adhesion and metabolic process categories. Fundamental differences were observed in the levels of expression of immune-related genes in response to intramammary infection in a strain (SA1, SA2, SA3) directed manner. Furthermore, comparative analyses of AS patterns in SA1, SA2 and SA3 revealed conserved ratios of the AS types amongst S. aureus infection. S. aureus infection quantitatively altered AS events in mice mammary gland in a strain specific manner. We observed AS events for >100

Erythroblastic Leukemia Viral Oncogene Homolog 2 (ErbB2) as a diagnostic biomarker of canine mammary carcinomas

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ErbB2 is a proto-oncogene that encodes a 185 kDa trans-membrane glycoprotein of tyrosine kinase receptor family. It is involved in a cascade of reactions which results in cellular growth and transformation. The over-expression of erbB2 is correlated to poor prognosis in canine mammary tumors. In this study real-time polymerase chain reaction was used to evaluate its expression and it was found to be upregulated (5.15 times) in complex canine mammary carcinomas. The gene was cloned and expressed in a prokaryotic system (E.coli) as 54 kDa recombinant fusion protein. The expressed protein was further purified by affinity chromatography and confirmed by western blotting. Hyperimmune sera were raised against the \expressed purified protein in rabbits and mice to standardize sandwich ELISAfor relative quantification of circulating protein in the sera of dogs with mammary tumors. Based on receiver-operating characteristics analysis, the

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Identi› cation and characterization of brain and testis speci› c homing peptides using phage display library

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In recent time new generation drugs have been advocated to combat disease/cancer but still could not score much over use of chemotherapy due to lack of tissue specific targeting capability of delivery systems. Therefore, there is an urge to search for homing peptides/molecular steerings to guide delivery systems for tissue specific off-loading of drugs. Each normal organ and pathological condition appears to contain organ- or disease-specific molecular tags on its vasculature, which constitute a vascular "zip code" system. In vivo phage display has been exploited to profile this vascular heterogeneity and identification of peptides that home specifically to various normal organs or pathological conditions. Having this knowledge, we have identified brain and testis specific homing peptides using 12mer Phage display library following four rounds of in-vivo biopanning approach. The identified peptides were synthesized employing Fmoc chemistry, purified and labeled at their N-terminal end with FITC to test their homing ability both qualitatively by fluorescence microscopy and quantitatively by flow cytometry. Screening for homing ability clearly revealed that all the identified brain and testis homing peptides preferentially bound to the N2a and LC540 cells and primary cells of brain/ testis in comparison to liver, spleen, heart and kidney.

These peptides when tagged to cell penetrating peptide limited the delivery to specific cells. Gold nanoparticles as vehicle/contrast agent when conjugated with peptide showed target specific in-vivo biodistribution in respective organs wherein preferential localization (increase in HU unit/CT value) in brain and testicular tissue as compared to other organs was observed. The targeting specificity of AuNP-peptide meets or exceeds the amount of gold that is required to induce x-ray contrast to function a tissue specific CT molecular imaging agent.

In summary, we were able to identify two novel brain homing and 9 testis homing peptides as steering for tissue specific targeting. A brain homing peptide having repetitions

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ORFeome of equine infiuenza virus: State-of-the-art clone resource for functional genomics

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The Influenza A viruses are RNA viruses which undergo frequent mutational changes due to non proof reading activity of polymerase gene rendering vaccines ineffective. Further the functional aspects of frequent changes in the genes and their in vivo interaction with host defence mechanism is enigmatic. Equine influenza caused by H3N8 is one such flu virus which continuously threatens the equine population globally. India faced a large epizootic of EI in 2008-09 which led to huge economic losses to the industry. Recent break-through viz. development of ORFeome facilitates expression of large number of proteins and allows large-scale, high-throughput proteome analysis. This will help in context of influenza viruses in understanding the disease pathogenesis as well as developing effective prophylaxis through studies of the proteomes. Such library is used as resource for research in the areas of protein-protein interactions, host-pathogen interactions and thereby deciphering the mechanism of disease pathogenesis, drug & vaccine development.

In this context, Gateway Clone Library of ORFs of EIV isolated from 2008-09 outbreak in Katra, Jammu was generated. All 10 major ORFs (HA, NA, NP, M1, M2, NS1, NEP, PA, PB1 & PB2) encoded by EIV were amplified by two rounds of PCR using ORF-specific primers having lambda phage attB sites. The amplicons were cloned into Gateway vector-pDONR221 by homologous recombination; recombinant clones were selected by antibiotic resistance and suicidal action of ccd gene of the vector and validated by sequencing the inserted ORFs. To check the utility of the developed library in recombinant protein production, 3 ORFs (NP, M1 & NS1) were shuttled into gateway prokaryotic destination vector - pDEST15. The confirmed construct (pDEST15-ORFs) were subsequently transformed into BL-21 (DE3) cells, recombinant proteins were expressed upon stimulation with IPTG and confirmed the expressed proteins by SDS-PAGE. Another major application of the gateway ORF library is to develop yeast-two-hybrid expression library by shutting ORFs into yeast vectors for studying protein-protein interactions, thereby understanding the virus host interactions. During influenza virus infection, polymerase proteins are know to interact together and form complex - responsible of transcription and replication of viral RNA. Further, NP protein causes inhibition of innate host defence against virus, through inactivation of RNAdepended protein kinase (PKR) by dissociation of p58IPK from HSP40. For studying these interactions, two ORFs (PB1 & NP) were subcloned into bait vector-

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Emergence and spread of antibiotic resistance genes in environmental bacteriophages

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The ecosystem is continuously exposed to a wide variety of antimicrobials through wastewater treatment plant (WWTP) effluents, agricultural runoff, animal related and anthropogenic activities, which may contribute to the emergence and spread of antibiotic resistance genes (ARGs). The contamination of pristine components of ecosystem with ARGs may create increased opportunities for their transfer to naive microbes and eventually may lead to their entrance into human food chain. Transduction is a significant mechanism of horizontal gene transfer in naturally occurring environments which has traditionally been underestimated as compared to transformation. We explored the presence of ARGs in environmental bacteriophages with special reference to host bacteria of veterinary importance in order to recognize the contribution of phages in spread and persistence of ARGs in environmental settings. Sewage and soil samples were collected from areas of animal interventiaon and were used to enrich bacteriophages against variety of bacteria by incubation at 37°C overnight under shaking conditions followed by centrifugation at 10,000 rpm for 10 min and filtration. The bacteriophage activity was detected using spot assay on nutrient agar seeded with host bacteria. The filtrate was serially diluted and plated upon enrichment host by double-agar layer technique to obtain separated plaques, one of which was purified grown in bulk and concentrated using PEG8000.

Individual concentrated phage isolate was purified by extraction in chloroform and accessioned in Veterinary Type Culture Collection, NRCE. Phages were characterized partially and their DNAwas used to amplify 16s rDNAto detect any contaminating host DNA followed by PCR detection of antibiotic resistance genes including: TEM-bla, OXA-2, Int1, Int2, Int3, TetA, TetB, TetW, QnrA, QnrS and mecA. It was observed that although other ARGs

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Whole genome analysis of bovine rotavirus strain carrying novel genomic constellation with evidence of multispecies reassortment

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Group A rotaviruses (RVAs) are of major concern in causing gastroenteritis in both animals and human worldwide. The most common G- and P-genotypes isolated from bovine population include G6, G8, G10 and P[1], P[11]. Recently, we identified an unusual strain (M1/09) in a male cross bred bovine calf suffering with gastroenteritis whose VP7 genotype could not be explicitly classified with sequencing. The examination of the complete genome sequences of this strain revealed a typical genotype constellation G3-P[11]-I2-R2-C2-M2-A11-N2-T6-E2-H3. The VP7 gene displayed most nucleotide sequence identity with simian G3 genotype strain RVA/Simian-tc/USA/RRV/1975/G3P3 (73.8%). However, VP4 gene clustered with Indian porcine strain RVA/Porcine-wt/IND/AM-P66/2012/G10P11. In addition, VP6, NSP1 and NSP4 genes were identical or nearly identical to Indian bovine strains (RVA/Cow-wt/IND/B-72/2008/G10PX, RVA/Bovine-wt/IND/B85/2010/GXPX, respectively). The remaining four gene segments (VP1, VP2, VP3 and NSP2) were closely related to RVA/Human-wt/ITL/PAI11/1996/G2P4 (93.5%), RVA/Caprine-wt/CHN/LLR/1985/G10P12 (88.8%), RVA/Human-tc/SWE/1076/1983/G2P2A6 (93.2%) and RVA/Human-wt/AUS/ CK20003/2000/G2P4 (91.2%), respectively. The minimum spanning network clearly indicated segregation of the RVA isolate (M1) from G3 and G16 network, suggesting emergence of a putative new genotype. It was also evident from the parsimony splits network, where the M1 isolate showed striking distance from G3 and G16 RVA isolates with establishment of an independent haplogroup. Presence of isolates from more than one origin in a single haplogroup is an indication for the possibility of inter species transmission between human and other animals. The genealogy of VP7 genes from RVA isolates of G3 and G16 elucidated the occurrence of several nucleotide substitution events in due course of time but these mutations were random as evidenced by Tajima's D test. We could not found evidence of statistically significant positive selection pressure in the analysis. Furthermore, we tried to determine the ancestral isolate among selected

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In silico identi› cation of a novel peptide epitope and generation of antibody for sensing buffalo Luteinizing Hormone

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Buffalo has emerged as the "black gold" of India and proved itself as the favouritemilch animal of farmers.While accurate and timely detection of estrus is pre-requisite for successful artificial insemination program, lesser overt or weak signs of estrus in buffaloes has been one of the main reason for the highest percentage of the conception failures during the first service. Presence of luteinizing hormone (LH) in the blood and urine has been one of the most common and measurable changes that occur during estrus and prior to ovulation.In this context, ovulation prediction kit is well adapted and user friendly in humans and based on LH surge for predicting the timing of ovulation. Sensing any analyte in biological system is quite a difficult task, as biological fluids and matrices are complex mixture of different compositions. Gold nanoparticles (AuNPs) have been used in the design of biosensors and possess excellent user friendly characteristics. Utilization of peptide conjugated nanoparticles for the design and development of sensors is very rare and there has been no report for use of peptide conjugated gold/silver nanoparticles for affinity based biosensing in buffalo luteinizing hormone. Therefore, in the context of above, present study was undertaken. In this work,a new peptide sequence (luteinizing hormone peptide, LHP) has been identi?ed using several bioinformatics tools. LHP has been synthesized and characterized. We also describe a novel strategy for the fabrication of a sensor for detecting luteinizing hormone (LH) of buffalo using the peptide and antibody

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Extraordinary diversity in the zinc › nger domain of the speciation gene PRDM9 but multiple disruptive mutations in its paralog PRDM7 in ruminants

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PRDM9 is the sole hybrid sterility gene identified so far in vertebrates which contributes to the speciation process. PRDM9 gene encodes a protein with an immensely variable zinc finger (ZF) domain that determines the site of meiotic recombination hotspots genome wide. It is quite a fascinating gene since there is a strong variation in PRDM9 in the number of encoded zinc fingers both within and across species, which points to strong selective pressures. In this study, the terminal zinc finger domain of PRDM9 and its paralog PRDM7 was characterized for the first time in five ruminant species (cattle, yak, mithun, sheep and goat). Extraordinary variation was observed in the number and sequence of ZF domains in the analyzed species. The number of ZF repeats varied from 6 to 12 in different ruminants studied. Ruminant zinc fingers were found to be diversifying under positive selection and concerted evolution, specifically at positions involved in defining their DNA-binding specificity, consistent with the reports in other vertebrates such as mice, humans, equines and chimpanzees. Remarkable diversity in the zinc fingers suggests that PRDM9 may

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Indirect ELISA kit for sheep and goat brucellosis

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India is a rich niche of ovine germplasm, accounts for more than 6.8% and 20% of world's sheep and goat population respectively with diverse cross and indigenous breeds. Brucellosis is an important reproductive disease in sheep and goats, characterized by abortion, stillbirths and reproductive failure with an estimated economic loss of INR 2122 and 1818 per animal in sheep and goats respectively. Early detection and segregation of infected animals from the flock are important to control the transmission disease in animals and to humans.

In the present study, indirect ELISA for the detection of anti-brucella antibodies has been developed using smooth lipopolysaccahride (sLPS) antigen of B. abortus S99. Hyperimmune sera against Brucella sLPS was raised in two brucellosis-negative adult sheep. Pre-immune sea was used as the negative control. The moderate positive control was prepared by diluting strong positive sera with pre-immune sera at 1:500 dilutions. ELISA protocol has been standardized using laboratory standardized antigen extraction protocol and optimization of antigen, blocking buffers, serum and conjugate dilutions. Sera samples showing Percentage Positive values of 54 and above in comparison to control positive were considered brucellosis positive. A total of 374 and 626 sera samples from goats and sheep respectively were subjected to RBPT and indirect ELISA tests. On comparison with RBPT 95.66% and 96.33%, relative sensitivity and specificity respectively were recorded with validation score of 90% to 95%.

Using the developed indirect ELISA kit for sero-diagnosis of brucellosis in sheep and goats, a study on spatial prevalence of brucellosis in small ruminants of the country was undertaken by screening a total of 8904 samples [sheep (n1)-4868, goat (n2)-4036] from

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Polymorphism of prolactin gene and its assoication with egg production performance in Kadaknath hens

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Present study was carried out to study egg production performance and polymorphism of Prolactin gene at 24 bp indel locus at promoter region (PRL24). Egg production performances were recorded as age at first laying (AFE), Body Weight at First Egg (WFE), Mean Egg Weight (MEW) and Total No. of Eggs at 90 days of laying (TEN). DNA was isolated from 2-3 of Blood of 20 birds collected from wing vein. PRL24 locus for indel polymorphism was amplified by PCR and the product was resolved on 6% native PAGE for genotyping. The AFE (d), WFE (Kg), MEW (g) and TEN of Kadaknath hens in the present study were found to be 188.00±0.71, 1.26±0.03, 42.83±0.21 & 37.75±0.59 respectively. The prolactin gene locus PRL24 showed two alleles I & D and three genotypes: II, ID & DD. The frequencies of I and D alleles at this locus were 0.55 & 0.45 respectively. The birds of D allele had a significantly ($P<0.05$) better TEN than birds of I allele.

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Understanding the Circulating Indigenous Strains and Innate Resistance
Potential of Indigenous Livestock - To Enable Better Control Strategies

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India is a rich reservoir of genetic diversity. It is known for its indigenous animal wealth that are being reared in diverse environmental conditions and nutritional status and also exposed to a wide variety of pathogenic insults. Most of the indigenous breeds anecdotally claimed to exhibit better resistance to tropical diseases with supportive scientific evidence. In addition, with respect to some viruses the circulating strains of the pathogens are unique to our country and there is dearth of information on the whole genome sequence of the indigenous strains to gain more understanding on to the relatedness and also on the genotypes that are currently circulating in this country. In this context, I would like to classify our research in the two major areas:

a) Native breeds are more disease resistant

The role of innate resistance: Our research in the past five years had provided sufficient evidence to bring out the importance of our native breed of livestock (especially Kanni breed of goat and the endangered breed of buffalo the Toda) with respect to the contribution of Toll-like receptors (TLR) to innate resistance (their levels and the different types in the different tissues) and the data generated substantiates the need for suitable programes to conserve our native germplasm. Our salient contributions included generating novel information on the 10 different TLR types in goats (Raja et al., 2011), expression profiling of the TLRs in different tissues of goats (Tirumurugaan et. al., 2010), higher levels of TLR expression contributing to increased resistance in Toda buffaloes (Vignesh et. al., 2012; Dhanasekaran et. al., 2013) and the role TLR's in contributing to species specific resistance / susceptibility (Dhanasekaran et. al., 2015)

b) Whole genome sequencing and genotyping of indigenous viruses to generate

information on the circulating genotypes The birds reared in the commercial sector are regularly vaccinated against different diseases and the Newcastle disease virus (NDV) is widely prevalent in many countries with the virus isolated from 241 species of birds. The severity of the disease depends on the species of bird affected and the infecting pathotype (lentogenic or mesogenic or velogenic). Our research in the past five years with respect to this important disease has provided the following salient things which include reporting the

Novel Technologies and Informatics for Improving Livestock Health and Productivity Transcriptome analysis to study host pathogen interactions

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Systems biology, or more specifically network biology, is driven by the gradual realization that a single gene is seldom accountable for a discrete biological function. In response, contemporary biology has evolved a battery of methods to survey the global features of the cells, from DNA, RNA and proteins to small molecules. Transcriptome analysis, enabled by technologies such as oligonucleotide microarray or RNA -sequencing, is a simultaneous interrogation of gene expression by measuring the transcriptional activity on a global scale. Microarray as well as the genome project, were the first forays into the realm of systems biology. The major problem to be addressed of these technologies is the sheer volume of information obtained. Also, the expression level change of a gene may be corollary to change in another gene and may not be the direct cause of the cellular phenotype therefore demanding additional information to place these genes in context. Interactome analysis is a study of interactions between the biological molecules on a global scale. High-throughput mapping of protein interaction allows the global survey of protein interaction of organisms. The resulting maps of proteome-wide protein interactions are called protein networks.Topological features of the protein networks have been demonstrated to reflect the functionality of the interacting genes. Protein network analysis will place the genes identified in microarray/RNA-seq experiments in a broader biological context. Since protein networks reflect the functional grouping of these interacting or coordinately induced/suppressed genes, the roles of the subsets of co-expressed genes may be resolved using the combined data. By integrating functional genomic and proteomic mapping approaches, biological hypotheses can be formulated with increasing levels of confidence. Steady progress has been made to map the molecular interactions between transcription factors (TFs) and the genes that they regulate. Several TF-gene interactions have been identified for human and model organisms, providing a foundation for identifying cell or tissue specific regulatory networks.

Identifying Differentially expressed genes and Functional enrichment: The major goal of microarray or RNA-Seq experiments is to determine which genes are differentially expressed between samples. Oligonucleotide arrays have become popular to study gene expression in a wide range of organisms. Among the most popular platforms, Agilent technology microarray uses 60-mer oligonucleotide probes,and the targets can be labeled with single or dual-color dyes. In addition to the commercially available arrays for model organisms, custom arrays can be printed increasing the flexibility of the system. However, microarray data analysis poses challenges for many biologists. Bioconductor has a collection of R packages written specifically for microarray data analysis and annotation provides excellent functionalities for researchers to analyse their data. However, use of the Bioconductor packages requires the ability to understand and write R scripts. GeneSpring is another software which helps in analysing data to identify differentially expressed genes.

RNA-Seq is a powerful technology for analyzing transcriptomes that is predicted to replace microarrays. Leveraging recent advances in sequencing technology, RNA-Seq experiments produce millions of relatively short reads from the ends of cDNAs derived from fragments of sample RNA. The reads produced can be used for a number of transcriptome analyses, including transcript quantification, differential expression testing, reference-based gene

annotation, and de novo transcript assembly. RNA-seq analysis tools generally fall into three categories: (i) those for read alignment; (ii) those for transcript assembly or genome annotation; and (iii) those for transcript and gene quantification. obtain differentially expressed gene across conditions. TopHat and Cufflinks package takes care of all the three and gives a list of differentially expressed genes across conditions. RSEM (RNA-Seq by Expectation Maximization), is another user-friendly software package forquantifying gene andisoformabundancesfrom single-end or paired-end RNA-Seq data. There are several other approaches for identifying DE genes in an RNA- seq experiment viz. DESeq, edgeR, baySeq, BBSeq , EBSeq etc.

Functional enrichment of the differentially expressed genes in different pathways can be done by using the Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.7) and enrichment of Gene Ontology (GO) terms among differentially expressed genes can be analyzed using g : Profiler.

Interaction network among the differentially expressed genes: The Biological General Repository for Interaction Datasets (BioGRID) is a curated biological database of protein-protein and genetic interactions created in 2003. It provides a comprehensive resource of protein–protein and genetic interactions for all major model organism species. BioGRID currently holds 347966 interactions (170162 genetic, 177804 protein) curated from both high-throughput data sets and individual focused studies derived from over 23 000 publications in the primary literature. In this repository, protein-protein interactions in human are well defined to construct the protein-protein interaction network with the differentially expressed genes.The complete interaction network can be visualized in Cytoscape 3.2.0.

Interactome in goat against PPRV: Peste des petits ruminants (PPR), is an acute transboundary viral disease of economic importance, effecting goats and sheep. Live attenuated vaccine of Sungri 96 is widely used in Northern India against PPR. This vaccine virus, isolated from goat works efficiently both in sheep and goat. The molecular mechanism was investigated through host-vaccine virus interactions by infecting the peripheral blood monocuclear cells (PBMCs) isolated from goat with Sungri 96 vaccine virus. The transcriptome data generated from infected goat PBMCs using Illumina Hiseq-2000 resulted in 120 million high quality 100-base paired-end reads. The expression data generated from RSEM and cufflinks was analyzed using DESeq2, edgeR, and EBSeq; and cuffdiff2, respectively, to identify 4149 DE genes. Initially, a cutoff of 2 fold was taken to select 2000 genes for gene ontology enrichment analysis using DAVID and G-profiler. These genes were extrapolated on the available comprehensive resource of protein–protein and genetic interactions database (BioGRID) and connectivity within them was established. Finally, 163 differentially expressed highly connected genes(DEHC - connectivity ≥5 and fold change ≥3) were represented in a network using Cytoscape 3.0.2. Transcription factors regulating these DEHC genes were identified using MEME and TOMTOM. The gene expression signatures were validated by Real time PCR. On GO enrichment analysis of all the DE genes, a statistically significant number represented - immune signaling pathways. antigen processing and presentation, spliceosome, chemokine and JaK-STAT signaling pathways, MAPkinase cascade, and apoptotic processes. Network analysis revealed that the protein - protein interaction network among differentially expressed genes is significantly disrupted in infected state. Several genes encoding TFs that govern immune regulatory pathways were identified to co-regulate the differentially expressed genes. This study provided insights into the host - PPRV vaccine virus interactome which further indicated that transcriptome analysis could be a novel way to study the HPI.

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Point of Care Nano-diagnostics for Pathogens and Parasites

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Development of the ability to design protein molecules will open the path to the fabrication of devices to complex atomic specifications, thus sidestepping obstacles facing conventional micro-technology. This path will involve construction of molecular machinery able to position reactive groups to atomic precision. It could lead to great advances in computational devices and in the ability to manipulate biological materials. K. Eric Drexler

Molecular Engineering: An approach to the development of General Capabilities for Molecular Manipulation, Proc. Natl .Acad .Sci. USA 1981, 78, 5275

There is class of microorganisms which is potentially very harmful for health of humans and animals and may be the cause of different infectious diseases. Worldwide, infectious diseases account for nearly 40 % of total 50 million estimated deaths annually. There are numerous ways of detection of these microbial pathogens such isolation and identification of culture, ELISA, PCR and biosensors. Of late, biosensors approach for detection of pathogen has got greater attention from researchers and agencies controlling the spread of disease at hospital, field and point of attack. Hence, development of portable, sensitive, rapid, real-time biosensor technology with immediate 'on-the-spot' interpretation of results is well suited for our intended purpose. Biosensors are analytical devices that allow the detection of biomolecules in a label-free, real-time, specific, highly sensitive format. Lateral flow assay(LFA) is another class of detection system for point of care diagnostics and now available for several diseases and markers.

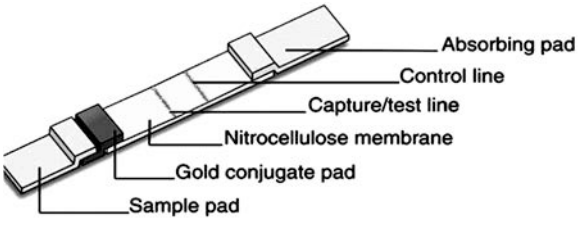

Fig.1: Design of typical lateral flow strip having its various components

This assay can be used for qualitative as well as for quantitative evaluation of analytes and easy visual judging of the antigen–antibody reaction using gold nanoparticle. The lateral flow assay strips were prepared using nitrocellulose membrane, sample pad, conjugate pad and absorption pad for PPR virus detection. There is dearth of literature on rapid diagnostics in relation to parasitic infection, it indicates that a lot of efforts to develop field level rapid diagnostics for parasites of economic importance is the need of hour. IVRI Izatnagar, a premier institute has taken up research for developing a chromatographic strip test for Babesia Gibsoni (Raina, O K Banerjee, P S & Praveen Singh).

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Information technology enabled spatial epidemiology based approach for control and eradication of Hemorrhagic septicemia

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Hemorrhagic septicemia is an economically important disease of cattle and buffaloes. Routine vaccination can successfully prevent the occurrence of the disease. However, desired level of herd immunity is not achieved due to various myths about vaccine in the mind of livestock owners as well as failure of state animal husbandry departments to put an effective vaccination based control program , in place.

Development of early warning systems for HS may help in timely vaccination of the population at risk and thereby prevent the occurrence and spread of the infection. Geographical Information System (GIS) based tool of information technology can be used for spatial epidemiological studies and for development of early warning system For creation of sentinel network for disease surveillance ,GIS tools can be used for identifying critical epidemiological parameters. Disease hot spots may be identified with tools of geostatistics and GIS may also assist in creation of buffer zone around the location of ana outbreak Current understanding of epidemiology of the disease, role of mathematical modeling of disease progression in a sentinel and simulation models for likely spread of the disease are discussed with a view to develop an approach of strategic vaccination against HS.

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Potential applications of single-domain antibodies ('Nanobodies') produced by phage display technology

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Genetically engineered 'functional' antibody fragments are increasingly being produced since 1990s for a range of potential applications in the fields of diagnostics, therapeutics, theranostics and life science research. Nanobodies or single-domain antibodies (dAbs) are the smallest antibody molecules produced by phage display technology and are derived from 'heavy chain immunoglobulin' variable-domain genes of camelids and sharks. Nanobodies against several clinically and biotechnologically important antigens have already been produced and feasibility of diverse applications demonstrated. Conventional ELISA using nanobodies have been developed for detection of microbial antigens, toxins, poisons and venoms. Nanobodies have also proven robust diagnostic reagents for biomarkers of a number of diseases. Small size, solubility and stability are the unique features that make nanobodies suitable for immobilization on microchips for microarrays and use as biosensors. Nanobodies-based biosensors include those for rapid diagnosis of Foot-and-Mouth Disease virus, staphylococcal enterotoxin B, ricin, botulinum toxin A, etc. Nanobody-modified nanoparticles for the selective capture of a single bacterial species, such as Staphylococcus aureus have been developed.

Therapeutic and theranostic applications of nanobodies have been reported for various cancers/tumours with known biomarkers, and antiviral, antitoxin and antivenom therapies. Anti-tumor necrosis factor-α nanobody has shown promising results for treatment of rheumatoid arthritis. A novel approach utilizing a nanobody conjugated to trypanolytic factor has been reported for treatment of trypanosomosis in humans. Several of the nanobody-based drugs are currently in the development pipeline and some have reached phase III of the clinical trials. Unique structural features of nanobodies have allowed development of novel constructs as enzyme catalytic site-inhibitors, thereby making possible the treatment of some metabolic disorders. In addition, virus-resistant and salt-tolerant transgenic plants expressing 'intrabody' (nanobody expressed and effective within cytoplasm) against the target protein antigens have been produced. We have constructed the phage display library of dAbs derived from Indian desert camel and isolated anti-E. coli endotoxin and anti-S. aureus beta-hemolysin dAb clones. Neutralization of Gram-ve endotoxin by nanobody has been demonstrated in the chicken embryo and the mouse models in our laboratory, and further investigations are being done.

Of three categories of reagent antibodies i.e., polyclonal, monoclonal and recombinant sequenced antibodies, currently in use for research, the recombinant antibodies have proven as the most reliable and reproducible. A large group of world-renowned investigators, who met in USA, proposed to phase-out the former two categories during the next decade in order to save money, materials and time. In conclusion, various formats of bio-engineered antibodies with superior features and known sequences will most likely replace several conventional antibodies for their wide applications in diagnosis, treatment, proteomics and life science research in the coming decades. Veterinary medicine and animal health improvement programmes will definitely draw enormous benefits from the developments in recombinant antibody production technologies.

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Transcriptome analysis: Deciphering the Interactome

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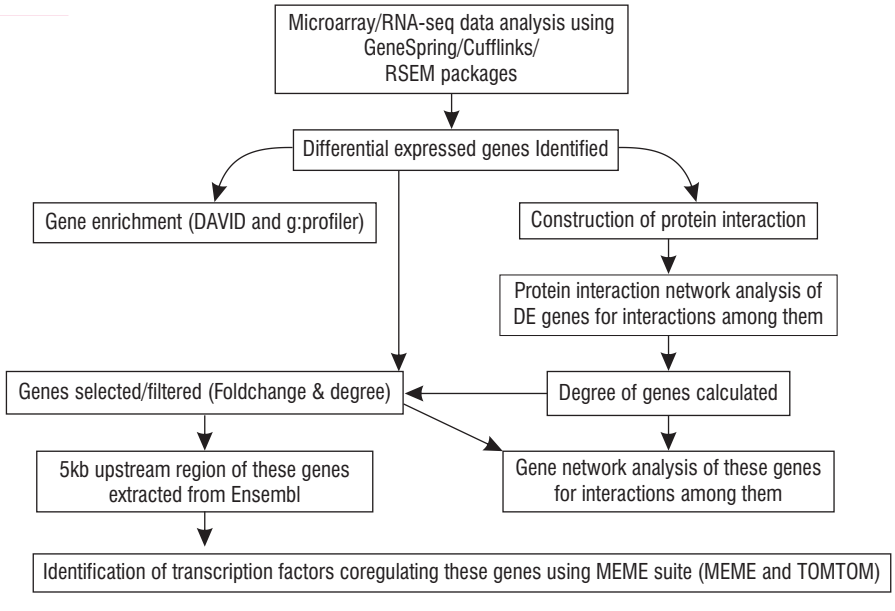
Systems biology, or more specifically network biology, is driven by the gradual realization that a single gene is seldom accountable for a discrete biological function. Transcriptome analysis, enabled by technologies such as oligonucleotide microarray or RNA -sequencing, is a simultaneous interrogation of gene expression by measuring the transcriptional activity on a global scale. The major problem to be addressed of these technologies is the sheer volume of information obtained. Also, the expression level change of a gene may be corollary to change in another gene and may not be the direct cause of the cellular phenotype therefore demanding additional information to place these genes in context. Analysis of genome-wide differential RNA expression provides researchers with greater insights into biological pathways and molecular mechanisms that regulate cell fate, development, and disease progression. These interactions between the biological molecules on a global scale constitute the interactome. High-throughput mapping of protein interactions allows the global survey of protein interactions in organisms resulting in proteome-wide protein interactions called protein networks.Topological features of the protein networks have been demonstrated to reflect the functionality of the interacting genes. Protein network analysis will place the genes identified in microarray/RNA-seq experiments in a broader biological context. Since protein networks reflect the functional grouping of these interacting or coordinately induced/suppressed genes, the roles of the subsets of co-expressed genes may be resolved using the combined data. By integrating functional genomic and proteomic mapping approaches, biological hypotheses can be formulated with increasing levels of confidence. Steady progress has been made to also map the molecular interactions between transcription factors (TFs) and the genes that they regulate. Several TF-gene interactions have been identified for human and model organisms, providing a foundation for identifying cell or tissue specific regulatory networks.



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Nano-immunobiologicals: associated interference with standard cytotoxicity tests

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A range of immunobiologicals are being produced ranging from more complex such as proteins, polysaccharides to relatively simple recombinant peptides. Many of these products are becoming compositionally more subunit so as to make them safer, less reactogenic and less toxic. However, these minimalist preparations are ending up as less effective. In case of vaccine candidates, they are proving to be less immunogenic. Whether a complex biological-origin preparation (having a potential to be more reactogenic) or a minimalist preparation (with less immunogenicity), nanotechnology has shown a broader promise. The use of nanoparticles to capture, conjugate, and deliver immunobiological preparations has been gaining momentum. To ascertain the safety of biologicals, mandatory assays are required before biologicals could enter advance stage of testing in a target host. However, interactions of nanoparticles with biologicals could alter their properties and interfere with interpretations of in vivo, ex vivo & in vitro. This presentation would focus on associated interference of nanoimmunobiologicals in these assays and approaches to tackle them.

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Identification of novel splice variants of meiotic recombinase DMC1 gene in ruminants

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Disrupted meiotic cDNA1 (DMC1) recombinase plays a pivotal role in homology search and strand exchange reactions during meiotic homologous recombination. In the present study, full length coding sequence of DMC1 gene was sequence characterized for the first time from testicular tissue of four ruminant species (cattle, buffalo, sheep and goat) and phylogenetic relationship of ruminant DMC1 with other eukaryotes was analyzed. DMC1 gene was found to encode a putative protein of 340 amino acids in cattle, sheep and buffalo and 341 amino acids in goat. Comparative analysis of cDNA sequence of four ruminant orthologs revealed limited sequence divergence at both nucleotide and amino acid level indicating a high degree of evolutionary conservation. Functional feature prediction by SMART and PDBsum revealed significant identity of cattle, buffalo, sheep and goat DMC1 protein with humans as well as other animal species. DMC1 protein in ruminants has 2 domains: Domain I which contains DNA binding HhH motif and domain II which has ATPase domain. In cattle and sheep, novel alternatively spliced mRNAs with skipping of exons 7 and 8 were isolated in addition to the full length transcript. Novel transcript variants with partial skipping of exon 7 and complete skipping of exon 8 were found in sheep and goat. These deletions lie in the domain II region of DMC1 protein. However, HhH motif and C-terminal region of DMC1 protein were found to be conserved in all transcript variants of DMC1. The presence of these variants was validated by amplifying cDNA isolated from testis tissue of ruminants using two oligonucleotides flanking the deleted region. It can therefore be speculated that these novel splice variants represent proteins with diverse biological functions or with altered functional activities.

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Patinformatics for Technological Competence in the Area of Proteo-Genomics for improving Livestock Health and Productivity

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Patents are granted to provide monopoly to a researcher in lieu of access of information to other researchers. Since patenting is a sign of state of progress of knowledge, strategic mining of information for the field of proteo-genomics was observed by retrieving information for patent databases namely Espace, USPTO http://us.mg2.mail.yahoo.com/dc/blank.html?bn= 397.8&.intl=in&.lang=en-US - _ftn5 and WIPO maintained Patent Database. One of the offshoots of patent system is open access of technological information available. Proteo-genomics is one of the evolving fields where having a competitive edge gives researcher cud to chew, and build upon existing , state of art technology, which otherwise would take years to diffuse to reach the laboratory. An initiative was taken to analyse patents related to proteo-genomics for existing technological advances having potential to be used for livestock health and productivity. Novel and inventive information is available in patent documents that were strategically mined using International Patent Classification for retrieving and refining information having application in livestock sector. The present paper deals with the information search related to patents by using patent scope in the field of proteo-genomics. The patent information shall be discussed that could be used to enhance technological competence by creatively working around a technology for identifying and analysing peptides for ultimate diagnosis and treatment. The paper also discusses ecosystem of innovation that can be harnessed to increase production by licensing-in technologies created by innovators and also by licensing-out technologies that have been created by researchers in public and private funded institutes. The paper addresses the gaps and innovation clusters/hotspots for strategically developing a line of R&D to address livestock health and productivity.

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Differentiation of capripoxviruses at species level by a newly developed PCR assay

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Sheeppox and goatpox are economically important diseases of sheep and goats, caused by genus Capripoxvirus of the family Poxviridae. The SPPV and GTPV show high antigenic similarity with each other and therefore the serological assays are not useful to differentiate them at species level. It has been reported that at least some strains of SPPV and GTPV can infect both sheep and goats. Recently, molecular based assays have been employed to differentiate SPPV and GTPV at species level. In the present study, one of the ORFs in the terminal genomic region has been targeted for differentiating SPPV and GTPV at species level. A total of six known positive capripoxviruses i.e. three each of GTPV and SPPV strains were screened with a newly developed conventional PCR assay. The PCR assay employed in the present study could be able to differentiate SPPV and GTPV, GTPV strains yielded amplicon size of 182 bp whereas SPPV yielded 206 bp in size. The assay could be able to detect up to 1000 copies of the viral DNA. Further, the assay was found to be specific for capripoxvirus only. The conventional PCR assay developed in the present study could be useful for the detection and differentiation of capripox as well as to study molecular epidemiology of sheeppox and goatpox.

IgY antibodies - Potentials for research diagnostics and human nutraceuticals

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Just as immune protection is transferred in utero in mammals or passively by a lactating mother via colostrum, hens passively transfer protection to their young by secreting immunoglobulin and other immune factors into its egg for use by the hatching chick.Since the discovery of egg yolk antibodies, now called immunoglobulin Y (IgY), this has been harnessed to produce antigen-specific yolk antibodies for numerous applications in the medical and research fields. The appeal of sensitive and specific diagnostics is always in demand, IgY antibodies comes with some of answers to key questions which might have impact to basic research. Most mammalian proteins exhibit enhanced immunogenicity in chickens than in mammals due to phylogenetic distance and thus raise antibodies of higher avidity. This also makes production of antibodies against conserved mammalian proteins more successful in chicken than in mammals. In addition, comparison to mammalian IgG, chicken IgY has less cross reactivity with mammalian proteins hence provide better specificity.

One of the most valuable and promising areas of IgY research is for passive immunization to treat and prevent human and animal diseases. Passive immunity is the transfer of active humoral immunity in the form of ready-made antibodies from one individual to another. Being an ingredient in our regular diet, poultry eggs are considered generally safe. The production of large amounts of IgY in a cost-effective manner is key to its successful use for passive immunization. The alarming increase of resistant microbes (bacteria, fungi, protozoa) is today one of the biggest threats to both mankind and environment. The frequency of antibiotic resistance organisms has been on the rise at an alarming rate against a backdrop of decreasing numbers of new antibiotics being developed and added to the market. Simple and effective natural remedies of which IgY comprises the most potent and easily generated substitute to antibiotics. Passive immunotherapy by antigen-specific IgY acquires a special value as a tool for infection control and immunologic research with global commercial application for applications in numerous medical and research fields. Recently, successful progresses have been achieved through industrialization of IgY technology. IgY has been shown to provide a safer, more efficient and less expensive method for managing disease-causing pathogens.

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Opportunities for improved serodiagnosis of Paratuberculosis in animals

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Paratuberculosis or Johne's disease is a chronic, infectious, granulomatous enteropathy caused in domestic and wild ruminants by Mycobacterium avium subsp. paratuberculosis (MAP). This organism has also been reported to be associated with cases of Crohn's disease, a chronic multifactorial inflammatory bowel disease in humans. Johne's disease is widespread throughout the world including India and causes huge economic losses to the livestock industry.

The lack of a reliable, sensitive, specific and rapid test for the detection of Mycobacterium avium ssp. paratuberculosis (MAP) is a major barrier impeding the effective control of this economically devastating disease in animals. The diagnosis of MAP is difficult because of the organism's fastidious slow growth in vitro and the lack of a specific diagnostic test that is sensitive enough to detect most subclinical yet infected cattle that are intermittently shedding organisms.

The presently used diagnostic tests such fecal culture and PCR are incapable of detecting subclinical MAP infections. Poor specificity of intradermal skin test and in vitro cell mediated immunity based assays has limited usefulness because of antigenic cross reactivity with other mycobacteria. The most commonly used diagnostic test for MAP is ELISA, which is used to detect the 30% in subclinically infected animals because promising immunodominant antigens are lacking. Hence studies are warranted to search for novel antigens for the development of easy to perform robust serological tests.

Comparative genomics and PCR based analysis of the complete genome sequences of M. a. paratuberculosis revealed several putative coding sequences. Efforts have already been made in our laboratory identify and select several MAP specific ORF's. The gene from each coding sequences (eliciting cell mediated and humoral immune responses) were cloned and expressed in E. coli. The immunoreactivity of the in expressed proteins have been determined by western blot analysis. The cell mediated immune response were studied by Delayed type hypersensitivity (DTH) using T cell specific recombinant antigens.

The antibody response to various recombinant proteins was carried out by ELISA. However, no single antigen is consistently a target for an antibody response with high sensitivity Therefore, the generations of multiantigenic proteins as novel antigens from various epitopic regions have also been explored for developing ELISA based assay with higher sensitivity jeopardizing the specificity.

Despite of development made so for using the serodiagnostic assays, the efficient lab-free diagnostic devices for the diagnosis of paratuberculosis is not yet available.

Therefore, in the present talk the currently available and recently developed diagnostic methods along with the contribution made in our laboratory highlighting the potential benefits of lab and lab-free methods for the diagnosis of paratuberculosis will be discussed.

Biomolecules infused nanomaterials in making rapid affordable diagnosis

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Nanotechnology that deals with materials having single unit of 1-100 nm dimension, presents a great opportunity to develop rapid affordable diagnosis. Considering the novel characteristics of nanomaterials from gold nanoparticles and their plasmonic shifts to iron oxide nanoparticles and changes in magnetic properties which can be varied with infusion of biomolecules/or bimolecular interaction at the interface of these nano-sized materials have helped in the detection of pathogens,toxins,antigens and nucleic acids with impressive detection threshold.

Despite advancements made in pathogen identification in past, some of the gold standard diagnostic methods suffer limitations associated with laborious sample preparation, bulky instrumentation and slow data readouts. New rapid affordable field deployable diagnostic methodologies are urgently required in point of care applications. Biomolecules such as antigen, antibodies, nucleic acids, oligonucleotides and peptide nucleic acids (PNA) can be infused in nanomaterials either by simple adsorption or by chemical conjugation to develop novel nanodiagnostic reagents that may facilitate pathogen detection even in remote rural areas. Application of biomolecule infused nanomaterials or carrying out bimolecular interactions on nanomaterial interface provided different biosensing platforms. Gold nanoparticles which undergo plasmonic visual colour changes when infused with specific molecules could provide visual sensors applicable in different formats for point of care diagnosis. These visual sensing systems have been used not only for successful detection but also for the quantification of pathogens and their genes.

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3AB3 recombinant protein based DIVA ELISA for screening of anti-non structural protein antibodies against FMD virus in Uttar Pradesh

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The recombinant r3AB3 based NSP-ELISA developed and standardized by the Project Directorate on FMD, Mukteswar, Uttarakhand was used in the present study to detect the antibodies against 3AB3 NSP of foot-and-mouth disease virus (FMDV) in the bovine serum samples collected from different districts of Uttar Pradesh. A total of 7580 bovine serum samples comprising of 3628 samples from buffalo and 3952 samples from cattle received as pre-vaccinated serum samples from 56 districts of Uttar Pradesh under 16th Phase of vaccination in FMD control programme (FMD-CP) from State Animal Husbandry department were tested. Out of the 7580 serum samples, 1868 (24.64%) serum samples were found positive for anti-3AB3 NSP antibodies. 25.18% cattle had r3AB3 specific antibodies as compared to 23.40 % buffaloes. For ease of interpretation entire state was divided into four different regions viz. Central, Eastern, Western and Bundelkhand region. The region wise analysis showed significantly high level of anti NSP-3AB3 antibody in Bundelkhand region (63.7%) followed by Central (40.4%) and Western (17.5%) Uttar Pradesh, suggesting high virus activity in the Bundelkhand region of the state bordering Madhya Pradesh. Eastern Uttar Pradesh recorded lowest anti NSP-3AB3 reactivity (8.3%). The study emphasizes the importance of r3AB3 based NSP-ELISA for FMD outbreak prediction with continuous surveillance and preventive measures in areas of high virus activity.

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A study on comparison of various diagnostic techniques for diagnosis of rabies in and around Bangalore

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The present study was carried out with an objective of comparative evaluation of different laboratory techniques for the diagnosis of Rabies, in rabies suspected brain tissue samples received at the institute, from in and around Bangalore. In the present study twelve rabies suspected brain tissue samples were subjected for Seller's staining technique, Fluorescent Antibody Test (FAT) and Reverse transcription polymerase chain reaction (RT-PCR) techniques. Sensitivity and specificity was calculated considering RT PCR as the reference assay. Seven out of twelve samples, nine out of twelve samples and ten out of twelve samples were positive for rabies by Seller's staining technique, FAT and RT PCR respectively. The percent positivity for the same were 58.33, 75 and 83.33. Sensitivity of Seller's staining technique and FAT were 76.92 and 90.90 per cent respectively. Specificity was 100 per cent in both the tests. Results of the study revealed that all the three techniques could be used for diagnosis of rabies but based on the comparative evaluation it was concluded that RT PCR to be the best among the three techniques used in the study, followed by FAT and Seller's staining technique. Hence RT PCR could be considered as a candid technique for diagnosis of rabies.

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Comparative evaluation of serological assays for diagnosis of Japanese encephalitis in swine

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Japanese encephalitis (JE) is a re-emerging mosquito borne flaviviral disease leading to thousands of deaths among children every year in our country. Swine, being an amplifier host, play an important role in epidemiology of JE. Therefore, detection of JE infection in swine is of immense importance for forecasting disease outbreak in humans with a view to devise timely public health interventions. The present study was undertaken with the aim to compare serological assays for diagnosis of Japanese encephalitis in swine. A total of 131 swine serum samples were collected from endemic regions of JE and screened by virus neutralization test (VNT) and in-house whole virus antigen based indirect IgG ELISA, while 102 of these serum samples were screened by haemagglutination inhibition (HI) test. Out of 131 swine serum samples screened by VNT, 58 samples were found to be positive and 73 were negative for JE antibodies, whereas 57 and 74 samples were found to be positive and negative, respectively by in-house indirect IgG ELISA. Further, screening of 102 swine serum samples by HI test, revealed 22 positive and 80 negative samples for JE. The OIE has pronounced VNT as a gold standard for detection of JE antibodies in swine serum and the same was used in the present study to calculate diagnostic efficacy of other serological assays. The diagnostic sensitivity of in-house ELISA was found to be 98.27 per cent as compare to 42 per cent of HI test. The diagnostic specificity of in-house ELISA and HI test was found to be 100 per cent and 98 per cent, respectively. It is concluded from the present study that whole virus antigen based ELISA can be a good alternative to Virus neutralization test due to its characteristics like ease of performing, less cumbersome procedure and shorter turnaround time.

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Seroprevalence studies on animal Leptospirosis in South Andaman

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Since the first report of Leptospirosis in Andaman and Nicobar island, this island is endemic for human Leptospirosis. As the animals are source of infection, continuous serosurveillance of animal Leptospirosis is emphasized. To know the seroprevalence of animal Leptospirosis 308 cattle serum samples and 121 goat serum samples from different parts of south Andaman. The serum samples screened for Leptospiral antibodies by Microscopic Agglutination test. The seropositivity in cattle was ( ) and goat ( ). The prevalent serogroup noticed in cattle were And goat were. The pattern of prevalence of serogroup is changed from the previous report of serogroups as prevalent.

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An outbreak of bovine popular stomatitis virus infection in buffalo calves

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Bovine papular stomatitis virus (BPSV) is a parapoxvirus associated with papular and erosive lesions on the muzzle, lips, and oral mucosa of cattle. The present report describes an outbreak of BPSV infection affecting buffalo calves at an organized buffalo farm in Hisar District of Haryana. The clinical signs included painful reddish papules, ulcers, and scabby proliferative lesions on ear pinna, head, lips and oral mucosa with a clinical course of 5-15 days. Within 10 days of the 1st observation, a total of 11 buffalo calves were affected out of 55 calves in a herd. Two (2) calves which were severely affected running with high fever, off feed and had erosion/small vesicles on the dental pad, gums, and tongue, and eventually died within 3 days.

Histopathologically acanthosis, spongiosis, and parakeratotic hyperkeratosis with adjacent focally extensive ulcers and mild inflammatory infiltrate were observed in the epidermis. Eosinophilic inclusion bodies were also observed in the cytoplasm of epithelial cells. Staphylococcus and streptococcus spp. were isolated from tongue and lung tissues of dead calves which confirmed the secondary bacterial infection. A polymerase chain reaction using a set of pan-parapoxvirus primers for the B2L gene performed on DNA extracted from scabs amplified a 594 bp product, which when sequenced, revealed similarities of 84%, and 83% with, Orf virus and Pseudocowpox virus respectively. A phylogenetic tree based on the B2L sequence was constructed, showing that the virus clustered with BPSV isolates. To the best of our knowledge this is the first report of BPSV infection in buffalo in India.

Porcine circovirus type 2 predisposes swine to infection with classical swine fever virus

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Porcine circovirus type 2 (PCV2) is a devastating pathogen of swine recorded worldwide. PCV2 is a non enveloped DNA genome virus with a diameter of ~ 18nm and genome size of 1.7 kb. The virus was first described in 1998 in farmed pigs in North America and now recorded worldwide. PCV2 associated diseases (PCV-AD), with a wide spectrum of clinical diseases, cause a significant impact on the economics of swine production. PCV2 infection and lesions are observed in multiple organs and the common manifestations are post weaning multi systemic wasting syndrome, respiratory disease, enteric disease and reproductive failure. The reproductive failure due to PCV2 infection is ascribed to the infection of embryos, fetuses and piglets, rather than the maternal tissue. PCV2 has a predilection for cardiomyocytes of the fetus. PCV2 infection causes depletion of B lymphocytes and CD4+ lymphocytes and causes characteristic pathognomonic lesions in the lymphoid organs, with lymphoid depletion and histiocytic replacement in follicular areas and histiocytic infiltration of parafollicular areas. Granulomatous inflammation and multinucleate giant cells in lymphoid organs are also observed often. Necrotizing lymphadenitis is also observed PCV2 infections. Lymphocyte depletion and lymphoid organ destruction by PCV2 causes immunosuppression. High levels of PCV2 viremia are correlated with damage to the lymphoid system and failure of the pig to develop antibodies against PCV2 and probably other infections and vaccines. In field conditions PCV2 infections are mostly found with co-infections. In the Indian subcontinent Classical swine fever virus (CSFV) and Foot and Mouth Disease Virus (FMDV) are recorded as the major viral pathogens of swine. In recent years PCV2 infection has been recorded in different parts of India. Interestingly, PCV2 infections recorded in India are mostly in cases of reproductive failure or neonatal mortality. However, in other parts of the globe, reproductive failure is only a part of the entire gamut of PCVAD recorded. The sub-tropical climate, lack of high-density pig farming and other unknown factors in Indian scenario could have influenced the lack of other manifestations of PCV2 infection such as post weaning wasting, respiratory and enteric diseases. An organized serological and PCR detection based survey of PCV2 infection along with sero-prevalence analysis of CSFV and Brucella Spp infections, the two other common etiologies for reproductive failure in swine, was carried out in swine farms in Tamil Nadu. In our observations, of the 438 samples screened 76% of the pigs were sero-positive for PCV2 and 61% were positive for PCV2 viremia. 82% of the PCV2 viremia positive pigs were also sero-positive for non-structural protein of CSFV. Whereas only 29% of PCV2 viremia negative pigs were sero-positivite for non-structural protein of CSFV. The data indicates that PCV2 infection predisposes the swine population to CSFV infection.

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Peste des petits ruminants virus and foot-and-mouth disease virus co-infection in goats: Long-term in vitro co-persistence of two acute pathogenic viruses

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A severe outbreak with a mortality rate of 51.63% in goats, clinical signs of which mimic with both peste des petits ruminants (PPR) and foot-and-mouth disease (FMD) was reported at a farmer's goat herd at Shahjadpur, Mathura, India. PPR virus (PPRV) and FMD virus (FMDV)-specific gene segments were amplified from clinical specimens by PCR. For virus isolation, African green monkey kidney (Vero) and baby hamster kidney (BHK-21) cells were co-cultured and infected with a single clinical specimen where cytopathic effect (CPE) was evident on 6th blind passage (BP). PPRV was plaque purified in Vero cells but no FMDV-specific plaques could be recovered both in Vero and BHK-21 cells. Anti-PPRV serum-treatment in the virus mixture resulted in entities (defective particles) that rapidly produced CPE but FMDV genome was undetectable. Our attempt complementing purified PPRV to defective virus particles-infected cells could not recover complete FMDV particles. Purification of FMDV from the virus mixture by transfecting viral RNA in cell culture is underway. To best of our knowledge, this is the first documented evidence that describes a naturally occurring mixed virus infection of FMDV and PPRV in goats as well as the ability of these two acute pathogenic viruses to infect and coexist in the cell culture system on long term passage.

Frequency distribution of picobirnavirus in diarrheic and non-diarrheic bovine calves

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Picobirnavirus (PBV) is a small, non-enveloped, bisegmentedvirus with dsRNA genome.It has been detected in both humans and animal neonates and causes enteric illness in animals. It is highly adaptable because of its wide host range and genetic diversity.It gives two segments in PAGE on silver staining. Based on analysis of animal strainsPBV has been classified into two genogroup as Genogroup I and Genogroup II.The biological outline of PBV transmission in environment is still mostly unfamiliar.During the study, a total of 240 fecal samples(diarrheic& non-diarrheic) collected from bovine calves (cattle calf & buffalo calf) during February 2015 to August 2015 from organized and unorganized farms (Uttarakhand & Uttar Pradesh) were tested for the presence of PBV using RNA-PAGE and reverse transcription-PCR(RT-PCR).Among the 240 fecal samples tested by RT-PCR targeting RNA-dependent RNA polymerase gene,43.75% (105/240) were found positive for PBV. In Genogrouping studies, 48.57% (51/105) samples belonged to GG-I and 25.7% (27/105) were identified as GG-II,and 26.66% (28/105) remained untypable.The occurrence of infection was found elevated duringrainy season (54.54%, 18/33) followed by summer (45.9%, 28/61samples)and winter season (39.7%, 18/33), respectively. Additionally 16.1%(17/105) samples showed concurrent infection of GG-1 and GG-II in bovine population.Interestingly, different isolates showed noteworthy sequence diversity.The result of this study confirmed the upward tendency of PBV in bovine population, thus in future requiring vaccine to manage PBV infectionsand to analyze the public health aspects of PBV infectivity, particularly its likely association with zoonosis.

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An outbreak of PPR in goats at Nilagiris hills of Tamil Nadu

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Peste des petits ruminants or goat plague is an economically important disease prevalent in India and the disease is under control due to extensive vaccination programme. Here we report an outbreak of the disease among goats in and around Ooty of the Nilagiris district in Tamil Nadu. The town Ooty extends over an area of 2545.4sq.km on the Nilagiris hills and the highest peak measures 2892 m above mean sea level, the mean temperature ranges from a minimum of 6.5ºC to a maximum of 20.9ºC. Mortality up to 35.71% was recorded in 11 different villages. The disease was diagnosed based on clinical signs, post mortem lesions and detection of PPRV by RT-PCR test. Goats were brought from two different markets of neighbouring districts and distributed among the local people. Vaccination history was not available. Within a week time of their purchase many animals were sick and slowly the disease spread over and killed 96 animals in a population of 345 animals. The disease was controlled by vaccination of normal goats (without clinical signs) and implementation of biosecurity measures. Few sick animals recovered by providing supportive therapy. This study highlights an outbreak of PPR in a hill station and measures taken to control the disease.

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Genotypic linkages of VP6 gene of human rotavirus isolates circulating in pediatric patients with acute gastroenteritis in Haryana indicated close proximity of antigenic epitopes with bovine strains

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The major capsid protein, VP6 specify the sub-group specificity of Rotavirus and high titre of antibody are generated against VP6 gene. A reverse transcription PCR was standardized to amply the complete open reading frame (1194 bp) of human group A rotavirus VP6 gene. The Viral protein 6 (VP6), encoded by 6th segment of genomic double stranded RNA, is the main target for rotavirus detection both by serological methods and molecular techniques. The genome segment 6 of local Rotavirus isolates from Haryana region were partially sequenced to analyze the variations within circulating rotaviruses and compared with vaccine strains. The VP6 protein in this study exhibited significant amount of genetic variations compared to Rotarix and 116E strain. The fact that the I2 genotype Haryana strains were more conserved with RotaTeq vaccine strains as compared to other vaccine strains available. Rotarix vaccine has been introduced in India and indigenous 116E will be available commercially very soon. Role of antibodies against VP6 is not confirmed although some workers have reported protective role of anti-VP6 antibodies. High percentage of amino acid substitution at antigenic region in circulating strains may affect the efficacy of these vaccines. Haryana strains share high amino acid identity with bovine strains of Indian origin as compared to other Indian human strains. This may suggest that reassortant process have taken place due to close proximity of human and bovine in socioeconomic fabric of Haryana. The VP6 genes from these circulating strains may be gradually evolving at different rates compared to other rotavirus gene segments, due to accumulation of antigenic changes caused by point mutations and reassortments. This was clearly shown by clustering of Haryana human strains with bovine strains of Indian origin.

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Exploration of cultural bacterial biodiversity and discovery of unusual animal pathogens

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The biodiversity of a small subset of bacteria of domestic animals with disease causing potential includes pathogens, commensals and opportunistic pathogens. There are also the reports of emerging and re-emerging pathogens which necessitates monitoring and surveillance of such organisms along with their metadata collection. In order to document and study such microbiota, their ex-situ conservation is a mandated objective of Veterinary Type Cultures Collection, which is also important to explore and utilize the genetic diversity they represent. This genetic diversity presents us with an opportunity to understand their application in veterinary medicine and public health. The isolation, characterization, and conservation of microorganisms from animals help us understand their molecular epidemiology and transmission dynamics and as resources for development of vaccines and diagnostics. VTCC has embarked upon exploration of such microbial diversity and their conservation and characterization, during which we have identified unusual or previously unreported animal pathogens. These microbes have been isolated from various diseased animal organs and samples from horse, poultry, buffalo, mithun and sheep. They have been characterized by phenotypic and genotypic methods and 16S rRNA gene sequence and phylogenetic studies. The identification is based on complete 16S rRNA sequence matched with cured ribosomal database.

Among the unusual hitherto unreported pathogens, significant isolates include Actinobacillus equuli, Bordetella bronchiseptica, Nocardia otitidiscaviarum, Escherichia fergusonii and Escherichia hermanii from horse, Mannheimia varigena, Trueperella pyogenes, Shigella sonnei and S. flexneri from buffalo, Moraxella ovis from sheep, Escherichia marmotae and Escherichia fergusonii from mithun and, and Lactococus garviae from poultry. In Gram-positive category, Streptococcus lutetiensis from horse and Streptococcus infantarius ssp. coli isolation from buffalo are reported.

Detection and identification of these unusual pathogens adds to new information on infectious disease epidemiology of domestic animals in India. The isolation and conservation of these isolates with their geographical location and metadata opens up new opportunities in molecular epidemiology, and genetic biodiversity studies. As microbes are in a continuous state of evolution, so availability of such novel authenticated strains can help us understand the genetic basis of virulence by comparative genomics and proteomic studies. In order to fight against the veterinary

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Advances in genomics and immunodiagnostics in fish disease diagnosis

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Frequent outbreaks of infectious diseases have been recognized as a major constraint to fish culture affecting both the economic development of fishers in many countries in the world. In India, increase in aquaculture production particularly in expansion into intensive and semi-intensive methods of production has been coupled by increased occurrence diseases in fish and shellfish resulting from high stocking densities and stress conditions that favours the occurrence and spread of infectious diseases. Successful aquatic animal health management relies on the accurate and rapid diagnosis of various diseases. The effective control and treatment of diseases of aquatic animals requires access to diagnostic tests that are rapid, reliable and highly sensitive. Direct culture of pathogens is also widely used. However, these methods are time-consuming, tedious costly and sometimes require live medium and, for shrimp and other crustaceans, cell lines suitable for virus culture have not been available. New assays from genetic engineering using nucleic acid based techniques has come boon to rapid and efficient diagnosis of pathogen in human and animal health and the same techniques have implications in the aquaculture industry. Techniques such as Polymerase Chain Reaction (PCR) can detect various bacterial, viral and fungal pathogens in the organism even in the sub-clinical stages of infection, which are otherwise difficult to detect. Diagnostic kits based DNA probes, are also effective in detecting the presence of nucleic acid sequences of pathogens from infected tissues. The techniques have been applied to detect different fish pathogens like Aeromonas hydrophila 7 other Aeromonas species, Pseudomonas species, Edwardsiella spp., Streptococci, Flavobacterium etc. and viralpathogens like Fish Noda virus, Koi Herpes virus, Spring viraemia of carp, Prawn noda virus (MrNV), White spot syndrome virus, Yellow Head virus, etc. These techniques have also been used for differentiation and characterization of many fish pathogens. Again, Immunological assays, including fluorescent antibody techniques (FAT) and enzyme linked immunosorbent assays (ELISA) using both polyclonal and monoclonal antibodies against WSSV and other pathogens are also presently available the diagnosis. These immunoassays selected for the identification of pathogens depends on a variety of factors since each method has its merits and disadvantages. Although such methods are useful, their sensitivity thresholds limit its use in environmental samples, especially where pathogen levels are extremely low. These techniques have revolutionized diagnostic procedures. Recent advances in immunologicaland DNA based techniques in fish disease diagnosis and characterization of pathogens assays have been discussed in the present paper in brief.

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Recent advances in diagnosis of Trypanosomosis in animals using immunological and molecular approaches

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Rapid and accurate diagnosis of trypanosomosis is extremely important for both in identifying animals for treatment as well as tracking the prevalence of disease. Under field conditions, the disease is diagnosed on the basis of clinical signs which are not sufficiently pathognomonic and using conventional parasitological diagnostic methods viz., microscopic examination of wet blood film, stained thick/thin smears, haematocrit centrifugation technique, dark-ground/phase-contrast buffy coat technique, mini-anion exchange centrifugation technique, lymph node biopsies, examination of joint fluid / cerebro-spinal fluid (in the case of nervous signs) supplemented with haematological, biochemical tests etc. These methods are less sensitive and subclinical form of disease often remained undiagnosed because of the periodically cryptic nature of the parasitaemia. Therefore, these methods are not adequate to know the epidemiology and magnitude of the disease in a particular region/ country. Attention has recently been focused on the development of more sensitive and specific serological, DNA based and molecular tests for the diagnosis of surra in animals. Sensitive serological methods include card agglutination test (CATT), latex agglutination test (LAT), ELISA, dot-ELISA, western blotting, IFAT etc. have been developed for detection of specific antibodies/antigens. Presently, for sero-prevalence studies of trypanosomosis whole cell lysate (WCL) antigen based ELISA is being used and the assay is also recommended by OIE. But, for preparation WCL antigen laboratory animals are required for propagation of sufficient number of the trypanosomes and procedures have also not been completely standardised. Therefore, uniformity of the assay is still the main limitation.

Various immunological and molecular approaches such as recombinant antigens, DNA based detection assays (PCR/nested PCR/multiplex PCR/qPCR/LAMP-PCR/DNA probes), nanobodies, RNA derived aptamers, synthetic peptide technology, biomarkers based assays, etc. have been reported with considerable degree of sensitivity and specificity for diagnosis of trypanosomosis. Recently, many recombinant antigens (Rotat 1.2, invariant trypanosome surface protein 75, tandem repeat proteins, HSP70, flagellar protein) based assays have been developed for diagnosis of trypanosomosis. The gold standard, to date, for detection of the Trypanozoon subgenus are the TBR primers based PCR test. Further, qPCR assay has added many advantages over conventional PCR methods, viz., simultaneous amplification and detection during exponential amplification, useful for monitoring the load of infection, lower carry over contamination due to closed tube operation, increased sensitivity due to fluorescent chemistry, high throughput analysis. The recently developed LAMP-PCR technique further simplified molecular detection of T. evansi with high specificity, efficiency, rapidity and capability to amplify billions of copies in less than an hour under isothermal conditions without need of sophisticated equipment.

Several poly-reactive anti-trypanosomal nanobodies have been generated in recent years using lymphocytes of T. evansi infected camel. It is expected that in future nanobodies will be generated that create access to unique locations,

such as crossing of the blood-brain barrier or and penetrate cell membrane. Thus nanobodies may prove useful tools for detection of T. evansi infection and specific anti-parasite drug targeting in near future. Aptamers are single stranded nucleic acid probes that can be selected from a large random library. They are analogues to antibodies in their mode of action and can be coupled to biotin or gold particles for development of lateral flow assay. RNA derived aptamers are generally high specific, thermostable and easy to use. The anti-trypanosome VSG-specific aptamers have been developed and showed affinities in sub nanomolar range, binding to structurally conserved epitopes of VSG. In recent years, phage display technique has been used as a powerful tool to identify mimotopes, small peptides that mimic linear, discontinuous and/or non-protein epitopes. The identification of mimotopes for epitopes of Trypanosoma may replace the native VSG proteins in antibody detection tests for trypanosomosis. The R&D activities are underway in many laboratories to search potential host-biomarkers using proteomic approach for development of sensitive and specific assays for diagnosis of animal trypanosomosis and there is hope for availability of uniform, sensitive and specific diagnostic assays using potent molecular platforms which may be used with ease at field level by different stakeholders in near future.

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Mixed infection of avian leukosis virus (ALV) and Marek's disease virus (MDV) among dead commercial chickens

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Avian leukosis virus (ALV) infection is a major cause of economic losses in the poultry industry by increased neoplastic and non-neoplastic mortalities, and significant production losses due to subclinical infections. In this study, we report prevalence of mixed infections of ALV subgroup A (ALV-A), and Marek's disease virus (MDV) in dead chickens of broiler, layer and other indigenous breeds, received from Central Avian Research Institute (CARI), Izatnagar, in the Post-mortem Hall, Indian Veterinary Research Institute (IVRI), Izatnagar, during mid-September 2013 till January 2014.

On systematic post-mortem examination, adult dead chickens, >14-week-old (n= 1,435), showed 58 (4.04 %) liver tumors with gross lesions of diffuse enlargements, or focal nodular growths of varying sizes with gray to creamy-white discoloration either in liver and spleen both (37/58, 63.79%); or liver alone (8/58, 13.79 %); besides 13/58 (22.41 %) nodular samples in liver and spleen both showing extensive autolytic/ degenerative changes. Younger dead chickens, <14-week-old (n= 1,536), did not show obvious gross lesions in any visceral organs including bursa of Fabricius.

Haematoxylin and eosin (H&E) stained tissue sections (45 nos.) of gray to creamy-white tumor foci revealed that 6/45 (13.33 %) gross lesions in liver and spleen both, and 3/45 (6.67 %) gross lesions in liver alone, indicated massive areas of uniform-sized lymphoblasts characteristic of B lymphoma of avian lymphoid leukosis (LL); and 7/45 (15.55 %) gross lesions in liver and spleen both, and 0/45 (0.0 %) gross lesions in liver alone, indicated generalized areas of multiple pleomorphic lymphoblasts including small to medium lymphocytes characteristic of lymphoma of MDV infection; besides 24/45 (53.33 %) gross lesions in liver and spleen both, and 5/45 (11.11 %) gross lesions in liver alone, indicated generalized area of uniform-sized proliferating lymphoblasts, alongwith pleomorphic lymphocytes ranging from small to medium lymphocytes, characteristic of both avian LL and Marek's disease (MD). However, this discrimination was not clear-cut. Many tissue sections could not be inferred discretely, whether they had areas of uniform lymphoblasts or pleomorphic lymphocytes. Thus, based on examination of all available H&E stained tissue sections, it was inferred that histopathologically it was inconclusive to confirm if it was only avian LL or MD or both; in particular in the present study, where 50 % tissue sections were found to have mixed infections of avian LL and MD.

Hence, molecular confirmation by polymerase chain reaction (PCR) assay of liver lymphomas (n = 44) with lesions characteristic of avian LL or MD, as compared to normal tissues, viz., liver, bursa of Fabricius and thymus, from freshly killed chicken no. M3070, was carried out using published primers against ALV subgroups and virulent MDV infections. Results revealed presence of PCR amplicons, viz., 1072 bp specific against endogenous ALV-E in all 44/44 (100 %) dead chickens; 229 bp specific against ALV-A only in 9/44 (20.45 %) dead chickens; 583 bp specific against virulent MDV only in 7/44 (15.91 %); and both 229 bp specific against ALV-A, and 583 bp specific against virulent MDV in 28/44 (63.64 %) dead chickens.

The study indicated that simultaneous infections of exogenous ALV-A and MDV against common background of genetic infection with endogenous ALV-E were playing a complex role in eliciting current trend of hepatic (visceral) lymphomatous lesions in majority of infected chickens.

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Bacteriological study of the wound of juvenile Hilsa (Tenualosa ilisha, Hamilton)

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Indian shad Hilsa i.e. Tenualosa ilisha (Hamilton, 1822) is a very popular food fish in South Asia including India due to its delicacy in taste and flavour. Moreover, due to high amount of omega-3 (n= 3) fatty acid content, this fish is healthy for cardio-vascular system of the body. Hilsa is mainly found in the river mouth and estuaries of India, Pakistan, Bangladesh and Myanmar and also in Tigris and Euphrates rivers of Persian Gulf area. There is very little information and report on diseases of this fish. In the present study, isolation and identification of different bacterial species from an ulcerative wound of recently dead juvenile Hilsa has been reported.

The juvenile Hilsa was collected from estuary and acclimatized in brackishwater pond. The acclimatized Hilsa were used for yard experiment. One juvenile Hilsa fish (Body weight 3.75 g, length 60 mm) showing the presence of ulcerative wound on the body surface was used for this study. Previously, the same condition was also observed in 6-7 fishes in the experimental yard. Death occurred in case of 2 fishes. After washing the area thoroughly with sterile normal saline, the wound area was swabbed with a sterile cotton swab. The organisms from swab were cultured in Tryptic soya broth and finally different dilutions in sterile normal saline were plated on Tryptic soya agar. Twenty different types of colonies were picked up from TSA, purified cultures was isolated and identification was carried both by conventional microbiological methods involving Gram staining and different biochemical tests and also 16S rRNA gene sequencing after isolation of pure genomic DNA. Following sequencing, the obtained sequences ware matched using Basic Local Alignment Search Tool (BLAST). The isolated different bacterial species have been identified as Micrococcus luteus, Exiguobacterium spp., Corynebacterium propinquum, Bacillus spp., Acinetobacter spp., Pseudomonas alcaligenes, Staphylococcous hominis and Bacillus oleronius. In some cases, organisms obtained from more than one colony were identified as same organism both by biochemical and sequencing methods.

Among different organisms isolated, Exiguobacterium spp., Corynebacterium propinquum, Acinetobacter spp., P. alcaligenes and B. oleronius have been reported to be associated with wound of human being and very often they act as opportunistic pathogens. Thus, more study is required to establish actual causative agent of the ulcerative wound of Hilsa through challenge trials.

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Myelin basic protein in cerebrospinal fluid: a diagnostic marker for neuroinflammation in dogs

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Neuroinflammation in dogs is a frustrating systemic illness and has association with canine distemper. Management of such condition is a clinical challenge. Myelin basic protein in cerebrospinal fluid of dogs with canine distemper may be a marker in assessing therapeutic response. We assessed this protein in CSF of dogs with CD associated neuropathy. A total of 47 CD infected dogs with neuropathy were used as subject for this study. Myelin basic protein was estimated in CSF using sandwich ELISA . In healthy dogs, MBP was estimated as 0.33 ±0.012 ng/ml in CSF. MBP concentration in CSF was found higher (2.39±0.06 ng/ml) in nondescript healthy dogs. CD infected dogs had significantly ($p \leq 0.001$) higher MBP (22.75 ± 2.19**) concentration in CSF. Interestingly, in CD infected dogs with chronic neuropathy had higher MBP concentration (32.15 ± 1.63) than CD infected dogs with acute neuropathy(13.35 ± 1.14).Therapy with neuroprotectants (Pyrinitol) has variable results; only 8.51%(4 out of 47) cases were completely recovered and 6.38%(3 out of 47) cases showed clinical improvement in the sense that they didnot show clinical recovery but their condition didnot deteriorate. The MBP levels in these dogs reduced significantly within 3 months of therapy. The data shows MBP in CSF could be used to assess therapeutic response in neuroinflammation

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A novel two-tube based multiplex PCR assay for the detection and differentiation of pathogenic Leptospira with specifically identification of five pathogenic serogroup

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Leptospirosis is a major contributor for worldwide zoonosis leading to global health issues. Early diagnosis of this neglected infectious disease is highly demand, which is requiredfor the prompt treatment of the disease and its control. Generally, to know the active infection, diagnosis of leptospirosis depends upon the isolation of leptospires from clinical specimens or the demonstration of serodiagnosis in paired acute and convalescent serum samples. Individual conventional or multiplex PCR assays have also been developed, but all have some limitations which have restricted their widespread use for the detection of non-pathogenic and pathogenic leptospira with serovars or serogroup levels. In order to overcome these limitations,

In this study, for easy screening of leptospira in the clinical samples for identification or determination of the active infection, a multiplex PCR was developed, which saved lot of time as against individual PCR applications in disease diagnosis. This novaltwo-tube based multiplex PCR assay for simultaneous detection and differentiation of pathogenic leptospira was standardized initially with two primers set Lept 1 & 2 (targeted at 331bp in 16S RNA) and Lig B 3 and 4 (targeted at 434bp in lig B gene). Further, this two-step PCR was expanded to multiplex PCR assays with simultaneously targeting specific gene dtDP-4-DHR,dehydrogenase,carbamoyl transferase, glycosyltransferase, glycosyltransferase of some pathogenic leptospira of endemicserovars representing serogroup Canicola, Sejro, Hebdomadis, Icterhaemorrhagiae, Gripppotyphosa belongs to Interrogans species of Leptospira which specifically yield 341bp, 319bp, 656bp, 590bp and 352bp respectively. Using 18 pathogenic reference Leptospira serovarsand one non-pathogenic Leptospira serovar patoc DNA, the assay was standardized and found specific and sensitive for detection of pathogenic PCR. Further, as a preliminary approaches developed assay was evaluated for detection of Leptospira by DNA extracted from Microscopic Agglutination Test (MAT) positive serum samples in the clinical samples of the human (PUO cases n=45), bovine(n=27) and canine (n=12) along with rat reservoir samples (n=26). The developed PCR technique is able to differentiate between saprophytic and pathogenic species and subsequently identifying the five pathogenic Leptospira serogroups levels for initial identification or providing early diagnosis for active infections in order to provide timely prevention and control of Leptospirosis.

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Detection of avian mycoplasmosis by conventional and molecular techniques in poultry in Tamil Nadu state

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Avian Mycoplasmosis' is an important threat to poultry farming, caused by several 'pathogenic Mycoplasma organisms like Mycoplasma gallisepticum (MG) and M. synoviae (MS) are the most important as they are involved in causing respiratory diseases and loss of production in birds (OIE, 2014).Avian mycoplasmosis causes substantial economic losses directly through embryo mortality (5-20%), chick mortality (5-10%), slow growth (8-25%), condemnation of carcass (5-10%), and production drop (8-20%).In the present study , the Mycoplasma gallisepticum was isolated and characterized from Poultry in TamilNadu state. 110 poultry farms were visited with the clinical signs of mortality(5-10%), Drop in egg production, Swelling of one or both infra orbital sinuses,Closure of one or both eyes, Breathing through partly open beak,Reduced feed intake,Swelling of hock joint and the necropsy findings includes Lung congestion,Frothy exudates in trachea,Airsacculitis,Yellow flakes in the abdomen and Salpingitis and 790 specimen were collected from both live ailing and dead birds .Swab samples from conjunctival sinuses,oropharynx,esophagus,cloaca,trachea and phallus were collected from clinically ill birds . Trachea, lungs,air sacs , spleen and yellow flakes and heart blood swab were also collected from dead birds and smears were also prepared and stained with giemsa stain to demonstrate the presence of bacterial organisms . Bacterial growth with yellowish discolouration could be observed in PPLO broth and fried egg colonies in PPLO agar. Mycoplasma organisms could be observed from both the culture under stereomicroscope.Confirmation was done by Growth inhibition test and digitonin inhibition test as identified as Mycoplasma gallisepticum .Further , all the samples were subjected to Species specific Mycoplasma gallisepticum PCR and the PCR product amplified at 185 bp was considered positive for Mycoplasma gallisepticum.In conclusion,,the avian mycoplasma was detected by conventional and molecular techniques from the specimens collected from a spectrum of respiratory diseases in poultry and found that the PCR is a rapid, sensitive and cheap method for early diagnosis of Avian mycoplasmosis than the conventional techniques which can help poultry farmers to avoid severe economic losses due to avian mycoplasmosis

Lab-to-Land technology: A pen-side diagnostic tool for brucellosis infection in multiple livestock species

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Brucellosis serves as a major barrier to socioeconomic progress, food security, livestock and public health in most of the endemic countries including India. It is a multiple species disease and the major challenge in its control is early detection of infection by a simple diagnostic tool which can be performed beside an animal to provide on spot decisions to the owners at farm level, buyers at markets, clinicians at hospitals and researchers at diagnostic laboratories. The present study highlights development, standardization and evaluation of lateral flow assay (LFA) for diagnosis of brucellosis in multiple livestock species. Brucella sLPS antigen from B. abortus strain S99 sprayed on nitrocellulose strip with Protein G conjugated colloidal gold nano-particle as detecting reagent was used to develop test devices. In the first stage of evaluation, test has shown 0.86, 0.79 and 0.8 kappa ( ) agreements with both RBPT and iELISA for a pre determined panel of 600 coded serum samples of bovines, small ruminants and swine (200 each species). The overall diagnostic sensitivity of 87.20 to 91.20% and specificity of 91.60 to 95.60% with a high diagnostic accuracy (89.8 - 93.4%) were recorded. In second stage evaluation with field serum samples (bovine-309, smallruminants-298, swine-136), up to 90% test agreement for bovines and swine and 80% agreement with small ruminants, was observed with the protein G iELISA and RBPT tests. The utility of assay devices were also evaluated as field test in investigation of brucellosis outbreaks in buffaloes and swine showed diagnostic accuracy greater than 95%. These diagnostic features strongly suggest use of newly developed LFA as an alternative tool even to the simplest RBPT test in Indian context. The utility of test can be greatly exploited at farms, slaughter houses, market places, veterinary hospitals and as pre-purchase test for screening brucellosis in multiple livestock species. The test offers an additional complementary measure facilitating quicker and early diagnosis and will act as important tool for the ongoing brucellosis control program of India.

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Viability PCR to detect the most-probable-number of probiotic bacteria from commercial preparations

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Molecular diagnostics that could detect only viable cells from such samples are much sort after following the introduction of viability PCR (V-PCR). We have used a photoactivable nucleic acid intercalating dye that selectively permeates membrane compromised cells which upon light activation (using an indigenously built in device) results in an irreversible DNA modification and inhibition of amplification in PCR. In this study, we optimized V-PCR using a candidate L. plantarum probiotic strain and the technique performed efficiently in detecting live cells from an admixed suspension of live and dead (ratio of live to dead cells 108:102 to 102:108 respectively). Application of V-PCR on different probiotics strains (*B. coagulans, L. plantarum* and *L. fermentum*) revealed a strong positive correlation in its performance across the three strains tested both in an admixture of different concentration of live and dead cells and also at different dilutions respectively (r=0.93 to 0.98). We obtained five and four commercial probiotics indicated for human (ranged from 8.3 x 107 to 1.66 x 108 viable counts) and animal use (ranged from 3 x 106 to 1012viable count/g) respectively available from market shelf and tested for the recovery of total viable bacteria by plate method and also by V-PCR. We could observe a 1 to 2 log reduction in the total viable count in preparations indicated for human use while among veterinary products two of the preparations correlated with the listed counts and two of them showed a 5 log reduction in the total viable count (included Saccharomyces sp. that are not measured in the plate method) The reduction in the viable count could be due to the decreased stability of the Lactobacillus species in the preparations as the viable counts were lower in the selective MRS agar. A semi quantification approach using arbitrary density units of the universal 16S rRNA PCR amplicons following V-PCR to quantify the viable organism count from commercial probiotic products revealed a similar counts as that of total viable count except for a 4 log reduction in two of the veterinary preparations that listed 1012 CFU/g. Screening several commercial probiotic preparations indicated for human and animal health will enable us to develop this technique as a routine to monitor the viability at the point of use rather than at manufacturer's site.

Pasteurella multocida infection modulate inflammatory and immunological responses in mice by altering the expression of virulent genes

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Pasteurella multocida are Gram-negative coccobacilli causing various diseases in animals. Its pathogenesis is complex involving interaction between host and bacterial virulence factors. In this study we identified the role of cytokines and toll-like receptors in P. multocida infection. Three groups of BALB/c mice were setup. The first group received 2x102 CFU/ml P. multocida serotype B:2 (challenge group), the second group was vaccinated with formalin killed alum adjuvant P. multocida (vaccinated group) and the third control group received PBS. Equal numbers of animals from different groups were sacrificed to collect lung and liver tissues at various times of infection. Different cytokines IL-2, IL-4, IL-10, IFN-γ and TNF-α, and TLRs, TLR-1, -2, -4 and -6 were selected for regulatory effects of P. multocida infection in mice. The time-course of release showed statistically significant elevations of these cytokines in lung and liver tissues during early hours of infection. The relative fold change expression of cytokines and TLRs showed higher levels of IL-4 and IL-10 than IFN-γ, IL-2 and TNFα in challenge group when compared with vaccinated and control groups. The expression of TLR2 and TLR4 was also upregulated as compared to other TLRs. These results suggest the dominance of Th2 cytokines over Th1 cytokines in protective immunity against P. multocida infection. Additionally, the pathogen-associated virulence factors showed statistically significant upregulation of iron aquisition gene HgA and filamentous gene PfhA most prevalent in P. multocida serotype B as compared to less prevalent SodC. These virulence genes facilitated the colonization and invasion of host leading to the stimulation of host inflammatory response. Our findings suggest that P. multocida infection modulate inflammatory and immunological responses by altering the expression of its virulent genes.

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Isolation and molecular characterization of leptospira sp strain isolated from canine by PCR and 16s rRNA gene sequence analysis

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Leptospirosis is an emerging bacterial zoonotic disease, worldwide in distribution. Dogs play a major role in this zoonosis by acting as carrier and contaminating the environment by excreting the leptospires in the urine. A Labrador dog suffering from jaundice and haematuria showed seroreactivity to Leptospira interrogans serogroup Australis,(1:800) and Ballum (1:200) on microscopic agglutination test.

Urine sample collected by catheterization examined by dark field microscopy and cultured in EMJH medium for isolation of leptospires. The organism isolated and presumptively identified based on its morphology and motility. The pathogenic nature of the leptospira isolate confirmed by amplification and detection of outer membrane protein LipL32 (756bp) and LipL21 (561bp) by multiplex PCR and virulent marker Loa22 (257 bp). The speciation of isolate was carried by the robust molecular method, 16sRNA gene (1430 bp) sequencing and analysed with Basic Local Alignment Search Tool (information available at www.ncbi.nlm.nih.gov/BLAST). The relatedness of isolate with other leptosipra strain was performed by the phylogenetic analysis, it is closely related to the other L. interrogans sp. The serogroup of the strain was identified as L. interogans serogroup Canicola using hyperimmune serum by microscopic agglutination test. The pet owner was advised to take protective measures to prevent the zoonosis.

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Isolation and identification of an antimicrobial resistant persistent cell culture contaminant as Achromobacter spp.

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Laboratories dealing with cell-cultures face a constant threat of microbial contamination, especially if resistant bacteria enter the system. Various inhibitory agents are incorporated in cell-culture media to prevent the contamination, however the bacterial contaminants are difficult to characterize and there is no option left other than completely discarding the cultures infected with bacteria and starting afresh following aseptic techniques. However, monitoring of contaminating antimicrobial resistant strains may help in future control. We report isolation, identification, and characterization of an antimicrobial resistant strain of Acromobacter spp. consistently affecting primary cell-culture derived from amniotic tissue of buffalo (Bubalus bubalis). Pure colonies obtained on SBA were subjected to phenotypic and genotypic characterization for identification. Antimicrobial sensitivity was determined by disc-diffusion method on Muller Hinton Agar No 4. The isolate was resistant to more than one class of antimicrobials. Molecular identification of isolate was performed by amplification of 16S rRNA gene and clone was sequenced and phylogenetic analysis was carried out. The strain also harboured the genes responsible for resistance to antibiotics, as detection of antibiotic resistance genes viz. bla-TEM, OXA-1, OXA-2, Int1, Int2 and Int3 was also carried out. Among six antibiotic resistance genes detected by PCR, the isolate showed strong amplification of OXA-2 (encoding for class D beta lactamase) and Int1 (integron1). MIC (minimum inhibitory concentration) of commonly used cell-culture antibiotics gentamicin sulphate and streptomycin sulphate was determined against the Acromobacter spp. Gentamicin sulphate and streptomycin sulphate exhibited very high MICs against Achromobacter spp. strain (MIC90 of 0.016–0.256 mg/mL) for both antimicrobials. Achromobacter species have been shown to be resistant to multiple antibiotics and assessment of microbial cell-culture contaminant further will help development of antibiotic replacement strategies for continuous cell-lines.

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Equine influenza virus: An insight into evolution and interspecies transmission

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Influenza in horses is known to be caused by two subtypes of viruses viz. H7N7 and H3N8. Equine influenza viruses of the H7N7 subtype have not been isolated since 1980 whereas H3N8 viruses continue to circulate in equine populations throughout most of the world and are essentially enzootic in the USA and Europe. The evolution of the Influenza viruses is mainly driven by the mutation rate and herd immunity. During replication the lack of proof reading by viral RNA polymerase allows the replication errors resulting in point mutations during transcription causing antigenic drift. This is an adoptive mechanism of the influenza viruses for their survival and evading the host immune response. Influenza A viruses are renowned for their capacity to cause epidemics on a nearly annual basis due to these mutations. In addition to the linear evolution of the influenza viruses, the segmented nature of its genome allows reassortment to take place resulting in rapid virus evolution (antigenic shift) and emergence of new strains which may have potential to cross the species barrier. Earlier it was hypothesized that H3N8 subtype viruses evolved as a single lineage however, later in 1996 it was reported that there were two distinct Eurasian and American branches of H3N8 influenza virus circulating in the equines since 1990. The viruses within the American branch have further diverged into three lineages: South American lineage, a Kentucky lineage, and a Florida lineage. These multiple evolution pathways are not due to geographic barriers, as viruses from different lineages co-circulate at a given time. Further evolution of the Florida sublineage has resulted in the emergence of two groups of viruses viz. clade 1 and 2. The epizootic in India occurred subsequent to China (2008) and Mongolia (2007-08) and the virus in all these outbreaks belonged to Clade 2 of Florida sublineage. The disease reemerged in India in 2008 after a gap of 20 years and spread to 13 states of the country.

Influenza virus infection is initiated by the binding of the HA to the sialic acid receptors present on the surface of the cell which have 5-N-Acetylneuraminic acid (Neu 5Ac). Sialic acid generally occur in α2,3 or α2,6 linkages with other sialic acid moieties. Human influenza viruses preferentially bind cell surface oligosaccharides that contain the 5-N-acetylneuraminic acid- 2,6-galactose (Neu5Aca2,6Gal) linkage, while avian and equine influenza viruses bind Neu5Aca2,3Gal. Pigs, however, express substantial amount of both forms of sialic acids, and it is believed that both avian and human influenza viruses can attach to the appropriate receptor and infect pigs potentially allowing them to serve as "mixing vessels" for the generation of reassortant influenza viruses. Generally, individual influenza viruses are host- and species-specific due to specific receptor binding property of the viruses. However, mutagenic changes and re-assortment often resulting in cross-over of the species- specific barriers/habitats of the viruses thus posing a continuous threat of emergence of new strains capable of infecting the new host species. This species-jumping has been documented among various species notably avian to pig, human, equine and pigs to human and vice versa. Equine influenza is no exception to this and transmission of equine influenza virus (H3N8) has been documented in dogs and pigs besides recently experimental transmission has been reported in cats. Thus, continuous and rigorous influenza virus surveillance is necessary to identify and characterize currently circulating viruses and new variants arising out of the outbreaks, which will help in knowing about the ecological distribution of the viruses, early disease diagnosis, vaccine strain selection, disease forecasting and developing control strategies.

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Equine piroplasmosis-an insight into interacting molecules and novel drug targets

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Equine piroplasmosis, more commonly known as biliary fever, is an acute, sub-acute or chronic tick-borne disease of equidae (horses, donkeys, mules and zebras), caused by an intra-erythrocytic protozoa Theileria equi and Babesiacaballi. The disease has attained a world-wide importance due to wide distribution of its various tick vectors. Both T. equi and B. caballiusually share the same vectors and are closely associated in an enzootic region. Equine babesiosis has posed a threat to the international movement of horses, because horses from a Babesiafree zone, when introduced into an endemic area, suffer clinically from the disease resulting into death of large number of animals. During Atlanta Olympic games (1996), limited number of Babesiapositive horses were allowed to participate in equestrian sports events and kept under rigorous piroplasmosis control 'Twenty Point Plan' Protocol.

We identified immunodominant polypeptides in T. equi.On SDS-PAGE, 16 polypeptides with molecular weight (Mr) in the range of 112–17 kDa were obtained from the WM and CM antigens. On immunoblotting with high titred serum collected from donkeys following two immunizations with a killed T. equi merozoite immunogen, 11 polypeptides were observed in theWMand CM antigens (Mr 112–18 kDa). Of these, four polypeptides (Mr 112, 45, 33 and 18 kDa) were identified as most immunoreactive. Besides these, a 28 kDa was observed as strong immunoreactive protein in WM and CM antigens. Further we investigated the cellular localizations and expression patterns of equi merozoite antigens (EMA) -1 and -2 of T. equi during its asexual erythrocytic-developmental cycle using anti-EMA-1t or -2t mono-specific mouse serum. Indirect fluorescent antibody tests demonstrated that EMA-1 and EMA-2 were not expressed in all the erythrocytic-developmental stages of the merozoites and that these two antigens were co-expressed during the early developmental stages. Additionally, it was shown that EMA-1 and EMA-2 were mutually expressed on the surface of extra-erythrocyticmerozoites and also that the intra-erythrocyticmerozoites shed only EMA-2 antigen in the infected erythrocytic cytoplasm or inside the membrane surface. These findings facilitate our understanding of the biological roles of merozoite surface proteins of T. equi and our investigation for new drug targets.

Additionally, only the EMA-2 is shed into the cytoplasm of infected erythrocyte or inside the erythrocytic membrane during their early developmental stage. We initially performed West-Western blot analysis on Triton X-100-insoluble erythrocytic skeleton collected from a healthy horse, using a glutathione S-transferase (GST)-tagged recombinant EMA-1t or EMA-2t of T. equi. The results indicated positive interactions of actin and band 4.1 molecules in the equine erythrocytic skeleton only with the recombinant EMA-2t. Subsequently, we carried out GST pull-down assay using the recombinant antigens against solubilized lysate of equine erythrocytic skeleton, and confirmed the co-precipitation of actin molecule with EMA-2t, but not with the EMA-1t. The interaction of EMA-2 with host erythrocytic actin indicated its role in the pathobiology of T. equiinfection within host erythrocytes.

During asexual development within erythrocytes, T.equi parasites synthesize considerable amounts of membrane through the phospholipid metabolism.Phosphatidylcholineand phosphatidylethanolamine are the major PL of the infected erythrocyte, representing about 85% of total PL. Phosphatidylcholine is the most abundant phospholipid in

apicomplexa parasite and is synthesized mainly by the Kennedy or CDP-choline pathway. Choline kinase is the first enzyme in the Kennedy pathway (CDP-choline path).Mono and bis quaternary ammonium salts were good candidates for mimicking the choline structure.Compounds which possess one (or two) quaternary ammonium ions, such as choline and long lipophilic alkyl chains, were found to be very potent against P. falciparum within the micro- and picro-molar range. In another in-vitro trial we planned to target phospholpid metabolism of T.equi by quaternary ammonium bromide salts. For that we choosenDMB, Dodecyltrimethyl ammonium bromide, Decyltrimethyl ammonium bromide as choline kinase inhibitor.In our study we found that Dodecyltrimethyl ammonium bromide and Decyltrimethyl ammonium bromide were significantly inhibit the growth of parasite. At 1 µm concentration it inhibited the multiplication of parasite significantly. Both this compound show significant effect from first 24 hours onwards. In our PBMC cytotoxicity evaluation these both compound have high cytotoxicity effect. In haemolytic assay also supports its cytotoxicty effect at more than 500 µm concentration.DMB showed promising significant inhibitory effect at more than 28 µm concentration. In PBMC cytotoxicity evaluation it having 8% toxic effect at 1000 µm where its IC 50 is only 14 µm. In haemolytic assay it do not have any haemolytic effect. So, it promising significant inhibitory effect with minimum pbmc cytotoxicity and null haemolytic activity. Heat shock protein 90 inhibitor study is under processing. Both NBC and APPA do not have any cytotoxic and hemolytic effect as per our study. More data will be discussed during the presentation.

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Evolution of avian influenza viruses: Indian and global perspective

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Avian influenza is caused by Type A Influenza viruses (IAVs) of the family Orthomyxoviridae. Over a period of one decade, the currently circulating H5N1 highly pathogenic avian influenza (HPAI) virus that emerged from a goose in 1996 in China has evolvedinto tengenetic clades (clade 0-9) and emergence of a dozen 2nd, 3rd and 4th order clades and variant groups within them has been reported. Co-circulation of H5N1 and H9N2 subtypes in multiple poultry species in South East Asia and frequent reassortment among them has resulted in extensive diversification of H5N1 virus. The H5N1 virus, that resulted in direct human infection in Hong Kong in 1997, was a triple reassortant with HA gene from gs/GD/96-like H5N1, N1 NA gene from H6N1 and the internal genes from H9N2 viruses. The recent H7N9 virus emerged in March 2013 in China most likely by two separate reassortment events involving viruses from wild bird (subtypes H7N3 and H2N9 or H11N9) and poultry (subtypes H9N2).In India, H5N1 HPAI viruses have been reported almost every year since its first report in 2006. Analysis of the outbreaks strains revealed presence of diverse H5N1 viruses from four genetic clades; clade 2.2 detected during 2006 and 2007, clade 2.2.2.1 detected during 2008-2010, clade 2.3.2.1a detected in 2011 and continued till early 2015, and a new clade 2.3.2.1c detected during late 2014 and early 2015. Reassortments have also been detected in a subset of clade 2.3.2.1a viral isolates in which the PB1 gene derived from a low pathogenic avian influenza (LPAI) H9N2 virus. The new clade 2.3.2.1c is a reassortant in which the PB2 gene is derived from LPAI H9N2 virus. Another novel reassortant has been detected in clade 2.3.2.1a that derives its PB1, PA and NS genes from H9N2 virus and the remaining genes from gs/GD/96-like virus. Emergence of drug-resistant H5N1 strains has been reported. The H9N2 LPAI virus detected in India was a reassortantG1-like sublineagevirus. In the present scenario, continued surveillance for IAVs in poultry, wild birds and other hosts and timely reporting of genome sequence data is critical to quickly identify new genetic clades and assess their impact on animal health.

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Porcine enteric rotavirus infections: Etiology and genotypic distribution with impact assessment

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Diarrhea is the second most important health problem after respiratory infection which affects young ones mainly. Diarrhea is major cause of loss in animal husbandry sector. Piggery sector more frequently encounter the diarrheal episodes. It occurs most frequently during two critical life spans including neonatal life in the farrowing shed and post weaning. It is mainly a result of infections due to viral, bacterial and parasites. Among the several causes of gastroenteritis, virusesare known to play more significant role.However, despite appreciable improvements in managemental practices, diarrhea is still a common and costly syndrome of neonates. The technological advancements in the field of diagnostics and metagenomics of the enteric environment have further enhanced the identification of new and novel viruses. Worldwide, rotavirus (RV) is considered as one of the main causes of diarrhea affecting young animals of different host species. Classification of rotaviruses into nine distinct serologic groups designated A to I is based on the capsid VP6 protein. Group A rotaviruses (RVA) are most often responsible for diarrhea in piglets. Due to the difficulty in diagnosis of diarrhea caused by other rotavirus groups, non-group A rotaviruses are rarely reported. Rotaviruses were first discovered in feces of pigs by Rodger in 1975. Since the discovery of first group A rotaviruses in the feces of diarrheic piglets in the 1970s, several serologically distinct non-group A rotaviruses, such as RVB, RVC, RVE and RVH, have been encountered in feces of piglets around the world. Still, little attention has been paid on the circulation of rotaviruses of different groups in Indian swine population. Less sensitive assays fails to identify rotavirus groups B and C in comparison to group A directly in fecal samples because less number of group B and C virus particles are being excreted during the acute period of the infection. In contrast, prevalence studies in many countries around India reported relatively frequent occurrence of RVs infections. At this moment it remains unclear to which extent RVA, RVB, RVC, RVE and RVH are circulating in the Indian swine population. Diagnostic assays for these viral species will be setup in the near future as well, and their occurrence and pathogenic importance will be further exploited in longitudinal field studies in Indian suckling piglets and piglets after weaning. In depth assessment of the impact of these viral infections on the animal health and production, itbecomes indispensable and thus necessitates the development of precise diagnosticsfor apprising the diagnostic algorithms of enteric pathological conditions. During the presentation, some important differentials of diarrhea in the young piglets and the impact of coinfections with rotaviruses will be presented.

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"Mycobacterium tuberculosis on steroids"

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Tuberculosis (TB) remains one of the world's most important infectious diseases, with an estimated 10 million cases and 2 million deaths per year. Although the current treatment of drug-susceptible TB is effective, treatment duration is exhaustively long (6-9 months) and therapeutic agents are highly toxic. Further, due to the emergence of multi drug resistance (MDR) & extremely drug resistance (XDR) strains of Mycobacterium tuberculosis (Mtb), the etiological agent of TB, and a sudden increase in the incidence of human immune deficiency virus (HIV) in the population, TB, a seemingly treatable disease, is turning out to be a major public health hazard. One of the most challenging aspects of tuberculosis treatment is the presence of a slow growing, non-replicating, metabolically inactive "persisters" population inside host that requires extremely long treatment regimen. Studies shows that targeting these persisters by altering the metabolic state of dormant Mtb could increase the effectiveness of antibiotics and shorten treatment duration. We have earlier demonstrated that although Mycobacterium tuberculosis (Mtb) eats cholesterol throughout the infection process, it becomes essential only during the later chronic stage of infection. The essentiality of cholesterol during the persistent stage of Mtb infection is very intriguing. We hypothesize that cholesterol modulates critical metabolic and signaling pathways. We predict that this re-wiring significantly decreases Mtb replication by reducing its metabolic activity. These metabolically arrested non-replicating persisters are refractory to anti-mycobacterial treatment and predicted to be the main reason behind mycobacterial persistence. We have scientific evidence showing cholesterol grown Mtb has a lower metabolic activity and replication rate. Microarray based transcriptional profiling experiments revealed Mtb grown in cholesterol i) generates a transcription signature indicating

reduced growth rate ii) up regulates genes required for growth in low oxygen (hypoxia) condition iii) differentially regulate genes of unknown function possibly regulating cholesterol mediated regulation of growth in Mtb. Currently, we are characterizing Mtb genes predicted to be critical for regulation and utilization of cholesterol. Our long-term interest is to decipher the metabolic pathways and signaling network involved in Mtb persistence. A mechanistic insight on mycobacterial persistence will increase the repertoire of potential TB drug targets. The knowledge, information and expertise thus generated would guide the research community towards new unexplored frontiers of TB research.

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Changing pattern of bovine group A rotavirus in Kashmir, India

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Bovine group A rotaviruses are important cause of diarrheal diseases in neonatal calves. A total of 790 faecal samples from calves aged between 0 to 6 months were examined for the presence of different G and P genotypes of rotavirus circulating in bovines during the study from 2002 to 2014, in Kashmir Valley. Group A rotavirus was detected in 109 (13.9%) faecal samples. Genotyping analysis revealed G10, G6, G3, P[11] and P[5] to be the predominant types. The most common type of combination detected was G10P[11] (40.3%) followed by G6P[11] (12.9%). The prevalence rate of G10 and P[11] decreased from 60% to 35.3% and 100% to 67.6%, respectively. Genotypes G6, G3, P[1] and P[5] which were not previously reported from Kashmir, India, were detected for the first time. Novel combinations, G6P[11], G3P[11],G10P[5],G3P[5],G6P[1],G6P[5], G6+G8P[11] were also observed for the first time. Slight increase in the mixed infection from 3.2% to 8.10% was also recorded. Fluctuations in the predominant types, emergence of new types and possible genetic reassortment events suggest the non-stable epidemiological picture and the need for the continued surveillance of the circulating types to ensure the suitability of vaccination programme.

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Multiple introductions of a reassortant H5N1 avian influenza virus of clade 2.3.2.1c with PB2 gene of H9N2 subtype into Indian poultry

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During November 2014 to January 2015, mortalities in ducks and turkeys in Kerala, and ducks in Chandigarh were reported. Influenza A(H5N1) virus in the dead birds/swabs was confirmed by molecular tests and virus isolation. Nucleotide sequence of all eight gene segments of six isolates (representative from each outbreak epicenter) were determined and compared with available sequences. Analysis of the HA gene revealed presence of multiple basic amino acids near the cleavage region, which is characteristic of HPAI viruses. This was corroborated by IVP index of 2.96/3.00 for one of the isolates from Kerala.To know the source of the virus phylogenetic analysis was carried out. In the HA gene phylogeny, all the H5N1 isolates grouped into a new clade 2.3.2.1c which was not detected earlier in India. Within clade 2.3.2.1c, H5N1 isolates formed two distinct groups with high confidence; one group (group A; bootstrap value100%) is comprised of duck and turkey isolates from Kerala indicating a separate introduction of the virus. However, the second group (group B; bootstrap value 87%) is formed by one isolate each from Kerala and Chandigarh along with a falcon isolate from Dubai. All the Indian and Dubai isolates showed close relationship with H5N1 viruses isolated during 2012-2014 in Jiangsu province of China and Vietnam indicating probable ancestor. In the PB2 phylogeny, all the Indian isolates grouped with H9N2 viruses isolated during 2007-2013 in China and 2009-2014 in Vietnam indicating reassortment of the PB2 gene. All the remaining six genes grouped with H5N1 viruses. The Jiangsu H5N1 virus which is closely related to the Indian H5N1 viruses is also a reassortant virus with PB2 gene of H9N2 virus. From the grouping pattern in the tree, it can concluded that the outbreaks in Kerala, Chandigarh and Dubai were due to simultaneous introduction of virus, which includes second introduction into India from China. Such simultaneous spread of infection over a long distance indicate that migratory birds as the most probable carriers of infection. Evolving of such H5N1 ressortant virus with internal genes of H9N2 virus may give rise to novel strains, with potential to cause pandemic and hence continuous monitoring and targeted surveillance are required for preparedness.

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Heterologous expression of exposed outer domain of 86 kDa outer membrane protein (OPR86) of Pseudomonas aeruginosa

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Pseudomonas aeruginosa is an opportunistic pathogen of human and animal being causing nosocomial infection in immune-compromised individual. It has antibiotic-resistant biofilms covering composed of polysaccharide matrix which makes difficult for its therapy. Therefore, there is need of vaccine development against P. aeruginosa consist of immunogenic protein antigens. Currently, there is no vaccine available which is effective against the biofilm but Opr86 (outer membrane protein) of P. aeruginosa homologous to outer membrane protein of other gram negative bacteria, might serve as vaccine candidate.The Opr86 gene of 2,394 bpencodes 798 amino acid having two peptide fragments: o-opr86 (exposed outer domain opr86) and p-opr6 (periplasmic domain opr86). It was reported, antisera against o-opr86 inhibit biofilm formation in P. aeruginosa. A 459 bp oligonucleotide encoding o-opr86 fragment was amplified, cloned in pJET1.2/blunt vector, and sequenced. The sequence was submitted to NCBI (accession number KF646784) after bioinformatics analysis. Further o-opr86 fragment was subcloned in pET302/NT-His expression vector in E coli BL21 (DE3) pLysSRosseta-gami 2 cells. The positive clone was induced by IPTG (0.6mM) for heterologous expression of recombinant protein (o-opr86) of 18 kDa size.Temporal expression kinetics of expression was studied by SDS-PAGE analysis. The recombinant protein was purified by using Ni-NTA affinity chromatography. Purified protein was further confirmed by SDS-PAGE and Western blotting.

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Prediction of functional changes in equine Mx protein and association with susceptibility vis-a-vis resistance against Equine Influenza

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Equine Influenza (EI) is a highly contagious and rarely fatal respiratory disease of horses, donkeys and mules. High mutation rates due to segmented genome make the virus unpredictable in nature and therefore, considered a threat for human pandemic. The general approaches to combat EI include vaccination and appropriate management practices including biosecurity. Vaccination does not always prevent infection due to variability of the vaccine strains with virus in circulation. Genetic variations between hosts within a population or between populations have been demonstrated to confer resistance against animal diseases. Mx protein has been shown to protect against influenza virus infection in mice, human, pigs and poultry. Peripheral blood mononuclear cells were isolated from EI positive horses and in contact horses with history of no clinical signs of EI and stimulated with IFN α/β. The total RNA extracted from these cells was reverse transcribed and Mx gene was amplified, cloned and sequenced. Diversity studies revealed polymorphism in nucleotide and deduced amino sequences of Mx gene. Sorting intolerant from tolerant (SIFT) algorithm was used to evaluate the impact of amino acid substitutions on the function of Mx protein. The substitutions predicted to be of functional significance in resistant and susceptible horses will be discussed. Comprehensive validation of these functional changes in terms of how these structural parameters correlate with the activation of unique host responses need further elucidation.

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Cytomegalovirus infection enhances the immune response to influenza

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Prior immunity to apparently unrelated pathogens can affect the pathogenesis of acute infections. The mechanisms of heterologous regulation in most circumstances remain poorly defined. In an attempt to assess the influence of prior infection on the immune response to the subsequent heterologous infection, Female 5- to 6-week-old control C57BL/6 mice were infected intraperitoneally with 4x104 PFU of murine cytomegalovirus (MCMV). For-coinfections, after MCMV infection, avertin (2, 2, 2-tribromoethanol)-anesthesized animals were challenged intranasally with 1x106 EID50 of HKx31 (mouse adapted) strain of influenza A virus. Spleen, BAL fluid, and lung samples were recovered from acutely and latently infected animals challenged with 1x106 EID50 of HKx31 at the various time points, for the evaluation of cellular and humoral responses.

We show that prior infection with latent virus such as (CMV) results in improved and more robust immune response to a new incoming acute infection such as influenza (IAV). This protection was dependent on IFN production by these mature anti-CMV CD8 T-cells. The acute IAV infection resulted in the attrition of MCMV specific T cell response. It is possible that the immunizations in large animal species and human subjects that are seropositive for latent and chronic infections can have an entirely different manifestations compared to the seronegative subjects. Moreover the new infections in already immunized individuals can result in the loss of the protective cells generated due to vaccination.

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Quantitative real-time PCR (qPCR) based diagnosis of benzimidazole resistance and comparison of various tests for its efficacy

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Benzimidazole resistance is known to be associated with SNPs (Single Nucleotide Polymorphisms) F200Y, F167Y and E198A in the beta-tubulin isotype 1 gene. Present study was designed to know the status of benzimidazole (BZ) resistance in goat flocks of Uttarakhand by faecal egg count reduction test (FECRT) and allele specific polymerase chain reaction (AS-PCR). Further, quantitative real-time PCR (qPCR) was used to identify predominant SNP, responsible for BZ resistance in Uttarakhand. Faecal samples were collected from various goat flocks and field animals. Faecal egg counts and larval culture were made from representative samples of all regions of state. Faecal culture and PCR-RFLP on beta tubulin isotype-1 gene before treatment showed prevalence of Haemonchus contortus, Teladorsagia circumcincta, Trichostrongyles spp, Oesophagostomum spp, and Bunostomum trigonocephalum. However, it was found that H. contortus was the predominant species after treatment. FECRT result showed all the farms/field samples harboured susceptible populations of strongyles. Total of 2341 H. contortus infective larvae from all regions of the state were genotyped for BZ resistance. AS-PCR results revealed 0.7% of H. contortus was homozygous resistant (rr), 97.4% homozygous susceptible (SS) and 1.9% heterozygous (rS) among different regions of Uttarakhand. The allele frequencies were 1.19% for resistant (TTC) and 97.86% for susceptible (TAC). High frequency of the resistant SNPs F200Y was detected among homozygous resistant population and no resistance was detected at F167Y and E198A alleles by qPCR. Our results suggest that the SNP F200Y is important for benzimidazole resistance in the studied populations and the status of BZ resistance is very low in all the parts of the state.

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Host-targeting antiviral agents

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Most antiviral agents so far approved, are pathogen-specific and select for resistance because virus can easily mutate the druggable target. Infection of cells with viruses results in the activation of a variety of intracellular signaling pathways which in turn create an antiviral state. However, viruses have been known to misuse these signaling pathways to ensure it's own efficient replication. These dependencies of the viruses on the host may be used to develop novel antiviral drugs. Nuclear factor-kappa beta (NF-κB), Raf/MEK/ERK, receptor tyrosine kinase and phosphatidylinositol 3-kinase (PI3K) are important signaling pathways that are known to be required for efficient virus propagation and have attracted some attention as suitable targets for antiviral interventions. However, there is a significant gap in our understanding about how animal viruses interacts with the host cell signaling pathways, characterization of which may help in developing novel antiviral therapeutics. Antiviral mechanism of some host-targeting inhibitor viz: NF-κ B, sarco-endoplamic reticulum calcium ATPAse (SERCA) and receptor tyrosine kinase (RTK) against a deadly animal virus i.e. peste des petits ruminants virus (PPRV) will be discussed. These studies are in preclinical phase and will likely lead to a paradigm shift in antiviral drug development in terms of minimizing drug resistance because the virus cannot easily overcome cellular functions by simply mutating its genome. Though at present time the antiviral strategy may not be cost-effective for livestock but it could complement emergency vaccination or be applied to treat valuable zoological collections and breeding stocks.

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Scope of genomic tools in enhancing animal breeding potential

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A pre-calculated animal breeding program emphasizes on selection of animals that have high breeding values for traits of interest to produce the progeny that performs better than average of the current generation. Traditionally, the genetic improvement of livestock breeds has been based mostly on phenotypic selection. In the last two decades, advances in molecular genetics have introduced a new generation of molecular markers for genetic improvement of livestock. This is focused on DNA based markers which have acted as versatile tools and found their own position in various fields including animal productivity. Eversince their development they are constantly being modified to enhance their utility and bring about automation in process of genome analysis. Polymerase chain reaction was a milestone in this endevour that brought about a new class of DNA profiling markers. DNA based markers are classified in three major groups: 1) Hybridization based DNA markers e.g. RFLP and oligonucleotide fingerprinting, 2) PCR based DNA markers e.g. RAPD, SSR/microsatellites, AFLP, 3) DNA chip and sequencing based markers e.g. SNPs. In the recent years microsatellites have been most popular in livestock genetic characterization studies. Untill recently, microsatellites were the markers used for mapping QTL for production and functional traits in farm animals and tightly linked markers are used in marker assisted selection in practice. These markers revolutionized the construction of genetic maps in most livestock species.But it still has limited implementation in practical breeding programs. The advent of high-density SNP genotyping combined with new statistical tools has resulted in whole-genome selection in dairy cattle. More than two million SNPs have identified in cattle and estimation of breeding values using high density SNP data has been implemented in cattle breeding programs in several countries and the research to implement genomic selection in other livestock species is underway. The high density SNP data also provides opportunities to detect QTL and to uncover the genetic architecture of quantitative traits. Results show that this genetic architecture differs between traits but for most of the traits, more than 50% of the genetic variation resides in genomic regions with small effects that are of magnitude that is expected under a highly polygenic model of inheritance. Results to date show that most traits of interest are indeed highly polygenic. Therefore genomic tools combined with statistical tools in the present era of molecular genetics has potential in increasing the breeding output of desired traits but it may take years to implement the genome selection breeding in most of the livestock species.

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The two faces of heterologous immunity: protection or immunopathology

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Immunity to previously encountered viruses can alter responses to unrelated pathogens. This phenomenon, which is known as heterologous immunity, has been well established in animal model systems. Heterologous immunity appears to be relatively common and may be beneficial by boosting protective responses. However, heterologous reactivity can also result in severe immunopathology. The key features that define heterologous immune modulation include alterations in the CD4+ and CD8+ T cell compartments and changes in viral dynamics and disease progression.

Heterologous immunity appears to be a common phenomenon among closely related pathogens, for example, different strains of influenza or DENV, or among different members of the same virus group, such as hantaviruses, arenaviruses, and flaviviruses. However, it also occursamong unrelated pathogens, including parasites, protozoa, bacteria, and viruses. Infections with distinct pathogens can occur concurrently or sequentially. Infections with differ-ent strains of the same pathogen or distinct pathogens are often classified as "coinfections" (when the two infections occurat the same time or within a brief window prior to the estab- lishment of the first strain in the host) or "superinfections" (where a second strain enters after the first strain is well established). For example in certain circumstances, immunopathologic features are more pronounced in young adults than inchildren, suggesting that prior infections in youngadults that have not occurred yet in most children may alterthe immune environment in the older patients. Alternatively,it is possible that primary immunization in children programsa distinct type of memory response that is less pathogenicupon challenge. Another situation where heterologous immunity is commonly observed is in persistently infected individuals who experience constant, low-level antigenic stimulation that alters their immunity to other pathogens.

Thus a well-regulated immune response to a plethora of pathogens, expression of an appropriate cytokine and chemokine milieu, and the activation of immune cells with regulatory activity are crucial for the survival of the vertebrate host in the setting of coinfections. The phenomenon of heterologous immunity has been shown to occur in mice and humans, and almost all types of immune cells play a role in this process. The general principles of heterologous immunity are currently being clarified, with the ultimate goal of being able to manipulate them to improve infection prevention, therapeutics, and transplantation outcomes. Some current approaches that modulate immune responses in a setting of multiple viralinfec-tions in vivo hold promise to manage microbe-induced immunopathology, and we anticipate that this will be a growth area in the field of therapeutics design and development.

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Cytoplasmic injection of murine zygotes with Sleeping Beauty transposon plasmids and minicircles results in the efficient generation of germline transgenic mice

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Transgenesis in the mouse is an essential tool for the understanding of gene function and genome organization. Here, we describe a simplified microinjection protocol for efficient germline transgenesis and sustained transgene expression in the mouse model employing binary Sleeping Beauty transposon constructs of different topology. The protocol is based on co-injection of supercoiled plasmids or minicircles, encoding the Sleeping Beauty transposase and a transposon construct, into the cytoplasm of murine zygotes. Importantly, this simplified injection avoids the mechanical penetration of the vulnerable pronuclear membrane, resulting in higher survival rates of treated embryos and a more rapid pace of injections. Upon translation of the transposase, transposase-catalyzed transposition into the genome results in stable transgenic animals carrying monomeric transgenes. In summary, cytoplasmic injection of binary transposon constructs is a feasible, plasmid-based, and simplified microinjection method to generate genetically modified mice. The modular design of the components allows the multiplexing of different transposons, and the generation of multi-transposon transgenic mice in a single step.

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Effect of sodium butyrate (histone deacetylase inhibitor) on donor cell physiology, those are used for production of handmade cloned buffalo (Bubalus bubalis) embryos

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Epigenetic aberrancies likely preclude correct and complete nuclear reprogramming following somatic cell nuclear transfer (SCNT) and may underlie the observed reduced viability of cloned embryos. In the present study, we tested the effect of the histone deacetylase inhibitor (HDACi), sodium butyrate on physiological status of donor cells. Briefly, Buffalo ear skin fibroblasts were derived from newborn adult female and characterized using cell type-specific markers, aim of characterization was to avoid specific effect of drug on selective cell type. To determine the type of somatic cells obtained from ear samples, the cells were stained for examining the presence of cytokeratin and vimentin. Ear tissue-derived cells were vimentin positive but cytokeratin negative demonstrating that these cells were of fibroblast origin. During each experiment, donor cells were seeded at a concentration of 1x105 cells per well on a 6 well culture plate. The cells were incubated in the culture medium containing selected dose of sodium butyrate (1mM/3mM/5mM) for 48 h, after that these were subjected to various physiological parameters such as cell morphology, cell proliferation and doubling time. All experiments were conducted on 5-8 passage numbers to minimize difference in results due to ageing caused by sub-passage. Our results showed that treatment of donor cells with sodium butyrate for 48 h resulted in altering cell morphology, proliferation and cell viability in dose response manner. Treated cells were enlarged in size, flattened and elongated in outlines, drug also increased population doubling time of donor cells. We have conducted limited experiment on cell cycle analysis of treated cells, and found that drug arrested cell in G0/G1 cell cycle stage, however further experiments are needed to validate effect of drug on cell cycle synchrony. Considering this in mind, our future experiments will be analysis of epigenetic status and embryos development of sodium butyrate treated donor cells. Till date our study demonstrates that the sodium butyrate modifies normal physiology of donor cells and its uses in SCNT may be beneficial for embryos development and minimize abnormalities in cloned calves.

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Immunolicalization of MSI1 and FNDC3B in water buffalo mammary glands

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Water buffaloes are the principle source of milk in south Asia. Mammary gland repeatedly undergoes the cycles of growth and regeneration during pregnancy, lactation and involution, it is assumed that buffalo mammary gland has mammary stem and progenitor cells that regulate gland growth and regeneration. In this study we analyzed percentage cellular composition, proliferation status and putative mammary stem/progenitor cell population. Identification of putative buffalo mammary stem/progenitor cells was attempted using immunohistochemical staining with Musashi1 (MSI1), an adult stem cell marker and fibronectin type III domain containing 3B (FNDC3B), mammary stem and cancer cell marker. Immunolocalization of MSI1and FNDC3B showed nuclear and cytoplasmic staining of alveolar and ductal mammary epithelial cells (MEC) and few stromal cells. The percentage of MSI1-positive MEC in non-lactating (3.31 + 1.11 %), lactating gland (2.73 + 0.78 %) and mastitic animals (3.30 + 0.97 %) were equivalent, indicating that the proportion of putative stem/progenitor cell population did not differ during various physiological stages. Likewise, the percentage of FNDC3B-positive MEC in non-lactating glands (12.40 + 3.22%) tended to be higher than lactating (8.19 + 2.71%) and mastitic glands (4.88 + 2.37%). In some cases, expression of MSI1 and FNDC3B was exceptionally high with high proliferative indices (37.6 + 2.4 %) - an indication of tumor cells of stem cell-like characteristics of buffalo mammary tissue. This is the first report on expression of MSI1 and FNDC3B in buffalo mammary gland but identification of buffalo mammary stem cells using MSI1 and FNDC3B requires further studies and functional validation.

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Molecular Docking studies of equine Chorionic Gonadotropin through insilico approach

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The heterodimeric glycoprotein hormone, eCG play imperative role in reproduction in equines. Equine chorionic gonadotropin (eCG) popularly known as Pregnant Mare's Serum Gonadotropin (PMSG) hormone was found in circulation of the pregnant mare during the first third of gestation period as early as 35 days of pregnancy. It is known to be similar to equine luteinizing hormone (LH), as both are coded by same genes but differentially glycosylated by the equine trophoblast cells. Although similar to eLH, it is one of the most heavily glycosylated hormone know till date and the prolonged half life in circulation owes to the property of its additional side chains. The reported amino acid sequence analysis was done by CLC sequence viewer, DNAStar, SCRATCH Protein Predictor and PSIPRED bioinformatics softwares. The 3D Homology Model (Tube diagram) of the eCG α-subunit illustrates the location of the three α- subunit loops – Loop 1, Loop 2 and Loop 3. This structure also illustrates the location of the region with sequences "CCFSRA" that probably may act as a binding antagonist. The eCG alpha protein was subjected to bioinformatics analyses including disulphide bond prediction, hydrophilicity/ hydrophobicity, antigenicity/ antigenicity propensity score. We also analyzed the eCG alpha protein sequence from a diverse selection of methods for secondary structure, hydropoathy, antigenicity, amphilicity, surface probability and flexibility its molecular docking with other drug molecules which may play role in elaborating the role played by α-subunit of eCG in the process of reproduction and maintenance of pregnancy in equines as well as other species. Ganirelix acetate (or diacetate) is an injectable competitive gonadotropin-releasing hormone antagonist (GnRH antagonist). It is primarily used in assisted reproduction to control ovulation. Ganirelix prevents ovulation until it is triggered by injecting human chorionic gonadotrophin (hCG). Insight into unique sites in alpha subunit will be useful in future studies based on equine gonadotropins. The in-silico analysis paves the way for future studies on eCG alpha using molecular docking hunt for agonist, antagonist or drugs or inhibitors for synergism, drug discovery etc.

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Hepatic gene expression profiling of rats reveals key enzymes involved in quinapyramine sulfate metabolism

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An aminoquinaldine derivative, quinapyramine sulfate (QS) is the commonly used and effective drug for treatment of trypanosomosis in domestic animals. The drug is administered to domestic animals in two or three divided doses at six-hourly intervals to reduce the toxic effects. A drug which affects certain metabolic process in a parasite often has a similar disruptive effect on the cells of the host. Drug-metabolizing enzymes (DMEs) are an important battery of proteins that are involved in drug metabolism, xenobiotic detoxification, and drug-induced toxicity. There is no single report on the induction as well as inhibitory effects of QS on DMEs by xenobiotics till date. Traditional enzymatic activity assays have been the primary tools for assessing drug metabolisms. However, these assays are time-consuming and require a large amount of experimental materials. High-throughput, simultaneous, and efficient measurements are currently lacking. Enzymatic induction is primarily mediated by increasing the transcription levels of drug metabolisms genes (DMGs), whereas downregulation of these genes is one of the mechanisms for enzymatic inhibition. Thus, assessing gene expression at the mRNA level is an important approach in identifying drug-induced effects on DMGs. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis is one of the most sensitive, reliable, and quantitative methods and is widely used to evaluate and measure gene expression at mRNA level. In this study, we employed a high-throughput method, the Rat Drug Metabolism RT2 ProfilerTM PCR Array, to profile phase I drug metabolizing genes in liver following administration of QS to rats at the recommended therapeutic dose. Here we report for the first time that, in response to quinapyramine sulfate, hepatic expression profile of metabolizing genes in rat revealed that the metabolism is mainly through oxidation and it has the greatest induction ability on flavin containing monooxygenase 1 followed by monoamine oxidase A and cytochrome P450 family enzyme.

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Seasonal variation in estrous cycle characteristics of Marwari mares in two different seasons as monitored by behavioral cues and ultrasonography

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Variations in the estrous cycle characteristics of adult Marwari mares were studied in two different seasons in semitropical climate. The ovarian characteristics were monitored by ultrasonography using rectal probe. Estrus duration and cycle length were monitored by behavioral cues (such as occurrence and intensities of attraction and receptivity to stallion, exposing the hind quarters to stallion, winking of vulva, etc.) as well as by ovulations observed by ultrasonography. Mares (n=10) were selected in each of the two seasons, i.e., winter (from November 2013 to February 2014) and summer (from April 2014 to September 2014) representing the short day and long day photoperiods. The estrous cycle length was significantly longer ($p<0.01$) in the short day photoperiods in winters than in summers (27.7 ±0.83 vs 24.50 ±0.50 days) but the duration of estrus was significantly($p<0.01$) longer in summer (10.07 ±0.41 vs 8.36±0.45 days). Mean preovulatory follicle size was not significantly different in the two seasons, although a higher size was observed in summer (47.34±0.7 vs 46.59 ±1.34 mm). The corpus luteum size also did not differ significantly ($p<0.05$) between the two seasons (32.47 ± 1.14 in summers vs 33.40 ± 1.34 mm in winters). During summer, the ovarian follicular size on the first day of behavioral estrus was observed to be 26.51±1.4 mm. The mares continued to exhibit estrous cyclicity in the first part of winter. However, during peak winter season, when the photoperiod got significantly reduced, mares exhibited erratic estrous cyclicity and ovarian quiescence which was not the case in the long day photoperiods in summer. It is concluded that long day photoperiod induces proper ovarian cyclicity in Marwari mares as compared to short day photoperiod.

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Hematological Profile of Hardhenu Strain of Cattle in Comparison to Sahiwal and Hariana Breed

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The Hardhenu is cross bred cattle strain evolved over last three decades in SAUs at Hisar. Proportionate blood profiling of this strain is HF 62.5% and rest from Sahiwal and Hariana Breeds. The average milk yield record is 3233 kg per lactation over average 305 days lactation period with 3.4% average fat percentage. This proves the sustainability of this strain for agroclimatic conditions of the region.

The nutritional status and metabolic profile(s) are based on physiological status that derives a lot on the hematological parameters. The metabolic status information for Hardhenu strain in contemporary literature is scanty. Thus, hemogram viz., Hb, TEC, PCV, MCV, MCHC and MCH were studied in this cross breed strain and two breeds, all evaluated at organized farm under same management conditions.

The total leucocytic count of Sahiwal & Hariana breeds (13.9 – 14.48 106/mm3) was significantly higher than cross bred calves for first two years of life. The TLC in age group of 2-3 years was significantly lower in Hariana breed. However, the TLC in 3 years & above age group was highest in Hariana & lowest in Sahiwal breed. The DLC profile was comparable as the farm livestock is by far healthy. The three breeds reveal comparable Mean Corpuscular Volume (28.18 to 48.95 fL). The Mean Hemoglobin Concentration revealed effect of age as values in the group of 3 years of age were significantly higher than in calves of age group of a year or less (8.53 Vs 13.48 pg). The Mean Corpuscular Hemoglobin Concentration was similar across breed and in respective age group(s) within a breed.

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Seminal plasma protein profile in indigenous jacks

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Seminal plasma proteins are secretary proteins originating mainly from the epididymis and the accessory sex glands. They are involved in remodeling of the sperm surface which occurs during sperm transit through the male genital tract and continues later at ejaculation. So far, there is no information on the seminal plasma proteins and their profile of the indigenous donkeys. The present study was aimed to document the seminal plasma proteins profiles in the indigenous jacks. Nine clinically healthy, docile, adult indigenous jacks of non-descript breed were used for semen collection. One ejaculate each from all the jacks was collected using artificial vagina (Colorado model). Gel portion of the semen was removed by filtration and immediately the seminal plasma was separated by centrifugation at 3000 rpm for 30 minutes to remove any cell debris followed by storage at -200C until further analysis. Twelve bands were observed in the range of 10.0 – 67.0 kDa (i.e. 10.0, 12.0, 15.0, 17.0, 18.5, 19.5, 31.5, 36.0, 40.0, 45.0, 56.5 and 67.0 kDa) in the seminal plasma of indigenous jacks using PAGE. The majority of these proteins were with a molecular weight <50 kDa. It can be concluded from this study that at least 12 bands of protein are present in seminal plasma of indigenous jacks.

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Importance of genomic tools for parentage testing in equines

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The genetic heritage of a registered horse is described by its pedigree. Owners highly value the ancestry records to provide information about their horses, expecting special potential as performance animals and as breeding stock. Recognizing that assurance of pedigree accuracy is central to their mission, all the major horse breed registries have adopted parentage testing programs worldwide to assure horse pedigree integrity, which is essential for breeding management plans. The most efficient genetic markers for such use are co-dominant markers that are inherited by Mendelian principles. Traditionally, in most laboratories, a combined battery of blood groups and protein polymorphisms has been used. Most of morphological markers are sex limited, age-dependent, and are significantly influenced by the environment. Protein markers show low degree of polymorphism. DNA testing techniques, now available and progressively adopted by most responsible laboratories, can greatly improve the success of parentage tests, providing an excellent alternative to traditional methods. They have the attractive features that they can be highly automated, require only small amounts of biological samples, are not restricted to fresh blood samples and the techniques involved are simpler and cheaper. Microsatellites or Simple sequence repeats or simple tandem repeats (STR) are highly polymorphic genetic markers with co dominantly inherited alleles that are relatively easy to score. Highly variable STR loci are common in mammalian genomes and can readily be typed by PCR followed by electrophoresis. A number of microsatellite markers have been used for parentage testing in several horse breeds in different studies. Parentage testing using molecular markers yields much higher exclusion probability (> 90%) than the testing with blood groups (70–90%) or other biochemical markers (40–60%). Implementation of these genetic markers for routine parentage testing has also become common procedure for parentage verification in developed countries. This provides an authentic proof of parentage in horses which is of utmost importance in further breeding programs and maintenance of germplasm. In India, the demand of such test is increasing, getting the parentage verified from the available sources costs too high, this necessitates the development of such facility in India.

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Expression of recombinant equine interleukin (IL) -4 and interleukin (IL) -10 and its effect on cytokine production by equine peripheral blood leukocytes

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Interleukin (IL)-4 and IL-10 are known to be the most potent cytokine that can initiate Th2 cell differentiation, promote humoral immune responses, enhancing class II expression on B cells. Studies on equines immune system and responses to pathogenic invasion are limited by the scarcity of horse-specific reagents. The aim of this study was to evaluate the recombinant horse IL-4 and IL-10 in terms of their ability to induce cell proliferation and to stimulate the secretion of other cytokines. The coding sequences of mature horse IL-4 and IL-10 were expressed in Escherichia coli and insect cells and purified. SDS-PAGE analysis displayed a molecular weight of 15kDa for IL-4 and 19kDa for IL-10. Biological activity of recombinant IL-4 and IL-10 was assessed by lymphocyte proliferation assay and real time RT-PCR. For lymphocyte proliferation assay, peripheral blood mononuclear cells were cultured at a density of 2×105 cells/ well in 96 well culture plate and stimulated with purified recombinant cytokines at different concentrations ranging from 250-1500ng/ml and with mitogens concanavalin A (5µg/ml), phytohaemagglutinin (10µg/ml) as positive control. Cell proliferation assay was performed by XTT assay after 72 h of post stimulation. Optimum and significant cell proliferation was observed at 250ng/ml concentration of recombinant IL-4 and IL-10. Real time RT-PCR revealed that, recombinant IL-4 was able to induce IL-10 transcripts by 128 fold and IL-2 & IL-18 by 32 fold, IFN-γ by 8 fold. On the other hand, recombinant IL-10 induced transcription of IL-4 by 256 fold, IFN-γ by 128 fold, IL-2 by 64 fold, IL-18 by 8 fold. Thus, recombinant IL-4 and IL-10 promote the development of a Th2 response. A functionally active recombinant equine IL-4 and IL-10 has been produced which will be useful for future immunological studies in horses.

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Expression of TLR4, TLR9, TNF-α and IL-1β in lungs following exposures to poultry barn air

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The present study was conducted on 40 healthy male mice aging 7-8 weeks to investigate the effects of single (one day) and multiple exposures (6 and 24 days) of poultry barn air on lungs of mice. The animals were divided into four groups viz. three treatments and one control (n=10; each group). Among the treatments groups, Group 1 was exposed for single day, Group 2 for 6 days (Monday to Saturday) and Group 3 for 24 days (4 weeks, Monday to Saturday). At the end of exposure period, five animals from each group were challenged with LPS @ 80µg/ animal and the remaining with NSS @ 80µg/ animal. Single and multiple exposures of 6 days resulted significantly increased ($p<0.05$) in the expression of mRNA of TLR 4 and IL-1β mRNA. The expression of TLR4 was down regulated after multiple exposures of 24 days whereas, expression of IL-1β mRNA was further increased after multiple exposures of 24 days. There was almost 10 and 50 folds increase in the expression of TLR9 while 2.5 and 3.5 folds increase in the expression of TNF-α mRNA following single exposure and single exposure followed by LPS challenge, respectively. Multiple exposures of 6 days down regulated the expression of TLR9 and TNF-α mRNA whereas there was increased expression following 24 days exposures. The data suggested altered pulmonary immune response following exposures to poultry farm air.

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Identification of polymorphism in TLR4 genes and their association with occurrence of Paratuberculosis in cattle

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Paratuberculosis (PTB), caused by Mycobacterium avium subsp. paratuberculosis (MAP), is a chronic, infectious, granulomatous, inflammatory bowel disease primarily infecting domestic ruminants leading to persistent diarrohea, progressive wasting and eventually death. TLR4 has been implicated in cellular recognition of mycobacteria, binding cell wall components including lipoproteins. The present study was undertaken with to investigate the presence of polymorphism in TLR4 gene and to evaluate association of these SNPs with occurrence of paratuberculosis in cattle. For this 213 cattle belonging to four breed groups and four different farms were subjected to Johnin PPD, ELISA test (indigenous as well as Parachek kit method), faecal microscopy and faecal culture for detection of presence of bovine paratuberculosis infection. Based on the screening results 51 animals each could be assigned to case and control population. All the further investigations were done on these 102 animals. Two SNPs in viz rs8193046 and rs8193060 associated with TLR4 gene were validated by amplifying two fragments and digested them with RE AluI and AciI respectively. Both SNPs were found to be polymorphic in case: control population. The association study was carried out by PROC LOGISTIC procedure of SAS9.3. The SNP rs8193046 yielded three genotypes viz. AA, AG and GG with genotype frequencies in case and control population were 27.45, 68.63 and 3.92 and 50.98, 29.41 and 19.61 respectively. These genotypes were significantly ($P<0.01$) different in case than control population. The ODDs of AA and AG genotypes verses GG genotype were 2.69 (0.52-14.04; 95 % CI) and 11.67 (2.276-59.78; 95 % CI) respectively. AA and AG genotype had more prevalence than GG genotype in case population.The SNP rs8193060 also exhibited three genotypes viz. CC, CT and TT with genotype frequencies in case and control population as 27.45, 47.06 and 25.49 and 45.10, 17.65 and 37.25 respectively. These genotypes were significantly ($P<0.01$) different in case as compared to control cattle population. The ODDs of CC and CT genotypes verses TT genotype were 0.89 (0.34-2.35; 95 % CI) and 3.90 (1.376-11.04; 95 % CI) respectively So CT genotype had more prevalence than CC and TT genotype. The linkage disequilibrium (LD) of different loci was tested using the Chi square probabilities, which revealed that both SNPs, were significantly associated with occurrence of paratuberculosis in cattle, tightly linked to each other.

Cloning and characterization of partial chicken Mx1 gene

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The blood samples from White Leghorn chicken were collected; polymorphic blood mononuclear cells were isolated and cultured in RPMI-1640 medium supplemented with fetal calf serum. The total RNA was isolated after induction with chicken interferon and partial cDNA of 225 bp was amplified with using a set of forward (- 5' TAT CGA GAA TTC ATG AAG GCC TAT TTC ACT G- 3)', and reverse (- 5' TCC GAT AAG CTT CTA GTA TTG GTA GGC TTT G -3') primers. The amplified product was cloned in pGEM-T Easy vector and sequenced. The nucleotide sequence was submitted to NCBI GenBankdata (Acc. No. - JF932501) and subjected to nucleotide BLAST analysis. The comparative sequence analysis revealed 33 mutation sites among WLH, RIR, Blackbone (wuji), Comtaier (native breed of china), quail and turkey species. Out of them, 23 were marked as singlet on variable sites and 10 were marked as parsimony informative sites. Only two of the mutations (rs313590198 and rs317224711) were reported earlier in public dbSNP database. However, within the chicken breeds, a single nucleotide variation at 23rd position could only be identified. At 23rd position, RIR have guanine and other breeds of chicken have adenine. The Mx1 gene reported to lack antiviral activity in WLH breed due to the presence of guanine at 23rd nucleotide position. But in our present study we found adenine at 23rd position which additionally confirmed that Mx1 gene of WLH breed have positive antiviral activity. Taking nucleotide substitution rate into consideration, Jukes Cantor model with lowest BIC score (362.702) was found to be the most suitable model for genetic distance estimation with 1000 replicates of bootstrap value. In the phylogenetic tree, the RIR was found to be in different cluster from other breeds of chicken due to the single nucleotide substitution of guanine in place of adenine. Other species viz. turkey and quail were present in different cluster due to their separate evolutionary lineage.

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Single Strand Conformation Polymorphism (SSCP) in Caprine mLYS (Intron-III) gene and its effect on serum lysozyme activity and SCC

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Lysozyme, a cationic hydrolase enzyme found in serum and body fluids elicits nonspecific bacteriolytic activity. The present investigation was undertaken in Indian goats with objectives to identify the allelic variants of lysozyme gene affecting serum lysozyme activity and somatic cell count (SCC). A total of 200 animals of two breeds (Barbari and Marwari) were screened by SSCP for a 230 bp fragment spaning over partial intron 3 to exon 4. A total of six genotypes (AA, BB, AC, AD, AE and AF) and six alleles (A, B, C, D, E and F) were observed, of which AD (frequency 0.44) and AE genotypes (frequency 0.34) were highest in Barbari and Marwari goats, respectively. The genotypes AE and AF were absent in Barbari goat, whereas, Marwari breed was devoid of allele AC. The mean somatic cell counts were lowest in AC (1.10±0.43) and AA (2.18± 0.31) genotypes in Barbari and Marwari goats, respectively. Likewise, mean serum lysozyme activity were highest in FF (2.62 ± 0.31) and AA (1.79± 0.21) in Marwari and Barbari goats, respectively. Although mean SCC and serum lysozyme activity varied in different genotypes but the difference were statistically non-significant. Differences at total 12 positions among the alleles were found in intronic region with no exonic variation. Allele A had maximum similarity with allele E (99.6%) and allele F (99.6%). Inter species comparison of goat with other species revealed that goat was having more similarity with Buffalo than cattle. Allele A was found to be much closer to buffalo followed by cattle as compared to other allele. The phylogenetic tree revealed that the alleles C and D formed a cluster and are closer to each other followed by allele E, F, B and A. Cattle and buffalo also formed a cluster and were equally distant from all the alleles of goat.

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MHC Class II DRB gene polymorphism in Rohilkhandi goats

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The MHC plays a central role in immune system and the DRB region that forms the antigen binding groove is one of the attractive candidate genes for association studies. In general, associations between the MHC-DRB1 gene and disease resistance are focused primarily on the second exon because of its high level of polymorphism and its important functional significance. Keeping in view the importance of this region, the 283 bp region of DRB1 exon 2 was amplified in Rohilkhandi goats by using a set of Forward (5'-TAT CCC GTC TCT GCA GCA CAT TTC-3') and Reverse (5'-TCG CCG CTG CAC ACT GAA ACT CTC-3') primers. The amplified product was then digested with TaqI restriction enzyme. The data on polymorphism was then analyzed using SAS 9.3 software package. The restriction digestion gave three genotypes AA (283/283 bp), AB (283/163/120 bp) and BB (163/120 bp) with frequencies 0.069, 0.319 and 0.612, respectively and two alleles A and B with frequencies 0.228 and 0.772, respectively. The polymorphic information content (PIC) value was low (0.290) with low level of heterozygosity (0.310), as less number of heterozygotes were observed. The Chi-sqaure test for Hardy Weinberg Equilibrium (HWE) showed the locus was in HWE ($P<0.05$). The data on polymorphism can be further utilized in association studies, in explaining the effect of genotypes on various indicator traits/phenotypes.

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Peripheral blood mononuclear cells of brucella infected goats showed more expression of tlr 4 and ifn γ.

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Toll like receptors (TLRs) recognize the unique pattern (PAMP)present on Brucella and alert the immune system by stimulating the inflammatory cytokines, andplay important role in the pathogenesis of brucellosis. In present study, weinvestigated differential expression of TLRs and inflammatory cytokines in the Peripheral blood mononuclear cells (PBMC) of Brucella infected goats. we collected blood fromrecently aborted goats and normal goats which were screened for B. melitensisby serum agglutination test (SAT), isolation of organisms, and the confirmatory identification of Brucella by PCR using 16S rRNA and Omp 31 genes as target. Total RNA was extracted from PBMC and, cDNA was synthesized and amplified by quantitative SYBR Green Real Time PCR by using highly specific primers. Analysis of Real Time-PCR results were done by comparative Cq method. Transcriptional expression of TLR 2, 4, 9 and IL 1,12&IFN γ were detected in PBMC. The mRNA expression of TLR 4 was found more and highly significant ($P<0.01$) (5 fold) as compared to TLR 2 &TLR9 (2 fold& 3 fold resp.)whereas among cytokines IFN γ expression was significantly high(7 fold) then the IL 1 and IL 12 (2 fold &5.66 fold resp.) in PBMC. Therefore, we found that B.melitensis induces significantly higher expression of TLR 4 & IFNγin PBMC because of its initial localization in bloodDue to more immunological involvement of TLR4 & IFN γcan be used as adjuvant or as marker for early diagnosis of caprine brucellosis.

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Expression and secretory profile of homologous fetal fibroblasts and Wharton's jelly feeder layers used for propagation of buffalo embryonic stem cells

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The present study examined the expression and secretory profile of four important signaling molecules BMP-4 (Bone morphogenetic protein 4), FGF-2 (Fibroblast growth factor 2), LIF (Leukemia inhibitory factor) and TGF-β1 (Transforming growth factor β1) in homologous buffalo fetal fibroblasts (BFF) and Wharton's jelly (BWJ) cell layers at different passages (0, 2, 4, 6, 8). Both BFF and BWJ feeder layers were successfully expanded upto 8th passage. Signaling molecules (BMP-4, FGF-2, LIF, TGF β1) and pluripotency associated transcriptional factors (Oct-4, Nanog, Sox-2, klf-4, cMyc, FoxD3) were successfully localized in the BFF and BWJ cell monolayers via immunocytochemistry. Quantitative real time PCR was done to determine the expression profile of signaling molecules at various passages. In order to analyse the secretory profile, medium was replaced by knockout Dulbecco's Modified Eagle's Medium (KODMEM) and serum replacement media (SR). Conditioned media (CM) from different passages (2, 4, 6, 8) was collected after 72 hours. Protein detection through ELISA for BMP-4, FGF-2, LIF and TGF-β1 was done. The effect of mitomycin-C (MMC) treatment on the expression profile of signaling molecules in the selected passages of BFF and BWJ revealed that MMC modulates the expression levels of these aforesaid molecules. Furthermore, the alteration caused by MMC treatment in expression profile varies with time. Expression and secretory profile revealed that CM collected from second passage of BFF and sixth passage of BWJ could be more appropriate for quality propagation of embryonic stem cells (ESCs) as aforesaid passages exhibited optimal levels of important signaling molecules.

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Dynamics of mitogen stimulated cytokine gene expression in peripheral blood mononuclear cells of indigenous and exotic breeds of pigs

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The current study was undertaken to analyze the mitogen stimulated cytokine gene expression in PBMCs of local and exotic breeds of pigs reared in Mizoram. Total RNA was extracted from PBMCs,incubated with mitogen PHA-P, after different time periods using Trizol method.qPCR was performed to analyze the transcript variants of four cytokines; IL-2, IFN-γ, IL-4, IL-10 and beta actin (housekeeping gene).The expression kinetics of these genes in the PHA-P stimulated PBMCs of local and exotic pigs in vitro revealed a wide difference between and within the two breeds of the pigs.The IL-2 transcript levels of both the breeds increased several thousand folds when compared to that of IL-4 and IL-10.The increasing levels of IL-2, a Th1 cytokine, even after 24 hr of stimulation when compared to the other Th1 cytokine i.e. IFN γ indicates that IL-2 is the major cytokine that responds majorly to an external stimulus and leads to a cell mediated immune response in both pig breeds.Interestingly IL-4 expression levels, a Th2 cytokine, were also found to be increased to thousand folds (~4000 folds at 8 hr) in the local pigs, but not in the exotic pigs, suggesting that both Th1 and Th2 mechanisms might be operational in PBMCs of local pigs. However, the levels of IL-10 in PBMCs of both breeds were limited, showed that there was no regulatory mechanism was operational in PBMCs or IL-10 was not sufficiently expressed to regulate either Th1 response or Th2.Our study helps in understanding the early step in the molecular basis of disease susceptibility in pigs.

Genomics and proteomics in genetic testing of animals

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Genetic testing of individuals is useful for checking DNA for mutations and to know whether those mutations are pathogenic or not. The presence and amplification of genetic material (DNA/RNA) is usually done by PCR/RT-PCR and the amplified product (DNA/cDNA) scanned for any possible mutation by sequencing. Further, the emerging diseases are identified and tracked by the animal breeders through sequencing the pathogen's genome using genetic tests to guide their work. Our modern understanding of evolution and development is based on these genetic tests.

The genetic tests are normally performed just once and the result forms a permanent part of an individual's health record. The genetic testing answers i)"Any" mutation in "any" gene?, ii) "Any" mutation in a "particular" gene? and iii) "Specific" mutation in a "particular" gene? For DNA testing, DNA can be obtained from any cell in the body. But, for testing some particular protein/ enzyme, specific cell is picked up in which that gene is expressed. However, RNA is much less convenient to obtain and work with. mRNA's are generally unstable and so, samples are handled and processed with extreme care to avoid degradation of mRNA. Further, the gene of interest may not be expressed in any readily accessible tissue.

The other methods of genetic identification viz. detection of heteroduplexes, microarrays, detection of methylation in DNA, scanning methods based on single-strand conformation analysis/ polymorphism, and protein truncation test are also used in specific cases. Changes to highly conserved amino acids may well be pathogenic. For non-conserved amino acids, only extreme changes (introducing cysteine or proline in particular, having strong effects on the protein structure) have much likelihood of being pathogenic.

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Characterization of Salmonella enteric using PCR based Genotyping techniques

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A total of 32 isolates of Salmonella enterica belonging to six different serotypes, Untypeable and roughwere genotypically characterized for strain differentiation by various PCR-based techniques viz. ERIC-PCR, REP-PCR, and BOXAIR-PCR. The serotypes included in the study included S. Enteritidis(09), S. Typhimurium (05), S. Virchow (04), S. Gallinarum (03), S. Reading (02) and S. Altona (01). The study determined the clonality of the strains in order to track the source of a bacterial isolate and determine the variability in strains in the area. We found all the three techniques to be highly discriminatory in differentiating between and within the serovars of Salmonella enterica. The value of Discriminatory index was same for ERIC and REP-PCR (0.995) while that of BOXAIR-PCR was slightly on the lower side (0.985). The comparative analysis of the three techniques showed that REP-PCR was best to differentiate between the strains of the S. Enteritidis as all the isolates produced different fingerprints while ERIC-PCR and BOXAIR-PCR could not differentiate among all of the strains. Although, the results for Untypeable strains showed all techniques could differentiate among the six strains but BOXAIR-PCR was highly effective as it could show highest average genetic diversity of 66.86% compared to ERIC and REP-PCRs which had average diversity of 47.2% and 54.78% respectively. For S. Typhimurium isolates, ERIC and REP PCRs were highly discriminatory as compared to BOXAIR-PCR. The fingerprints for four S. Virchow isolates showed highest diversity by BOXAIR-PCR compared to ERIC and REP PCRs. The Gallinarum strains were differentiated effectively by BOXAIR-PCR as the genetic diversity of 51.7% was noticed compared to ERIC-PCR which produced only 40.9% diversity. Also, REP-PCR was not able to differentiate between two Gallinarum strains. Among the two Reading and Rough isolates, we found BOXAIR-PCR highly discriminatory giving an average diversity of 72.2% among isolates. Thus, the results of the study showed a combination of PCR based technique can be highly discriminatory to differentiate between various strains from different sources helping in epedemiological studies.

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Detection of coagulase gene polymorphism in Staphylococcus aureus isolated from cattle and buffalo mastitic milk

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Mastitis causes considerable economic loss to the dairy industry. Although several bacterial pathogens can cause mastitis, Staphylococcus aureus is probably the most lethal agent as it causes chronic and deep infection in the mammary glands that are extremely difficult to cure. Among numerous molecular techniques, coagulase (coa) gene typing is considered a simple and effective method for typing S. aureus isolates for epidemiological studies. Coagulase is an extracellular protein encoded by coa gene that possesses a conserved and a repeated polymorphic region that can be used to measure relatedness among S. aureus isolates. A typing procedure based on polymorphism of the coagulase gene (coa) was used to discriminate 32 Staphylococcus aureus isolates obtained from cattle (16) and buffalo (16) mastitic milk. All the isolates showed coagulase production on plasma obtained from various species of animals and human. Amplification of coa PCR products (400bp, 510bp, 600bp and 650bp) were produced by cattle isolates and five different products (400bp, 510bp, 600bp, 650bp and 680bp) by buffalo isolates. From four coagulase types in cattle isolates five restriction fragment length polymorphism (RFLP) patterns were obtained and from five coagulase types in buffalo isolates six RFLP patterns were obtained. The coa gene amplicon of 600 bp was produced by maximum number of isolates.

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Detection of bovine brucellosis by bruce ladder and Hinic real time PCR

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Bovine brucellosis, mainly caused by Brucella abortus, is an important zoonotic disease leading to reproductive problem in animals that causes tremendous economic loses in endemic areas. A total of 40 suspected samples from cattle and buffaloes comprising of fetal stomach contents (5), placenta (8), vaginal swabs (16) and uterine fluids (11) were processed for isolation of Brucella. Three samples (one foetal stomach content from cattle and one each of vaginal swab from cattle and buffalo) were positive for isolation of Brucella. All the three isolates were confirmed as Brucella abortus by using Bruce ladder PCR. Further these isolates were confirmed by employing Hinic real time PCR using Taqman chemistry that amplified desired length of product having a Ct value between 14 to16.

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Molecular detection of an outbreak of anthrax in ovine in Tamil Nadu

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Anthrax is a bacterial zoonotic disease caused by B.anthracis. Sporadic outbreaks are noticed in certain pockets of the state of Tamil Nadu. In 2015, March an outbreak was noticed in a flock with 70 sheep in Sastramangalam village, Kancheepuram district of Tamil Nadu. About 13 animals died within 2-3 days with clinical signs of fever, bleeding from nose and anus. A presumptive diagnosis, based on by clinical signs and demonstration of encapsulated bacilli in polychrome methylene blue stained blood smear was done.

Blood smear, impression smear from dead animal and soil from infected areas were collected, subjected for spore extraction. Amplification of B.anthracis specific chromosomal gene Ba813- 152bp, and plasmid marker, cap gene – 264 bp, lef gene – 385 bp from the blood smear and impression smear by multiplex PCR confirmed the outbreak as anthrax. The molecular diagnostic method reduces the potential risk of culturing the clinical sample for confirmative diagnosis and environmental spread.

Rapid diagnosis by confirmative methods enabled quick implementation of control methods, thus containment of outbreak.

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Intragenic variations among Indian isolates of Bluetongue serotype 9 demonstrate Genomic Diversity

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Genetic characterization of Indian isolates of BTV-9 serotype using molecular diagnostic tools was done.Two BTV suspected field isolates (ONG3 and ONG9) were passaged serially in BHK21 cell line. The genomic RNA isolated by GIT lysis method was run in polyacrylamide gel. cDNA obtained by reverse transcription was amplified using group (targeting ns1 gene) and type specific PCR (targeting vp2 gene). The PCR amplicons were then sequenced and analyzed using different bioinformatics tools.

Both isolates produced characteristic BTV like cytopathic effects in cell culture. The RNA-PAGE revealed a BTV specific electrophoretic migration pattern of genome segments as 3:3:3:1. The minor differences in migration pattern of some genome segments of both isolates indicated the prevalent diversity. The specific BTV serogroup specific PCR amplification confirmed the isolates as BTV. Both isolates were typed as BTV-9 based on specific amplification using type specific primers. The sequence analysis further confirmed the result of type specific PCR and revealed the presence of variations in the vp2 gene sequence of both isolates. In silico restriction digestion indicated a difference in restriction site in vp2 gene of both isolates. The information generated in the study could be a valuable tool to establish the evolutionary relationship and to help in selection of more suitable vaccine candidates upon correlation between genetic variations and corresponding virulence and prevalence of a particular virus type.

Two field isolates were diagnosed as BTV using cell culture, RNA PAGE, group and type specific PCR and sequence based analysis. The RNA-PAGE and sequence analysis indicated the presence of Intragenic variations among both isolates of BTV9. The information will be helpful to establish phylogenetic relationship and epidemiological studies.

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Microarray analysis of host and bacterial gene expression in Pasteurella multocida infected mice model

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Pasteurella multocida causes an acute septicaemic disease, Haemorrhagic septicaemia, in cattle and buffalo with case fatality of 100%. Mouse is an established experimental model to study the pathogenesis of P. multocida. In the present study mice were infected with P. multocida (1.67 × 104 cfu - i/p) to evaluate host response to P. multocida infection by microarray transcriptome analysis at onset and late stages of infection. The global gene expression changes in the host and bacteria were monitored in spleen, liver and lung. On analysis, 9863 and 7640; 10261 and 15972; and 17181 and 15929, differentially expressed genes (DEGs) were identified in spleen, liver and lung at onset and late stages of infection, respectively. These significantly ($P \leq 0.05$) differentially expressed genes with $\geq \pm 1$ fold change were used for functional annotation using g:Profiler. Also, 1724 genes of Pasteurella multocida with a fold change $\geq \pm 1$ were significantly expressed in all the tissues. Functional annotation of these DEGs in mice showed enrichment of key pathways, Toll - like receptor, NF-κB, MAPK, TNF, Jak-STAT and NOD like receptor signaling pathways. As several genes were found to overlap across different pathways indicating a crosstalk, the protein - protein interaction networks among the DEGs involved in these key pathways at both time points for all tissues were considered to interpret the global changes due to infection. These changes indicated that P. multocida through its outer membrane component LPS is recognized through a series of interactions with TLR4, CD14 and LBP to trigger intracellular signaling via a series of reactions involving MyD88, IL-1, IRAKs and TRAF6 leading to the activation of NFκB and MAPK pathways and consequent release of inflammatory cytokines (IL1β, TNF, IL-6, TGFβ and IL-10). The secreted IL-1 and TNF bind to their receptors to activate NFκB signaling and MAPK signaling pathways and IL - 6 activates JAK-STAT pathway to induce expression of proinflammatory cytokines. This cytokine (IL1β, TNF, IL-6, TGFβ and IL-10) storm and the surge of TNF at both time points and in all tissue results in disruption of vascular endothelium leading hemorrhages and death.

Canine parvovirus NS1 protein induced apoptosis in HeLa cells causes accumulation of reactive oxygen species and follows intrinsic pathway of apoptosis

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Certain viral proteins have the ability to induce apoptosis selectively in malignant cellswithout harming the normal cells. The non-structural protein (NS1) of parvoviruses plays an important role in viral replication and is thought to be responsible for inducing cell death. In the present study, we report that expression of CPV2.NS1 in HeLa cells arrests cells in G1 phase of cell cycle and the apoptosis is mitochondria mediated i.e. it follows intrinsic pathway of apoptosis as indicated by mitochondrial depolarization, release of cytochrome c and activation of caspase 9. Treatment of cells with caspase 9 inhibitor Z-LEHD-FMK reduced the induction of apoptosis significantly. The extrinsic pathway of apoptosis seems to play no role as we failed to detect activated caspase 8. We also report that expression of CPV2.NS1 causes accumulation of reactive oxygen species (ROS) and treatment with an antioxidant reduces the ROS levels and the extent of apoptosis. Our results provide an insight into the mechanism of CPV2.NS1 induced apoptosis, which might prove valuable in developing NS1 protein as an oncolytic agent for cancer therapy.

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Rapid detection of infectious bursal disease in chicken by reverse–transcription loop mediated isothermal amplification (rt-lamp)

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Infectious bursal disease (IBD), an immunosuppressive disease caused by infectious bursal disease virus (IBDV) that affects chickens, results in significant losses in the poultry industry. Reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) was developed in this study for the detection of IBDV by targeting the RNA Dependent RNA Polymerase (VP1) gene due to their conserved nature among the IBDV isolates. RT-LAMP amplicons were successfully detected by naked eye using SYBR Green I (green in positive samples) and the amplicons was subjected to Agarose gel electrophoresis which revealed ladder like pattern and were confirmed by sequencing. The sensitivity of the RT-LAMP was compared with VP1 based Real time RT-PCR, RT-PCR targeting VP1 and VP2 regions and biological assay by Chorio Allantoic Membrane (CAM) egg inoculation. It was observed that RT-LAMP was more sensitive than RT-PCR and egg inoculation technique and equally sensitive to real time PCR. The VP1 region showed great specificity with no cross reaction to other common avian pathogens such as NDV, IBV, DNA of MDV, S. typhi, B. abortus and E. coli. Out of 36 field samples tested for IBDV by the VP1 gene based real time PCR 32 samples (88.8%) were positive, whereas, RT-LAMP and RT-PCR detected IBDV in 32 (83.3%) and 29 (80.5%) samples respectively. Biological assay using egg inoculation technique detected IBDV in 21 among 26 samples tested (80.7%). The results indicated that RT-LAMP assay is a rapid, simple and sensitive assay suitable for less-equipped laboratories and can be utilized in field conditions as a screening test for rapid detection of IBDV.

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Isolation and characterization of an Indian H6N2 avian influenza virus

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We detected mixed infection of a duck with H6N2 and H9N2 avian influenza viruses(AIVs) in Kerala, during November 2014. Both the viruses were isolated and purified by limiting dilutions and cross-neutralization with subtype-specific reference antisera. All the eight gene segments of the H6N2 virus were sequenced and analyzed. Presence of a single basic amino acid at the cleavage region (323PQIETR*GLF331) of the surface glycoprotein hemagglutininindicated low pathogenicity to chickens which was corroborated by intravenous pathogenicity index of 0 (out of 3). Presence of amino acids Q226 and G228 in the HA (H3 numbering) indicated the virus has the affinity for 2,3 sialic acid receptor.There is no deletion of amino acids in the neuraminidase, a characteristic of influenza A viruses from water fowl/duck which are not adapted to chickens. To know the source of the virus, phylogenetic analysis was carried out.In the HA and NA gene trees, the Indian isolate grouped closely with duck strainsof H6N6 from Japan (A/duck/Yamagata/061004/2014) and H3N2 from Norway (A/Teal/Norway/10_1037/2010), respectively.The internal genes PB2, PB1, PA, M and NS were contributed by H2N3, H4N9, H5N3/H2N9, H10N8 and H6N6 avian influenza viruses, respectivelyfound in different duckspecies (mallard and tufted) in Eurasian region, except the NP gene which was contributed by H5N2 virus from a gull.The phylogenetic analysis indicated that the virus is a reassortant and is of waterfowl origin. The H6 subtype has played an important role in the emergence of H5N1 virus that directly transmitted to humans from poultry in 1997 in Hong Kong leading to one-third mortality by contributing the NA gene. This is the first report of H6 subtype of AIV in India.Since this virus was isolated during the investigation of outbreak of H5N1 in Kerala State along with H9N2 virus, there is an urgent need for continuous monitoring and targeted surveillance of migratory birds and ducks for the emergence of any reassortant virus with pandemic potential similar to Hong Kong/97 virus.

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Genus specific Real-time PCR using IS711 specific primers and Taqman probe chemistry for identification of Brucella

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A total of four cultures of Brucella spp. were isolated and identified biochemically from 100 samples comprising of foetal stomach contents, vaginal mucus, uterine discharges and placenta. Hinic Real-time PCR using IS711 primers and Taqman probe chemistry was employed on all the four isolates for the detection of Brucella spp. All the isolates showed Ct values between14-16 thus confirming the isolates to be that of Brucella spp. The sensitivity evaluation of Real-time PCR was performed using the genomic DNA of standard B. abortus S19 and found that Real-time PCR could detect upto 0.35fg of DNA of Brucella. The specificity evaluation of Real-time PCR was carried out by screening some commonly available spp. of bacteria (E.coli, Salmonella, Staphylococcus, Proteus, Streptococcus, Pseudomonas, Klebsiella and Pasteurella) and no amplification was observed. Real time PCR was employed directly on DNA extracted from forty clinical samples of uterine discharges (22), vaginal mucus (10) and foetal stomach contents (8) collected from repeat breeding cattle and buffaloes and from cases of abortion. By Real time PCR, three samples (two foetal stomach contents and one uterine discharge) were found positive for Brucella spp.

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Multiplex PCR assay for detection of different bacterial pathogens associated with reproductive disorders in cattle and buffaloes

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A multiplex PCR assay was developed for detection of Brucella, Leptospira, Listeria and Mycoplasma spp. associated with reproductive disorders in cattle and buffaloes. Four pairs of oligonucleotide primers chosen to amplify target DNA regions viz. 31kDa MEM protein in Brucella, 16S rRNA gene in Leptospira, hlyA gene in Listeria and16S rDNA in Mycoplasma spp. produced amplicon sizes of 223-bp, 331-bp, 456-bp and 270-bp respectively. No amplification was observed when the multiplex PCR was tested against commonly prevalent and related species of bacteria. The maximum detection limit of the multiplex PCR assay was 116pg for Brucella, Leptospira, Listeria and Mycoplasma spp. The developed assay was tested for direct detection of the agents in 30 clinical samples of uterine discharges and foetal stomach contents collected from aborted animals and animals with reproductive disorders. By multiplex PCR, out of 30, nine samples of uterine discharges were positive only for Mycoplasma and one sample of foetal stomach content was positive for Brucella, Leptospiraa nd Mycoplasma. The multiplex PCR appeared to be a rapid and ideal method for detection of all four organisms simultaneously in a single tube reaction and hence can be used for routine diagnostics.

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Detection and Genotypic Characterization of Rotavirus from Bovine Calves

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Rotavirus (RV) has been considered as one of the most important cause of severe gastroenteritis among human infants and the neonates of most farm animal species worldwide. The present study was envisaged to detect RV from faecal samples of bovine calves and to investigate its genotypic distribution among bovine population during the period from June 2014 to May 2015. A total of 196 faecal samples were collected from both diarrhoeic and non-diarrhoeic calves with age group up to 4 months from different places of Assam, India. Screening of the 196 samples by RNA-PAGE revealed 26 (13.26%) to be positive for bovine RV with characteristic migration pattern of group A RV. The same number of samples screened by RT-PCR revealed 71 (36.22%) to be positive for both VP7 and VP4 genes of group A bovine RV. RT-PCR was found to be more sensitive than RNA-PAGE. All non-diarrhoeic samples were negative for RV. Nested-multiplex PCR when carried out using type-specific primers of common genotypes, only 24 out of 71 samples were found to be typeable for G10P[11] RV genotype while 47 samples were either un-typeable or mixed type.

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Relative expression of proinflammatory cytokines in milk somatic cells of subclinical mastitis affected buffaloes

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Mastitis is a global problem affecting dairy animals and thereby impeding the development of dairy sector by incurring heavy financial losses. Subclinical mastitis is more important than its clinical counterpart because it is 15 to 40 times more prevalent, difficult to diagnose, and usually persist for longer duration in the herd. Early diagnosis of subclinical mastitis is extremely important to check its further progress to clinical cases and restricting substantial monetary losses. In present study, the expression profile of proinflammatory cytokines including TNF α, IL-1β, IL-6 and IFN γ were investigated in milk somatic cells of subclinical mastitis affected buffaloes using qPCR assay. Relative expression levels of target mRNAs were calculated by comparison with the expression of the housekeeping gene, β-actin within each animal. Highest relative transcript level of TNFα (44.93 ± 1.16) was observed followed by IL-1β (19.92 ± 1.78), IL-6 (4.72 ± 1.96) and IFN-γ (2.55 ± 2.23) from milk somatic cells of subclinical mastitis with respect to healthy ones. Lowest upregulation of IFN-γ in the present study attributed to the isolation of only Gram positive bacteria (staphylococci and streptococci) from subclinical cases. Changes in expression pattern of proinflammatory cytokines of mammary gland in healthy and diseased animals reflect intramammary infections. Monitoring of cytokines involved in the regulation of immune responses during the infection is useful in determining cytokine markers that could be utilized as a forecasting tool in the early diagnosis of subclinical mastitis.

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Oligomerization of recombinant non-structural protein 3 N-terminus (NS3Nt) of bluetongue virus by Coiled coil motif (CCM)

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Bluetongue, an endemic disease of small ruminants in India, is an arthropod borne non-contagious haemorrhagic disease caused by bluetongue virus (BTV) which is the type species of the genus Orbivirus within the family Reoviridae. The virus is a non-enveloped with ten double-stranded RNA (dsRNA) segments packaged within a three-layered icosahedral capsid of seven structural proteins (VP1-VP7). The genome of BTV encodes four non-structural (NS) proteins; NS1, NS2, NS3/NS3A, and NS4, which are involved in replication, maturation, or export of virions from infected cells. However, the role of each protein in pathogenesis is needs to characterized systematically. In the present study, the NS3 protein sequences of bluetongue viral serotypes were analyzed for the presence of heptad regions and oligomer formation. Bioinformatic analysis of NS3 sequences of all 26 BTV serotypes revealed the presence of at least three coiled coil motifs (CCMs). A conserved α-helical heptad sequence was identified at 14 to 26 aa (CCM-I), 185 to 198aa (CCM-II), and 94 to 116 aa (CCM-III). Among these, CCM-I occurs close to the N-terminus of NS3 and was presumed to be involved in oligomerization. Furthermore, the N-terminus of NS3 (1M-R117 aa) was over-expressed as a recombinant fusion protein in a prokaryotic expression system (Escherichia coli). The rNS3Nt fusion protein (~32 kDa) was purified by affinity chromatography using Ni-NTA cartridges under denaturing/renaturing condition. The purified protein was confirmed by SDS-PAGE and Western blot analysis. Biochemical characterization of recombinant NS3Nt protein revealed that it forms SDS-resistant dimers and high order oligomers (hexamer and/or octamer) under reducing or non-reducing conditions. Coiled coil motifs are believed to be critical for NS protein oligomerization and have potential roles in the formation of viroporin ring/pore either with six/eight subunits and this is the first report toward characterization of CCMs in NS3 of bluetongue virus.

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Development of Real Time PCR for Diagnosis of Brucella Sp.

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Brucellosis is one of the zoonotic diseases of major concern and can cause huge economic losses to livestock industry. Serological tests and bacterial isolation are considered as the gold standard assays for diagnosis of Brucella spp. but they are time-consuming, hazardous and lack specificity. To control and eradicate a disease, a confirmatory diagnostic method which is sensitive, quick and specific is the foremost requirement. Therefore, in this study three real time TaqMan PCR assay to detect (IS711 gene based) and differentiate B. abortus (BruAB_0168 gene) and B. melitensis (BMEII0466 gene) were designed and evaluated in singlex and multiplex formats. All assays were found to be highly specific. It was observed that there was no detectable difference in sensitivity of PCR assays between Brucella species using BruAB_0168 and BMEII0466 gene specific primers (0.02fg). By contrast, a relatively lower sensitivity was observed for the IS711detection among Brucella species (0.2fg). R2 value and efficiency of these assays ranged from 0.992 - 0.998 and 2.00 – 2.06, respectively showing that these assays are highly efficient. All of these assays were also tested and evaluated both in SYBR green and TaqMan formats. These qPCR assays were found 100 times higher sensitive than conventional PCR assays. In conclusion, the present study showed that the developed real-time PCR assays are more sensitive, specific, have high reproducibility and repeatability and are faster than serological and conventional PCR methods for detection of Brucella spp. and for differentiation of B. abortus and B. melitensis. However, protocols should be carefully validated on more numbers of Brucella infected and -free samples before being implemented in routine diagnosis of brucellosis.

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Cloning and expression of protease inhibitor, cystatin of Haemonchus contortus and its expression kinetics during infection

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Haemonchus contortus is most pathogenic and predominant species among gastrointestinal nematodes causing anaemia and morbidity in small ruminants. Widespread emergence of anthelmintic resistance in H. contortus has led to alternative approaches of control like vaccine development. The present study was carried out to express 15 kDa cystatin, a protease inhibitor of H. contortus in heterologous system and studied its expression kinetics during experimental infection. A 369bp sequence coding for cystatin was PCR amplified from the mRNA and cloned into a PGEMTeasy cloning vector to facilitate sequencing and characterization. Nucleotide sequence analysis revealed 369 nucleotides encoding a 122 amino acid polypeptide with a stop codon. Subsequently, the entire gene was sub-cloned into prokaryotic expression vector pET32a and expressed with thioredoxin as a fusion protein, molecular weight of 35kDa. The IPTG induction of recombinant clones resulted in the expression of cystatin protein as analyzed by SDS-PAGE and confirmed by western blotting using Ni-NTA conjugate and infected sera. Approximately 35kDa protein was observed in SDS-PAGE and western blotting. The recombinant cystatin protein was further confirmed by incubating with enzyme cystein proteinase. The expression kinetic of cystatin was studied using different days haemonchosis infective sera. It was found that cystatin was expressed in early part of infection.

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Isolation and characterization of porcine adenovirus (padv) for possible use as a vector in vaccine delivery

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Adenoviruses have transitioned from tools for gene replacement therapy to bona fide vaccine delivery vehicles because of their ability to elicit potent cell-mediated and humoral responses making them ideal for use against viruses and other intracellular pathogens. Adenoviral vector based vaccines are likely to play a significant role in overcoming these problems in the future. The advantages of vectored vaccines include efficient antigen presentation and induction of both humoral and cell-mediated immunity. Adenovirus vectors are highly efficient for gene transfer in a broad spectrum of cell types and species. Objective of our study was to isolate and characterize non pathogenic porcine adenovirus for use as a vector for vaccine delivery. We collected forty nasal swabs and fecal samples from clinically healthy piglets below two months of age from Pig Breeding Farm, Hisar. DNA was isolated from samples by TRI reagent and screened by nested PCR for porcine adenovirus using primers designed against hexon gene. Out of forty nasal swabs and fecal samples, twenty two nasal swabs and seven fecal samples were detected positive for porcine adenovirus (PAdV), amplifying 344 bp fragment of hexon gene. PCR positive samples were passaged in Porcine Stable (PS) and porcine kidney (PK-15) cell lines for viral isolation. PAdV could be isolated from two fecal samples in PK-15 cell line, producing characteristic cytopathic effects and intra-nuclear inclusions. The isolation was confirmed by PCR and both the isolates (PF-3 and PF-7) were detected positive for PAdV at passage 3. There non-pathogenic porcine adenovirus are being further characterized for their use as vector for vaccine delivery.

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Genetic confirmation of Escherichiacoliobtained from different sample of water in nearby places at Bikaner

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Escherichia coli is the important pathogenic bacteria for human and animals which mostly causes urinary tract infection, diarrhoea, pyogenic infection and septicaemia etc.In the current study, 58 samples of water were collected from different places of Bikaner. These were studied and examined for the presence of Escherichia coli. Out of 58 samples, 47 were confirmed as Escherichia coli by molecular detection by using the species specific primers and produced amplicon of 662 bp.Escherichia coli was confirmed in 47 out of a total of 58 samples with a frequency of isolation of 81.03 percent. The present investigation revealed that water is the major sources of Escherichia coli infection in animal and human beings.

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Phenotypic and Genotypic Investigation of Antibiotic Resistance determinants in Salmonella enterica isolated from Poultry

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An investigation was conducted to study the antibiotic resistance pattern among 32 isolates of Salmonella enterica obtained from poultry against various antibiotics belonging to different classes. The isolates were screened for the presence of genes associated with antibiotic resistance of β-lactam antibiotics viz. blaTEM, blaSHV, blaCTX-M, blaMBL , blaOXA, sulphonamides viz. sul gene, quinolones viz. gyrA, parC. The study also screened the isolates for prescence of antibiotic ressitance gene mobilizing factors viz integrons. The results of the antibiogram showed phenotypic resistance pattern varied from 68.7% to 100% against the classical β-lactam antibiotics like Ampicillin, Oxacillin and Penicillin. For cephalosporin class, resistance was observed in the range of 9.37% to 28% with the highest resistance found against the 2nd generation cephalosporin followed by Ist, 3rd and 4th. The study detected blaTEM and blaCTX-M genes behind the β-lactam resistance in a total of 14 isolates. Eight of the 14 isolates possessed blaTEM while 5 had blaCTXM. One isolate was found positive for both blaTEM and blaCTX-M. Similarly, more than 50% of the isolates were found resistant against Sulfafurazole, Nalidixic acid, Clindamycin and Piperacillin. We found an absolute correlation in 17 sulfafurazole resistant isolates with the molecular determinant governing sulfafurazole resistance (sul gene). The investigation of quinolone-resistant isolates showed the amplification of parC and gyrA genes in all of the resistant strains with some point mutations in parC gene. The study detected presence of integrons in a total of 15 isolates. Thus, the present study found a high prevalence of multidrug resistance among various strains of Salmonella which was attributed to a number of antibiotic resistance genes. The presence of integrons is a matter of concern as they help in quick dissemination of the antibiotic resistance genes.

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Prevalence of Latent equine herpesvirus infection among horses in India

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Among nine members of the family Herpesviridae EHV1 is the globally significant pathogen causing respiratory disease, abortion and rarely paralysis. The ability of EHV1 to establish life-long latent infection in lymphoid and neural tissues with periodic reactivation and shedding is central to the maintenance of these viruses in horse populations. During latency, expression of viral genes is highly restricted, with the expression of few or no viral proteins. In the present study trigeminal ganglia and lymph nodes were collected from nineteen healthy horses at necropsy to detect EHV1 infection, its state (lytic, latent) and strain (neuropathogenic versus non-neuropathogenic). Trigeminal ganglia and lymph nodes from all nineteen horses were tested for EHV1 DNA for viral infection using nested-PCR and real-time PCR targeting the glycoprotein B (gB) gene. Trigeminal ganglia and lymph nodes of 12 and 3 horses respectively, were found positive by nested-PCR while trigeminal ganglia of 16 horses and lymph nodes of 12 horses were found positive by real-time PCR. All the positive samples for EHV1 DNA did not show transcriptional activity of gB which refers to latent state of these horses. Viral strain differentiation showed that none of the horses was infected with a neuropathogenic strain. The findings of the study establish that latent infection of EHV1 is highly prevalent in equines in India and is the cause of persistence of infection in equine population.

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Study of gene expression encoding 16 and 18.5kDa Proteins from Mycobacterium avium Subspecies paratuberculosis (MAP)

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Effort have been made to clone and express gene encoding sequences of M.a.paratuberculosis to study their immune reactivity. Primers were designed for ORFs retrieved from MAP complete genome strain k10 (locus tag MAP 0862 and MAP 1087). The PCR amplified product of each gene fragments were cloned into E. coli expression vector pQE-30 and the resultant constructs were designated as pQE 441and pQE 501. The positive recombinant clones on induction with IPTG expressed the protein corresponding to 16 kDa and 18.5 kDa protein when checked on SDS PAGE. The Hexa-Histidine spacer was introduce during expression the protein, in order to purify protein using Ni-NTA. The yield of the purified His-16 & 18.5 kDa protein were about 15 mg/L and from induced E. coli cultures harbouring plasmid pQE 441 and pQE 501. Antigenicity of these proteins were evaluated by western blot using sera from a small number of cattle infected with MAP. The immuno proteomic analysis of culture filtrate (CF) and cellular extract (CE) of MAP revealed that serological tests may be improved by the use of MAP proteins derived from culture filtrates and not from cellular extracts. Development of sensitive serological tests for the rapid identification of infected animals at subclinical stage requires expression and characterization of proteins or secreted early from post infection of MAP. Polyclonal anti sera raised against purified His16 kDa and 18.5 kDa protein reacted with induced E. coli whole cell lysate harbouring pQE 441 and pQE 501 and also with purified respective proteins on western blot. There was no cross reactivity of sera with the expressed proteins demonstrating the specificity of the sera raised using recombinant protein were recognized by rabbit hyper immune sera of the MAP culture filtrates and also by serum from a goat with clinical paratuberculosis.

Prevalence, isolation and strain variation of Fusobacteriumnecrophorum from ovine footrot in Kashmir, India

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A total of 450 swab samples from footrot lesions of naturally infected sheep were collected in all the ten districts of Kashmir valley and examined for the presence ofFusobacteriumnecrophorum(F.necrophorum).The detection of F. necrophorumwas carried out by polymerase chain reaction (PCR) targeting the leukotoxin (lktA) gene. The averageprevalence of F. necrophorum in footrot affected sheep was found to be 26%, with the highest prevalence of 34.78% being recorded from the districts of Kulgam and Pulwama, with lowest(20%) in Baramulla district. While Bandipora, Ganderbal, Srinagar, Anantnag, Shopian, Budgam and Kupwara districts recorded a prevalence of 23.80, 26.66, 25.00, 21.11, 32.14, 29.16 and 23.33 %, respectively. Polymerase chain reaction – single stranded conformational polymorphism (PCR-SSCP) of lktA gene revealed the presence of four variants designated as JKS-F1/ F2/ F3/ F4. Out of these JKS-F3 variant was the most frequent (76.06%), followed by JKS-F2 (13.67 %), JKS-F1 (8.54%) and JKS-F4 (1.70%). This seems to be the first ever report on prevalence, isolation and strain variation of F. necrophorumfrom India.

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Analysis of the goat PBMCs transcriptome in response to infection with virulent Peste-des-petits Ruminants (PPR) Izatnagar/94 isolate virus

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Peste-des-petits ruminants (PPR) is a disease of global concern as it leads to huge economic loss to farmers of several parts of the world. After successful eradication of rinderpest, PPR gains wide spread concern for its eradication due to similarity of the causative agents. PPR is an acute, highly contagious, devastating transboundary list-A disease of OIE that mostly affects goats and sheep. It is characterized by erosive stomatitis, muco-purulent nasal discharge, pneumonia, gastro-enteritis, fetid diarrhea. PPR is caused by Peste des petits ruminants virus (PPRV) belongs to genus Morbillivirus of family Paramyxoviridae. To elucidate the molecular pathogenesis, global gene expression of PBMCs of PPRV infected goats was studied through RNA-Seq. Goats were infected with virulent PPRV Izatnagar/94 isolate and blood samples were collected in every alternate day for PBMCs isolation. The PBMCs samples just prior to death time point were sent for RNA-Seq. The data were analyzed through different bioinformatics tools which revealed a total of 5801 differential expressed genes (DEGs). Out of these 3467 and 2334 were upregulated and downregulated respectively. These genes were enriched for GO (Gene Ontology) terms and found to be involved in several biological processes such as apoptotic and immune system process. The important pathways enriched in response to infection with virulent PPRV were apoptotic signaling, B-cell activation, IFN.

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Semi quantitative estimation of differential expression of interferon-gamma gene in cattle

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IFN-γ cytokine is an important part of the immune response to several intracellular pathogens including M. bovis mediated through its effect on macrophage activation, inducing the release of nitric oxide synthase and increasing the expression of MHC molecules. The detection of mRNA is quicker and reliable method of demonstrating cytokine induction as part of the immune response against Mycobacterium tuberculosis infection in cattle. In the present study, semi-quantitative PCR based estimation of differential expression of IFN-γ gene was studied in Sahiwal, Tharparkar, Vrindavani and Murrah breeds at 2, 4, 6, 8, 10 and 12 post hour stimulation of whole blood with bovine PPD antigen, keeping GAPDH gene as housekeeping gene. For IFN-g gene, expression seems to be more at all the post stimulation hours in comparison to the pre-stimulation, however in the control group (without PPD stimulation), the IFN- γ expression seems to be low and consistent at all the stages of RNA collection in native as well as crossbred cattle. The expression of GAPDH seems to be consistent at all the stages of RNA collection and apparently not seems to differ between the control and stimulated groups in native as well as crossbred cattle. No differences could be observed between the cattle and buffalo either for IFN-g or GAPDH expression. Further differential expression of IFN-g was studied in 4 TB positive and 4 TB negative cattle at 2, 4, 6, 8, 10 and 12 post hour stimulation of whole blood with bovine PPD antigen, keeping GAPDH gene as housekeeping gene. Till 12 h post stimulation, no apparent differences for IFN-γ could be observed between the Case and control group, however subsequent results revealed the more expression of IFN-γ at 24 h of post stimulation with PPD antigen in TB positive animals in comparison to TB negative animals.

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Brucella abortus S19Δper mutant is moderately attenuated and mounted protective immunity to mice

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Brucella abortus is a Gram-negative, facultative intracellular bacterium that causes bovine brucellosis affecting reproductive system of cattle and buffaloes. The disease is of zoonotic importance. Brucella abortus S19 is a smooth strain used as live vaccine against bovine brucellosis. Smooth lipopolysaccharide (LPS) is responsible for its residual virulence and serological interference. Rough strains are more attenuated and are non reactive to conventional serological antigen. We developed a perosamine synthetase gene deletion mutant, S19Δper mutant which exhibited residual O-polysaccharide (OPS). Phenotypic studies revealed little variation from wild type S19 suggesting reduction in the chain length of OPS instead of its complete absence. Perosamine synthetase, encoded by per gene, is responsible for the polymerization of OPS. Deletion of per gene resulted in balanced attenuation of S19Δper mutant with optimum immunogenic properties. It mounted strong immune response in Swiss albino mice and conferred protection similar to S19 strain. S19Δper mutant immunized mice produced higher levels of IFN-γ, IgG2a and thus has immune response inclined towards Th1 cell mediated immunity. S19Δper mutant displayed more susceptibility to serum complement mediated killing and sensitivity polymyxin B. Sera from S19Δper immunized animals did not show agglutination reaction with RBPT antigen and thus could serve as DIVA (differentiation of infected from vaccinated animals) vaccine. S19Δper mutant inherited desirable qualities of both smooth and rough strain displaying remarkable resemblance to S19 strain with improved properties of safety, immunogenicity and DIVA capability for control of bovine brucellosis.

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Detection of novel avian rotaviruses in poultry birds and their zoonotic importance

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Rotavirus is one of the most important etiological agents of acute dehydrating gastroenteritis in the young animals and humans. The mode of transmission of virus is through Fecal-oral route. The disease is prevalent in the cooler winter months. Rotavirus belongs to Reoviridae family. The virus is non-enveloped, with icosahedral symmetry and genome having eleven segments of double stranded RNA. Due to segmented nature of their genome, these viruses can reassort their genes during co-infection. This causes the viruses to emerge with new pathogenic strains. This can cause animal viruses to broaden their host range and affect the humans as well. RVAs are distributed widely among animals, and diarrhoeic disease is caused mainly in young animals. Evidence of zoonotic transmission of rotavirus strains between animals and humans arose from several epidemiological studies and genetic analyses of RVA strains. Based on antigenic and genome sequence properties, five rotavirus groups (A–E) and two tentative groups (F, G) can be distinguished. Recently, a classification system into the eight rotavirus species A–H has been proposed, based on genetic data of genome segment 6. The present study describes the detection of avian rotaviruses from intestinal contents of dead birds. The samples were processed to isolate viral RNA using TRI reagent (Sigmal Aldrich). The screening of viral RNA using RNA-PAGE followed by silver staining indicated presence of Group A, D, F and G avian rotaviruses. Further, after screening the PAGE positive samples were transcribed and amplified using RT-PCR with rotavirus specific primers. The group A rotavirus were confirmed as G6P11 genotype.

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Demonstration of foot-and-mouth disease virus infection specific nonstructural protein-antibodies in a vaccinated herd comprising cattle, buffalo and goats in India

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A serological study employing 3AB nonstructural protein ELISA and liquid phase blocking ELISA to assess foot-and-mouth disease virus (FMDV) non-structural and structural protein antibodies (NSP- and SP-Ab) was undertaken through sampling from all resident animals of a vaccinated herd comprising 943, 377 and 211 cattle, buffaloes and goats, respectively in north India. A considerable, though disparate proportions of animals (61.2% cattle, 29.2% buffaloes and 29.9% goats) were found to be positive for NSP-Ab suggesting an exposure to FMDV. From the age-stratified analysis of NSP-Ab prevalence, the probable time point of virus introduction could be predicted in retrospect to be around 8 months before sampling. The proportion of animals showing ≥1.8 log10 titre against all three serotypes in the vaccine varied from 3.2% to 32.9% in different species indicating poor vaccinal herd immunity, which presumably might have been the reason for the outbreak in the farm.

Epidemiology of group Arotavirus in bovine population of northern states of India disclosesits high prevalence

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Rotavirus (RV) is one of the major causes of non bacterial gastroenteritis in humans and animals. Neonates are highly susceptible toRV infection across the globe. It is observed that the rate of viral infection and RV mediated mortality is high in developing countries as compared to developed one. Among different RVs, group Arotavirus are most pathogenic and cause critical fiscal loss to backyard and unorganized animal farmsin India. The present study was envisaged to assess the prevalenceof RVA infections in bovine population from northern India (UP & Uttarakhand). A total of 273 faecal specimens were collected from clinical cases of diarrhea in calves during three different seasons of 2015. RNA-viral electrophoresis followed by silver staining indicated genomic migration pattern (4:2:3:2) indicative of mammalian RVA. These specimens were screened for RVA infection using diagnostic RT-PCR based on gene encoding VP6 coat protein. Overall, 273 specimens were screened,where 140 samples were found positive for RVA in diagnostic PCRexhibiting prevalence of 51.28% infection in bovine populationfrom this region of country during all three seasons.The incidence was relatively higher in summer (54.4%, 68/125 specimens), followed by rainy (49.29%, 35/71) and winter season (48.05, 37/77), respectively. The sequence based genotyping of VP7 and VP4 genesis underway. Monitoring the episode of RVA in animals and its genotypingis important for the control of diarrhea outbreaks.Our study also suggestsan unmet need for the implementation of preventive measures to avoid viral induced morbidity and mortality in bovine population.The genomic data from these RVA isolates can be well utilized for designing vaccine candidates against the highly prevalent genotype in bovine population.

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Synthesis and Evaluation of Gold nanoparticles for applications in Animaldisease diagnosis

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Nanotechnology is now playing crucial role in the field of healthcare research, diagnosis and therapeutic applications. Numerous nanotechnology based assays, sensors have been developed for diagnosis which employ gold nanoparticles because of their unique physicochemical properties, good biocompatibility, easy preparation, stability etc. The gold nanoparticles are used in the detection of disease biomarkers, various pathogens, biomolecules, antigen/antibody assays.The application of gold nanoparticles help in developing methods which are fast, more accurate and cost effective.In the present study, the gold nanoparticles were synthesized and characterized for their applications in diagnostic assay. The colloidal Gold nanoparticles were prepared from tetra chloroauric acid (HAuCl4.3H2O) by the citrate reduction method.Trisodium citrate (Na3C6H5O7.2H2O) was used for the reduction of Au3+ to Au0. After the reduction process, wine red colored nanoparticles were obtained and characterized by UV-visible absorption spectroscopy, Zetasizer and transmission electron microscopy. The nanoparticles were prepared using different concentrations of reducing agent. The colloidal gold nanoparticles of 12-15 nmsize were synthesized with uniform size distribution. The synthesized gold nanoparticles will be used for the preparation of lateral flow/ dot blot assay for preliminary diagnosis of Trypanosomosis in animals.

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Canine parvo virus in dogs: molecular detection and haematological alterations

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In India, haemorrhagic gastroenteritis in dogs is a multiple etiologicaldisease commonly seen in all breeds and age groups. Disease is mainly seen in two prominent clinical forms: Enteritis form which is characterised by vomiting, diarrhoea,dehydration, fever and low WBC count and Myocarditis form which affects puppies less than 3 month of age leading to mortality. Aim of the present study was to develop a simple, specific and rapid method for detection of parvo virus directly from faecal swab taken. PCR assay was developed using self-designed primers targeting NS1 gene. The optimized reaction mixture contained 10µl of 2X top taq master mix, 1 µl of each primer (10pmol concentration), 2 µl of DNA extracted from faecal sample and nuclease free water added to make reaction mixture 20µl. PCR amplification was done with initial denaturation at 95ºC for 5 minutes, 35 cycles each of denaturation at 95ºC for 50 second, annealing at 55ºC for 45 seconds and extension at 72ºC for one minute followed by final extension at 72ºC for 10 minutesyielding amplified productsof size 202 bp. Test was performed onten faecal samples of dogs affected with haemorrhagic gastroenteritis, reported in TVCC. Out of ten,Four (40 percent) were found positive for CPV Virus using PCR assay.Haematological alterations in positive cases revealedAnaemia (Hb-8.75 g/dl), with significantly higher mean total leukocyte count (TLC- 26.25 m/mm^3).The differential leukocyte counts were 30 ± 7% neutrophil, 50 ± 9.08% lymphocyte, 7 ± 2.72 % monocytes, and 2.10 ± 3.01 % eosinophils.Diagnosis of CPV infection by conventional methods like virus isolation is practically not possible for clinical diagnosis of parvovirus, thusdiagnostic approaches based on molecular methods like PCR proved to be moreapplicablefor detection of CPV in clinical samples and can pave way for quantitative estimation of virus based on real time assay as well.

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Methicillin-Resistant Staphylococci in Livestock – A Duplex PCR Assay for its Rapid Detection

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Antimicrobial resistance is a growing crisis with the emergence and spread of methicillin-resistant Staphylococcus aureus (MRSA) a significant concern for both animal health and public health. Besides, in recent years an increase in the incidence of methicillin-resistant coagulase-negative staphylococci (MRCNS) has amplified the problem. Investigations of methicillin resistance is majorly dependent on phenotypic methods which shows discrepancy with genotypic methods. To meet the need of a reliable rapid molecular assay to detect methicillin resistant Staphylococci in isolates obtained from various livestock samples, this study aimed to standardize a duplex PCR assay for simultaneous detection of mecA (methicillin determinant) and Staphylococcus genus targeting 16S rRNA covering S. aureus and 8 CoNS species (S. chromogenes, S. hominis, S. haemolyticus, S. hyicus; S. sciuri, S. auricularis, S. simulans; S. epidermidis). Evaluation of the primers with 28 ATCC reference strains for Staphylococcus genus and ATCC 33591-Methicillin Resistant S. aureus affirmed the specificity of the PCR assays. The assay was validated with 42 mec A positive Staphylococci isolated from the livestock species and their handlers covering Cattle (426), Pig (57), Sheep (20), Goat (54) nasal samples with 707, 77, 25 and 88, Staphylococcus isolates. Further partial 16SrRNA sequencing of 42 mec A positive Staphylococci isolates showed predominance of S. chromogenes followed by S. haemolyticus.The results suggest that the technique can be used for accurate detection of Methicillin Resistant Staphylococci and thus can be adapted for testing bacteriological safety of milk, for field applications, and in laboratories handling clinical samples. In addition, the assay could have an important impact on the choice of appropriate antimicrobial therapy, based on detection of the mecA gene.

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Gene cloning, expression and purification of recombinant OmpLA protein of Pasteurella multocida B:2

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Haemorrhagic secpticaemia (HS), an endemic disease of cattle and buffaloes in India, is an acute, fatal and septicaemic disease caused predominantly by P. multocida serogroup B:2 strains. The disease has been reported from almost all parts of the country despite periodic HS vaccination. P. multocida is known to possess several virulence factors which could be targeted for development of either diagnostics or vaccines. Outer membrane phospholipase A (OmpLA) is widespread among gram-negative bacteria including P. multocida. OmpLA on the phospholipids is known to catalyze the hydrolysis of acyl ester bonds in phospholipids to yield lysophospholipids and free fatty acids. It is likely to be involved in the membrane disruption processes that occur during host cell invasion. In the present study, we analyzed the gene sequence encoding for OmpLA protein and the gene encoding for mature OmpLA protein of P. multocida serogroup B:2 (strain P52) was amplified (~816 bp) cloned in to pET32a vector and over-expressed in recombinant Escherichia coli as a fusion protein with N-terminus histidine tag. The solubility analysis of induced cell lysate revealed the partitioning of rOmpLA protein in to insoluble fraction. The recombinant OmpLA protein (~49 kDa) was purified by Ni-NTA based affinity chromatography under denaturing/renaturing condition. The purified OmpLA protein was analyzed on 10% SDS-PAGE, which indicated presence of two species, monomer and dimer. Recombinant proteins were confirmed by Western blot. Further characterization and utilization of rOmpLA protein either for diagnostic or prophylactic purpose needs to be evaluated.

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Cloning and Sequencing of Thioredoxin Reductase (trxB) Gene of Salmonella enterica serovar Typhimurium Isolated from Poultry

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The aim was to clone and sequence Thioredoxin reductase (trxB) gene of Salmonella Typhimurium (ST) strain E2375 and to construct a phylogenetic tree based on the information obtained after aligning the trxB sequence with other serovars of ST.

Salmonella Typhimurium (ST) strain E2375was procured from National Salmonella Centre, Indian Veterinary Research institute, Bareilly, India. Genomic DNA was isolated from the bacteria followed by amplification of Thioredoxin reductase(trxB) gene by PCR. The gene was restriction digested and cloned into a vector pET28c(+). The cloned trxB plasmid was transformed into NEB 5-alpha cells. The clones were confirmed by restriction digestion and by PCR with gene specific primers for trxB gene. The gene was sequenced and submitted in gene bank. After multiple alignment analysis of that sequence by BLAST, a phylogenetic tree was constructed with the help of MEGA4.0 software.

An amplicon of size 993 bp was produced after PCR by using ST genomic DNA as template. An insert of desired size was released following restriction digestion. The sequence obtained after custom sequencing was found to have 100 % homology with similar sequences of salmonella serovars taken from BLAST analysis. The boot strap value of 500 replicates of this analysis was found to be 99.

Thioredoxin reductase (TrxB) is a flavoprotein which acts as an integral part of Thioredoxin (Trx) system. This Trx system produces reducing equivalent in various oxidation-reduction reaction which ultimately targets in various metabolic processes inside cell. This system, particularly TrxB must be playing role in combating various stress conditions confronted by ST inside its host. The sequence of trxB was found to be highly conserved among the salmonella serovars.

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Intracellular Delivery of Histidine and Arginine Rich Cell Penetrating Peptides into different cell lines

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Most bioactive macromolecules, such as DNA and RNA, cannot permeate into cells freely from outside the plasma membrane. Cell penetrating peptides are a group of short peptide sequences that are able to transverse the cell membrane for mediating gene into living cells. In this study we demonstrate two cell penetrating peptides 5H-R9-C and 7H-R9-7H-C. These peptides are synthesized by solid phase methodology and labelled these peptides by FITC and purified them by RP-HPLC. FITC labelled peptides are efficiently internalize into HeLa and Vero cells confirmed by fluorescence and confocal microscopy and flow cytometry. Further gel retardation assay, Nuclease protection assay studies are performed to observe the N/P ratio of complex formation. These peptides deliver pDS Red plasmid DNA into the cells at the 1:20 N/P ratio, observed by fluorescence microscopy. By quantitation of flow cytometry it was observed that more than 20% cells are positive by the expression of pDS Red. These results suggested that these peptides appear to be a promising tool for drug delivery.

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Use of Dot-ELISA for detection of FAV-4 propagated in different cell cultures

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The detection of FAV-4 antigens is more relevant in the diagnosis of infection than quantification of its antibodies in serum because of the acute nature of HPS. In this study, the suitability of a routine serological test like Dot-ELISA for detection of fowl adenovirus antigens was attempted.

Dot-ELISA was standardized and used for detection of fowl adenovirus serotype-4 (FAV-4) propagated in vitro in various cell cultures (both primary cell cultures and cell lines) at different passage levels. Hyperimmune serum was produced against group antigen of FAV-4 culture for use in Dot-ELISA by bulk production of FAV-4 in chicken embryo liver (CEL) culture. The FAV-4 was concentrated and partially purified by ultracentrifugation using sucrose cushion gradient. The partially purified virus was injected into two rabbits for production of hyperimmune serum. The dot-ELISA was carried out to detect FAV-4 in infected cell culture supernatants. The cell cultures used were chicken embryo fibroblast (10 passages of FAV-4), CEL and CEK (5 passages) and Vero, Hela, McCoy and BHK-21 cells (all 8 passages). Dot-ELISA was performed on nitrocellulose membrane sheet of 0.45 nm pore size using chloroform treated known positive liver homogenate (50% w/v suspension) as positive control and chloroform treated uninfected liver homogenate (50% w/v suspension) as negative control. Hyperimmune serum raised in rabbit at 1:80 dilution was used as primary antibody and goat raised anti rabbit IgG HRPO conjugate at 1:1000 dilution was used as secondary antibody. Dot-ELISA could detect FAV-4 in infected supernatants of CEF cultures from 6th-10th passage level, CEL and CEK cultures from 2nd-5th passage levels and Vero cells from 6th-8th passage level. FAV-4 could not be detected in the cell culture supernatants of Hela, McCoy and BHK-21 cells in all eight passage levels. This data corresponds to the virus replication and CPE production in these cell cultures. In conclusion, dot-ELISA could detect FAV-4 virus antigens in infected cell cultures.

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Foot-and-mouth disease incidences in haryana during 2013-2015

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FMD is described by OIE as the most serious transboundary animal diseases imposing a great risk to global security. It is one of the most contagious and the most devastating diseases of farm animals and mammals (Royal Society, 2002), affecting more than 33 species of animals. FMD is endemic in India and neighboring countries. The economic losses in India due to FMD are estimated to be over 20,000 crore per annum (ICAR, 2013). During the period April 2013 to March 2015, upon investigation in the state of Haryana, only four FMD incidences were recorded: two in the month of February 2014 in district Rewari and one each in March and April 2014 in district Hisar. No FMD incidence was recorded during 2012-13 in the state. The species affected were Cattle only in both the incidences in February 2014, while it was Cattle and buffalo in March 2014 and pig in April 2014. A total of eight FMD specimens were collected during the period under investigation. All the samples were typed using sandwich ELISA and/or multiplex RT-PCR. Virus isolated from all the incidences belonged to FMDV serotype O. The R0 value for the animals in Rewari district and Hisar districts against FMDV serotype O after Extended FMD-CP Phase-V and FMD-CP Phase-XVI vaccination was 4.16 and 7.14, respectively which was suggestive of marginal protection for animals of Rewari and good protection for Hisar district against virus transmission in the event of FMD incidence in the surroundings. The animals that suffered from FMD in Rewari and Hisar had been purchased from Rajasthan with no record/ history of FMDV vaccination. However, the single incidence of FMD in pig was due to the fact that FMDV vaccination is not practiced in pigs.

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Single domain antibody selected from phage display library of Indian desert camel (Camelus dromedaries L.) neutralizes LPS of E. coli O153 in chicken embryo model

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Endotoxin or lipopolysaccharide [LPS], the major constituent of outer membrane of gram-negative (G-ve) bacteria is the major pathogenic factor of G-ve sepsis. Endotoxin-induced death is associated with the host's overproduction of proinflammatory cytokines.Endotoxin- neutralizing polyclonal and monoclonal antibodies has been produced, but they are not free from problems. Recombinant single-domain antibodies (dAbs), derived from camelid and shark 'heavy chain antibodies' by phage display technology, are of small size, stable and soluble, which make them suitable for in vivo applications. The objectives of this study were to express and purify dAb clone 26 in E. coli BL 21 cell and to studyin vivo neutralization of E. coliO153LPS by dAb clone 26 in chicken embryo model. The dAb clone 26 was cloned into the pET-303CT vector expressed in E. coli BL 21 cell in LB-Amp-broth. The level of expression was confirmed by SDS-PAGE followed by western blotting using mouse anti-his HRP conjugate (Invitrogen). The protein was purified by IMAC chromatography and concentration was measured by BCA method. E. coliO153 LPS CELD50 value was calculated by Reed and Munch method and was found 6.31µg/ml. Three different combinations of purified E. coliO153LPS and dAb clone 26 were mixed and inoculated into the 10-day old chickenembryonated eggs (n=7) through CAM route. Results of this study showed significant reduction in mortality in all the dAb clone 26 treated groups and 100 percent survivability in group receiving 10µg E. coliO153 LPS dose with 50 µg dAb clone 26. This study suggested that dAb clone 26 neutralized the lethal effect of endotoxin in vivo and could be further developed as a drug to prevent or treat G–ve sepsis in human and animal.

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A comprehensive study on seroprevalence of bluetongue virus in Haryana state of India

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Bluetongue (BT), is a non-contagious, economically important disease of ruminants (particularly sheep, cattle and some species of deer), caused by the bluetongue virus (BTV), If uncontrolled it can cause huge economic losses to the country. Hence, it is important to understand the sero-epidemiology of this disease to execute proper control programs. This study was conducted to find out the seroprevalence of the bluetongue virus in Haryana state of India. A total 816 serum samples, 421 of cattle and 395 of buffalo were collected from 80 villages of Haryana in 2014. The sampling was done randomly to get unbiased results. The samples were evaluated by a competitive ELISA (VMRD, USA). The seroprevalence of BTV in cattle and buffalo were found to be 75.29% and 93.41%, respectively. Analysis of the data and the results obtained, indicate that the BTV virus is circulating in the cattle and buffaloes population of Haryana state of India.

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Comparison of serological response to FMD virus antigens in FMD-hemorrhagic septicemia- black quarter combined vaccine and FMD vaccine alone in small ruminants

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Small ruminants have epidemiological importance in spreading FMD though, the disease is subclinical. Sheep and goat should be brought in per view of regular vaccination and combined vaccines can be cost effective. Data regarding comparative efficacy of monovac and combined vaccine is completely lacking in case of small ruminants. In the present study, a total 43, sheep (n=16) and goat (n=27) were divided in three groups separately. Group 1 received combined triovac vaccine (FMD, HS and BQ), group 2 received monovac (FMD only) vaccine and group 3 were used as control. The serum samples were collected on day 0, 30, 60 and 90. Pre vaccinated serum (day 0) samples were analyzed for NSP based 3 AB3 DIVA ELISA to rule out presence of circulating virus without any overt clinical symptom. All the serum samples (0, 30, 60 90 days) were tested by liquid phase blocking ELISA (LPB- ELISA) to compare the seroconversion to FMD serotype O, A and Asia 1 in both the vaccine formulations and control. Both monovac and combined vaccine group showed humoral immune response to all the three serotypes of FMDV antigen, comparing with control group which peaked on day 30 and remained unchanged upto 60 days and declined after 90 days. No significant difference was observed between the monovac and combined vaccine neither in sheep nor in goats ($p<0.05$). Species wise seroconversion comparison showed no significant difference for both types of vaccine formulations ($p<0.05$). It can be concluded that combined vaccines (FMD-HS-BQ) is equally effective as monovac vaccine for immune response against FMD virus but, effect of different formulation on seroconversion of antigens other than FMDV need to be evaluated first, before advocating combined vaccination in field condition.

Sero-prevalance of JEV antibodies in different domestic animals in the state of Assam

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Japanese encephalitis (JE) is a mosquito-borne zoonotic disease that has pigs as the major amplifying hosts. Japanese encephalitis virus (JEV) belongs to the family Flaviviridae of the genus Flavivirus. It causes a leading form of viral encephalitis in Asia with recorded affected human cases are around 50,000, out of which 10,000 died below 15 years of age. Till recent past most of the JE cases were confined to limited districts in upper Assam. However, in recent years incidences of JE were recorded in new areas where pigs are not dominating livestock species. Therefore, in the face of JE outbreaks a sero-survey was carried out in different domestic animals like pigs, cattle, horse and in goat for presence of JEV antibodies using HI technique. A total of 179 serum samples were collected from different species of domestic animals and 34/179 (18.99%) animals were identified as seroconverter. Highest JEV antibody was detected in horses (60.0%) followed by in pigs (22.44%). No antibody could be demonstrated in cattle as well as in goat sera. Interestingly, in certain districts where human JE cases were recorded none of these domestic animals are found serologically positive for JEV antibodies. To bring a logical conclusion on the role of domestic animals in amplifying the JEV more samples need to be screened at different seasons.

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Seromonitoring in bovines vaccinated against foot and mouth disease in six model villages of Mathura District, Uttar Pradesh

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To ascertain the sero-conversion of inactivated oil adjuvant FMD Trivalent vaccine in field condition, 6 model villages from different blocks of Mathura district were selected by Collaborating Centre of All India Co-ordinated Research Project on FMD, DUVASU, Mathura during 16th phase of vaccination under FMD-Control Programme in the state (2014-15). Vaccination was carried out by the animal husbandry official in all the animals in these six villages in strict vigil of the project staff. Twenty random cattle and buffaloes were selected from each village for the controlled study. Pre vaccination serum samples were collected prior to vaccination with inactivated oil adjuvant FMD Trivalent vaccine against FMD types O, A and Asia-1. Post vaccination samples from the selected animals were collected after one month. A total of 120 pre vaccinated and equal number of post vaccinated bovine serum samples comprising of 71 samples from buffalo and 49 samples from cattle were tested for antibody against O, A and Asia-1 serotype by single dilution-Liquid Phase Blocking ELISA developed and standardized by the Project Directorate on FMD, Mukteswar, Uttarakhand. The overall percent of animals demonstrating protective antibody titres (≥1.8 log10) against FMDV serotypes O, A and Asia-1 in pre-vaccinated cattle and buffalo was 65.8%, 53.3% and 49.2%, respectively. A significant rise in herd protection level was noticed after vaccination as the percentage of animals protected against serotypes O, A and Asia-1 virus after vaccination showed an increase to 78.3%, 67.5 % and 74.2%, respectively. The overall vaccination response against serotype O, A and Asia-1 is comparable in both cattle and buffalo and no significant difference could be observed. A higher level of anti 'O' antibody was observed both in pre and post vaccinated serum samples in comparison to serotype A and Asia-1.

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Production and Characterization of monoclonal antibodyagainstPasteurellamultocida

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Haemorrhagicsepticaemia (HS), a type of pasteurellosis, is an infectious and acute disease of domestic animals. The disease occurs mainly in Asian and African countries. Most isolates of P. multocida associated with HS, have been found to be B:2 type in Asia and E:2 type in Africa. In India, H.S. is prevalent in almost all states of the country with a high bovine mortality. In the present study, hybrids of Sp2/O myeloma cells of Balb/c mouse origin and B cells of Balb/c mouse immunized with PasteurellamultocidaB:2 (Hemorrhagic septicemia (HS) of cattle and buffaloes causing bacteria) bacterial sonicated antigen, were fused with chemical fusogen Poly ethylene glycol. A tetrapoloid cell line was thus developed with property of secreting anti Pasteurellamultocida monoclonal antibody and immortality. The hybridoma cells were cloned to achieve monoclonality of the antibody secreted by the hybridoma cells. The monoclonal antibody was produced in vivo in mice in ascites fluid.

The monoclonal antibody was characterized for its isotype by Mouse monoclonal antibody isotyping kit (Catalogue No. ISO-2), biological property and its epitope on the bacteria. The monoclonal antibody designated HS-1 belongs to isotypeIgG2b.This monoclonal antibody reacted with a 17.7 kDa protein band. Ruffoloet al., 1997 reported that 17.7 kDa protein identified type 4 fimbriae (PtfA Type 4 fimbriae, pili). Type 4 fimbriae pili (PtfA) are long, filamentous appendages that have been identified in many species of Gram negative bacteria. They are often key structures involved in the attachment of bacteria to host cell surfaces. Fimbriae consist of repeated fimbrial subunits which range in molecular mass from 15 kDa to 20 kDa and whose N terminal sequences are highly conserved. These can be classified into two groups, the classical type 4 pili and type 4-like pili (or type 4B). Type 4 fimbriae have been used as vaccines against ovine footrot and bovine keratoconjunctivitis caused by Dichelobacternodosus and Moraxella bovis, respectively.

Further, this monoclonal antibody proves to be an important tool in studying mechanism involved in attachment of bacteria to the host cell surfaces, interaction of appendages and can be used as vaccine agent as used in ovine footrot and bovine keratoconjunctivitis.

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Surveillance of Japanese Encephalitis Infection in Animal Population of North-East India

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Japanese encephalitis (JE) is a mosquito-transmitted viral disease of man and animals, caused by JE virus (JEV) belonging to genus Flavivirus of the Flaviviridae family. JEV causes clinical disease primarily in pigs and horses. JE is principally a disease of rural agricultural areas where vector mosquitoes proliferate in close association with pigs and birds. The most common symptom of JEV infection in pigs is the birth of stillborn or mummified fetuses, usually at term. By virtue of high levels and lengthy periods of viremia after infection, pigs are the principal hosts for viral amplification. JE occurs only sporadically in horses with low morbidity (0.045-0.3%) and fatality rates ranging between 5 and 30%. JEV infected equines may develop clinical signs of fever, anorexia, depression, impaired locomotion, impaired vision, paresis, paralysis, coma and death. In addition, antibodies to JEV have been observed in other animals like bovines, sheep and goat. JEV infection is highly endemic in North-Eastern region (NER) of India. Outbreaks of JEV occur frequently in human population; however, there is not much information available about the status of JEV infection in animals in NER. In the present study, we conducted a JEV serosurveillance in equines, pigs, goats and bovines in NER to know status of JEV infection. A total of 219 serum samples (20 equine, 138 pigs, 41 cattle and 20 goats) were collected during 2015-16 from NER and tested for JEV antibodies by hemagglutination inhibition (HI) and virus neutralization test (VNT). A total of 46 of 219 (21%) samples were positive for JEV antibodies. A total of 34 out of 138 pigs (24.6%) and 12 out 20 (60%) equines in NER were detected seroprevalence for JEV. None of the cattle and goat serum samples was positive for JEV antibodies. In addition, a multiplex reverse transcriptase PCR (mRT-PCR) was standardized for diagnosis and differentiation of Japanese encephalitis and West Nile virus infection in animals. The mRT-PCR is highly specific and sensitive assay and is being used for detection of JEV infection among animal and mosquito samples collected from NER.

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Comparison of Humoral response in calves following intranasal challenge with P. multocida B:2 and subcutaneous vaccination with formalin killed alum adjuvant vaccine

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Indirect Enzyme Linked Immunosorbent Assay (ELISA) was carried out to compare the differences in antibody response in serum and nasal secretions to intranasal challenge with P. multocida B:2 and to subcutaneous vaccination with formalin killed alum adjuvant vaccine of P. multocida. Four month old, nine calves, were divided into three groups of three animals each. Calves in Group 1 (Infected group) were challenged intranasaly with 5 ml of 3×109 cfu/ ml/ nostril, calves in Group 2 (Vaccinated group) were vaccinated subcutaneously with formalin killed alum adjuvant vaccine against P. multocida while calves in Group 3 (Control group) were the unexposed control. Serum and nasal secretions were collected at 0, 7, 14, 21, 28, 35, 49 and 63 days post challenge. Serum was subjected to indirect ELISA to determine the levels of IgG and IgM while nasal secretions to determine IgA antibody levels. IgG level in serum increased rapidly in infected group and reached to significantly high level at day 14 and remained high till day 28, before declining slightly the following days of the study. The IgG level in serum of vaccinated group increased gradually till day 35 and then increased rapidly to significantly high level on day 63. IgM levels increased gradually in both infected and vaccinated group compared to control group. IgA antibody level in nasal secretions in infected group increased rapidly to reach a significantly high level as early as day 14 post exposure and remained high throughout the study period compared to control and vaccinated group. IgA level remain almost same in nasal secretions of control and vaccinated group.

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Seroprevalence study of cattle brucellosis using Rose Bengal plate test and milk ring test

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Brucellosis is a severe disease of farm animals that has considerable animal health, public health, and international trade cost. Brucella abortus is common cause of brucellosis among cattle population. Brucella species are small, Gram negative, non motile, nonspore forming, rod-shaped (coccobacilli) bacteria. Cattle infected with B. abortus have high incidences of abortions, arthritic joints, and retained placenta. Rose Bengal Plate Test and milk ring test is used for screening and monitoring the dairy herds at regular intervals. Seroprevalence study of Brucella infection was carried out in cattle population of Vindhya region districts Rewa, Satna, and Sidhi in Madhya Pradesh. Total 240 serum samples and 240 milk samples from cattle of Vindhya region i.e. 80 samples from unorganised farm of each district were collected. Among 80 samples of each district Rewa, Satna and Sidhi 19(23.75%), 15 (18.75%) and 28(35.00%) were positive with RBPT and 4 (5.0%), 5(6.25%) and 2(2.5%) were positive with MRT respectively. In cattle out of 240 serum samples tested 62(25.83%) were positive by RBPT whereas 11 (4.58%) was found to be positive by milk ring test in 240 milk samples. Further 6(9.67%) out of 62(25.83%) RBPT positive cattle was found to be positive by milk ring test also. Considering the fact that RBPT is highly specific test the overall positive cases detected is 25.83% thus revealing that there is marked occurrence of Brucella antibodies in cattle population in Vindhya region, depicting the presence of Brucella infection in population and posing a threat for disease condition.

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Fluorescence Polarization Assay: A DIVA test for the diagnosis of brucellosis

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Fluorescence polarization assay (FPA) is a rapid homogeneous assay, which works on the principle of differential rotation of antibody bound antigen and unbound antigen in the liquid medium measured using plane of polarized light. In the present study, fluorescent polarization assay for the diagnosis of the brucellosis is standardized using o-Polysaccharide (from B. abortus S99), conjugated with fluorescence isothiocyanate-I (FITC) and purified by DEAE-Sephadex A25 column to yield tracer. Assay is standardized by optimizing various concentrations of tracer, buffers and serum dilutions. In first stage evaluation, a panel of 400 bovine serum samples (Positive=60, Negative=240 and Vaccinated=100) sera samples were screened to arrive a positive and negative cut-offs. ROC curve analysis using MedCalc 15.8 software has revealed 82.64% of area under the curve, with 88.89% and 93.75% of relative sensitivity and specificity respectively in comparison with RBPT.

In second stage evaluation, a total of 420 B. abortus S19 calfhood vaccinated serum samples collected on 0th, 14th, 28th, 45th, 60th and 90th days of post vaccination (Cattle=50 and buffalo=20 in six collection intervals). The samples were tested by RBPT, SAT, iELISA, c-ELISA, indigenously standardized FPA and commercially available FPA reagents. All the samples from day 14th–90th day were found positive by RBPT and iELISA with a varying SAT titres of 1: 2560, 1:1280, 1:640, 1:160 and 1:80 IU/ml on 14th, 28th, 45th, 60th and 90th day of post vaccination. In c-ELISA, animals were detected as positive till 45th day of post-vaccination and were found negative 60th day onwards as per manufacturers result interpretation. On the contrary, all the 420 control and vaccinated animals were FPA negative except 3 animals which were positive by both indigenously standardized FPA and commercial FPA reagents.

There was an 100% agreement between the indigenously standardized FPA and commercially FPA reagents was observed. These preliminary results clearly indicated FPA as a more potential DIVA test over cELISA as animals from day 14th of post vaccination can be differentiated from infected animals. In the current Indian situations, where the vaccination campaign for brucellosis are under operation, FPA could serve as a good diagnostic tool in resolving the antibody responses of vaccinated and infected animals. FPA test is OIE recommended cost effective test for surveillance of brucellosis and the test with DIVA status will definitely help to arrive accurate epidemiological status of brucellosis where brucellosis control programs is implemented.

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Development of peptide ELISA for serodiagnosis of Equine Herpesvirus

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Equine herpesvirus 1 (EHV1) is a contagious equine viral pathogen that causes significant economic losses to the equine industry and produces well documented syndromes of respiratory disease, abortion, neonatal foal death and myeloencephalopathy. There are 12 glycoproteins (gB, gC, gD, gG, gI, gH, gK, gL, gE, gM, gN, gp2) on the envelope which play a key role in the infection and immunity of EHV1 and out of these glycoproteins, gB, gC, gD, gG and gE are recognized as the most immunodominat antigens generating antiviral immune response against EHV1. During last decades, various tests for serodiagnosis of EHV1 have been developed like AGID, serum neutralization test etc. but these tests have a problem of cross reactivity with other members of Equid herpesvirus family. The synthetic peptides developed based on the sequences on antigenic epitopes can be used to develop peptide-based assays. The peptide-based assays offer several advantages over the use of the whole virus or recombinant protein as they were chemically defined, safe, non-infectious specific antigens as well as immunogens. In this study we identified a type specific epitope corresponding to immunogenic region of glycoprotein E of EHV1 using insilico analysis and designed synthetic peptides based on corresponding region and evaluated it for serodiagnosis of EHV1 by ELISA. The synthetic peptide was evaluated for n=70 (43 known positive and 27 known negative) EHV1 samples, out of which 33 (43.18%) and 37 (56.81%) were found positive and negative respectively, by peptide ELISA. In addition to these we have tested vaccinated EHV1 samples (n=48), all of these samples gives positive result in peptide ELISA. Further, development of synthetic peptide based ELISA is under progress.

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Faliure of Passive Transfer (FPT) of Immunity in Foals

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Foals are entirely dependent on antibodies absorbed following ingestion of mare's colostrum in the first few hours of life for protection against infectious diseases as they are born with negligible amount of antibodies. Mares produce colostrum only during the last 1 to 2 weeks of gestation as in this period; antibodies are actively transported from their blood and concentrated in the mammary gland. After nursing the colostrum, specialized cells that line the small intestine of the newborn foal absorb the antibodies and transfer them into the foal's blood. Absorption of antibodies by these specialized cells is greatest during the first 6 to 8 hours after birth and stops by 24 to 36 hours of age. Failure of passive transfer (FPT) of antibodies occurs in 10 to 20% of newborn foals. A foal greater than 24 hours of age is considered to have failure of passive transfer if circulating antibody levels are less than 400 mg/dl. A level of 400 to 800 mg/dl is considered partial failure of passive transfer and a blood IgG concentration greater than 800 mg/dl is considered adequate. The most common causes of FPT are poor quality colostrum and premature lactation. Mares that drip or run milk for several hours prior to giving birth are losing colostrum that is vital to the survival of the foal. Other causes of inadequate transfer of antibodies include failure of colostrum production (i.e. due to fescue toxicity), inability or lack of desire by the foal to nurse, prematurity, dysmaturity, foal rejection by the mare and failure to absorb antibodies that are ingested. Early testing of a newborn foal can detect potential cases of failure of passive transfer in time for oral supplementation with frozen colostrum to be effective. A timely diagnosis and early therapeutic intervention will often circumvent a life-threatening medical crisis in a young foal.

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Streptococcus uberis Induce Inflammatory Responses in a Mouse Intramammary Infection Model in a strain directed manner

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Streptococcus uberis causing mastitis is an emerging challenge to the dairy industry. The host response to S. uberis is poorly understood. In this study, two epidemiologically important strains of S. uberis (SU1ST439, sua+and SU2ST475, sua-) isolated from a subclinical case of mastitis with specific and unique multi locus sequence types (ST), Pulsed Field Gel Electrophoresis (PFGE) pulsotypes and virulence profiles were assessed by intramammary inoculation in lactating mice to uncover the host immune responses that could play a role in mastitis. Mouse mammary glands were challenged with live S. uberis strains, and tissues were collected at regular time intervals post-inoculation (2 h, 4 h, 8 h, 12 h, 24 h and 48 h). Temporal mRNA expression of key inflammatory mediators involved in the immune defense related to pathogen sensing, inflammation and immunity (IL 2, IL 4, IL 6, IL 12, TNFα, IFNγ, GMCSF, TLR 2, TLR 4, TLR 9, TLR 11, TLR 12, CD 14, IL 1β, RANTES, Lactoferrin, and CXCl1) was evaluated by reverse transcription and probe-based quantitative Real-Time PCR. Compared with the PBS control, the transcription of key inflammatory genes was observed to be differentially expressed in a temporal manner, with 24 h PI serving as a critical point for the deviating behavior (SU1 versus SU2). Relative mRNA levels were higher ($p<0.05$) in response to SU2 compared with SU1. Furthermore, the predicted gene ontology (GO) and pathway analysis of this tested pool of genes revealed diverse biological processes under the influence of these genes. By employing the gene expression data, we delineated the gene regulatory networks which showed that SU1ST439, sua+ did not significantly alter the expression of inflammatory genes, thereby favoring its persistence in the host environment; in contrast, SU2ST475, sua- elevated gene expression and effectively led to pathogen clearance or immune surveillance. Our results expand the available information and provide a firm foundation for further investigations to gain control over this frequently detected pathogen in mastitis.

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Disease resistance in livestock: challenges and opportunities

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Livestock health and well being have become increasingly important issues for livestock keepers and consumers. Animal health is influenced by many factors including genetics, nutrition, age, stress, management system, season, pathogen dosage, immunological background, epidemiology, animal biological status, and many other variables. These factors interact, thus confounding our ability to understand the mechanisms of disease resistance. Infectious disease adversely affects livestock production and animal welfare, and often has major impacts upon human health and public perception of livestock production. Breeding for improved disease resistance has become perhaps the major challenge facing animal geneticists. The most amenable endemic diseases to genetic selection are likely to be mastitis, bovine leukaemia, gastrointestinal (GI) parasitism, tuberculosis (TB) and paratuberculosis in cattle; and mastitis, GI parasitism and footrot in sheep. The benefits of successfully improving the resistance of animals to an infectious disease are manifold, including increased efficiency and productivity, and hence a reduced environmental footprint, improved animal welfare, reduced reliance on other disease-control measures and improved public perception. Further, despite its apparent benefits, its sustainability is often questioned due to the potential of pathogen or parasite evolution; and the role of host genetics within integrated disease-control systems is often unclear. Applications of integrated strategy for utilizing molecular markers help to assess genetic diversity, as a tool for designing disease genetics studies, and also for simultaneously detecting and exploiting genetic variation in resistance. This strategy could play a major role in understanding the genetic control of resistance to infectious disease and in solving practical issues that could potentially undermine the sustainable development of livestock production systems.

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Neonatal Fc receptor of IgG (FcRn) and their role in Failure of Passive Transfer (FPT) of Immunity

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In ruminants syndesmochorial type of placenta is present where as in primates including human haemochorial type of placenta is present. In syndesmochorial placenta there is no direct contact between foetal and dam's circulation and hence, there is very less transfer of immunoglobulins through placenta. Consequently, a new born calf contains negligible amount of immunoglobulins in its serum and depends on colostrum for maternal immunoglobulins. Ingestion of maternal antibodies via colostrum is essential for the well being and survival of calves. Main classes of antibodies/ immunoglobulins present in colostrum are IgG (86 %), IgM (7 %) and IgA (7 %). This transfer of maternal immunoglobulins from dam to calf through colostrum is called passive transfer of immunity. Any inadequacy to ingest or absorb maternal immunoglobulins is called failure of passive transfer of immunity which can be technically defined in terms of IgG concentrations as IgG levels less than 10 mg/ml in serum of calves at 24 hours age. The amount of passive immunity acquired by newborn ruminants depends upon several factors including age at initial feeding, the mass of colostrum ingested, concentration of immunoglobulins in colostrum and degree of selectivity exerted by intestinal epithelium. All the above mentioned classes of Igs are absorbed non-specifically through receptors present in Payer's Patches in intestines of newborn calves. These receptors are known as FcRn (neonatal Fc receptors). These are also found in mammary glands, placenta, vascular endothelium, blood brain barrier in CNS, kidneys and lungs. Crystal structure of FcRn revealed that it is a heterodimer of two polypeptide chains i.e., MHC class-I like heavy chain encoded by FCGRT gene located on 18th chromosome in bovines and β2 – microglobulin light chain encoded by β2M gene located on 10th chromosome in bovines.

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Serum Status of Trace Elements in Indigenous Donkeys from two donkey fairs

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Trace elements are dietary minerals that are needed in a very minute quantity for proper growth, development, and physiology of animals. Serum profile of trace elements (copper, cobalt, zinc and iron) was carried out on donkey serum samples collected from donkey fairs at Vautha, district Ahmedabad (Gujarat) and Shergarh, district Baran (Rajasthan). Serum copper and cobalt levels were significantly low in Vautha (25.6 µg/dl and 7.6 µg/dl) donkeys than the Shergarh donkeys (30 µg/dl and 44 µg/dl) while serum Zinc levels were significantly high in Vautha donkeys (35.5 µg/dl) than the Shergarh donkeys(25.2 µg/dl). No significant difference was found in serum iron level between Vautha (289 µg/dl) and Shergarh (235 µg/dl) donkeys. Vautha is situated in Gujarat near Dholka of Ahmedabad district and donkeys from various areas (Kutch, western Gujarat) of Gujarat are brought in the donkey fair for sale. In Gujarat, about 30 per cent of the soil contains available cobalt below the minimum level for the normal physiological requirements of grazing animals. Donkeys of Vautha also had significantly low (P value ≤ .001) level of cobalt than the Shergarh fair donkeys indicating soil deficiency of cobalt in the region. Shergarh is located in Baran district of Rajasthan and most of donkeys in this donkey fair were brought from adjoining districts of Madhya Pradesh and Baran district of Rajasthan. These area lies in central highlands (Malwa region) and 64% soil of this area is reported deficient in Zinc and this difference was observed in serum Zinc levels of donkeys, Shergarh donkeys were found with significant low Zinc level than Vautha. Iron levels were within the normal range as compared to other equines and did not vary between the animals surveyed at two different locations. Copper was low ($P<0.05$) in Vautha as compared to the levels in donkeys at Shergarh, however the levels were much below the normal range reported in horses. The higher level at Shergarh is possibly because of the comparatively higher level of copper in the soils of the Central (Malwa) highlands than the Western Plains and Kutch region. However, the causes of below normal levels of copper in serum cannot be attributed solely to the soil levels the other possible cause could be low level of nutrition to the animals as these donkeys were reared by poor donkey keepers and often used heavily in construction and load carrying work. Moreover, they were never dewormed. It is suggested that excessive work load along with the inadequate dietary supplementations and deworming, could have caused not only the copper deficiency but also that of cobalt and zinc.

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Freezability of kathiawari horse semen under field conditions

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Physical and morphological indices of Kathiawari horse semen were studied to generate baseline information and semen was cryopreserved for the first time. Vapor freezing technique was used to freeze semen under field conditions aimed at breed conservation and propagation using artificial insemination. The appearance of fresh semen was milky white to creamy and consistency of gel free semen was observed variably thin. Total semen volume, gel free semen and gel volume in semen was recorded 37.80±3.42 ml (ranging from 15 to 75 ml), 25.80±2.13 ml (ranging from 12 to 50 ml) and 12.0±2.22 ml (ranging from 2 to 35 ml), respectively. PH of semen was 7.05±0.04, ranging from 6.7 to 7.4. Total motility and progressive sperm motility in gel free semen was observed 82.0±1.51 and 77.0±1.51, respectively. Mean sperm concentration in fresh semen was 173.75±8.86 x 106 ml-1. The pre freeze motility in extended semen after 2 hour of equilibration at 4-50C was 69.64±1.18. The post thaw motility (PTM) observed with modified secondary extender was 35.62±1.99 (PTM 30 to 45%). Five Kathiawari horses were moderate to good (PTM 30 to 45%) freezer, whereas one stallion was poor freezer had PTM 5 to 10%. Freezability of semen from six Kathiawari horses was 80% (17 out of 21 ejaculates). The results of present study indicate that the semen from Kathiawari stallions can be cryopreserved at farmers' doorstep using vapor freezing technique for use in artificial insemination.

Detection of biofilm formation of canine persistent wound pathogens by Congo Red Agar Method

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Biofilm, community of bacteria attached in a biotic or abiotic surface are responsible for chronic diseases causing them resistant to normal drugs. 172 samples from different types of dog wounds of more than 16 days old presented to Teaching Veterinary Clinical Complex, Bhubaneswar were processed for their isolation and identification. Then they were processed for detection of biofilm formation by Congo red Agar method. The wound samples showed polymicrobial in nature and the predominant isolate was Staphylococcus intermedius. Congo Red Agar method detected average of 52 % as negative, 20.0% as moderate and 27.9% as strong biofilm producers suggesting that it is an easy and alternative method can be used for detection of biofilm production.

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Identification of mammary tumor homing peptides by in vivo biopanning using phage display peptide library

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Cancer is one of the leading causes of death worldwide. Despite significant progress made in cancer therapies, present conventional therapies are limited by their inability to discriminate cancer cells from normal cells leading to toxicity and narrow therapeutic index. Thus, there is a need for developing tumor specific delivery tools. As normal cells transform into cancerous cells their surface properties change, showing unique expression of certain antigens and receptors which can be attractive targets for cancer therapies. Screening phage display peptide libraries (PDPL) is a direct and fast method to identify novel peptide sequencesspecific for target tissues.In this study, tumour specific homing peptides were identified using PDPL by in vivo biopanningof rat mammary tumours induced by MNU and LA-7 cells in Wistar and Sprague Dawley rats respectively. In vivo biopanning was repeated for four roundsto enrich tumour specific phages.Finally, 60 pfus were selected randomly for sequencing to get homing peptides. The consensus peptide sequences were identified using Lasergene and Bioedit software. Tumour specificity of selected phage clones and peptides were studied by the analysis of in vivo phage affinity, competitive binding assay, immnocytochemical and histochemical staining.Two tumour homing peptides MT2 (TSLLGLS) and MT3 (KQSPPET) have ability to bind more than 75% to rat mammary tumour cells and 60% to canine mammary tumour cells. These two peptides can be further exploited to develop tumor specific delivery system.

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Study of chemokines of CXC family expressed during granuloma formation in mycobacterial infection in guinea pig

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Mycobacterium indicus pranii (earlier known as Mycobacterium w) has been used as an immunmodulatory agent in leprosy and tuberculosis by mediating the release of various cytokines and chemokines. CXC chemokines chemokines are involved in T-cell migration and stimulation of natural killer cells in Mycobacterium tuberculosis infection. In this study, the effect of heat killed M. indicus pranii (alone and in conjunction with chemotherapy) on disease progression was determined by colony forming units (CFUs) in guinea pig lung following their aerosol infection and the expression levels of CXCL1, CXCL8, CXCL10, CXCL11 and CXCL12 were studied by quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) and by situ RT-PCR in all the study groups of animals included; Control (NH), infection only (Rv), immunoprophylaxis (RvMw), chemotherapy (RvCh) and combination of immunoprophylaxis with chemotherapy (RvChMw). In the group where immunoprophylaxis was given in combination with chemotherapy, the CFU counts reduced significantly at 4th week post-infection as compared to animals that received immunoprophylaxis or chemotherapy alone. CXC Chemokine expression level were recorded significantly ($p<0.05$) at early and late stage of infection in immunoprophylaxis (RvMw) and in combination (RvChMw) group and our findings indicates that the differential expression of these CXC chemokine genes (CXCL1, CXCL8, CXCL10, CXCL11 and CXCL12) at different time point positively correlates with antitubercular treatment (at least with combination of immunoprophylaxis and chemotherapy). To conclude, this study showing that prior immunoprophylaxis and subsequent chemotherapy was found more effective in inhibition of growth of MTB and in production of chemokines associated with protection, in comparison with treatment alone in the studied groups against infection of tuberculosis. This study also showed that the expression of these chemokines of CXC family may positively correlate in granuloma formation.

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Evaluation of raw chicken sausages incorporated with blend of clove and holy basil essential oils during refrigeration storage

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The anti-microbial activity of blend of clove oil (CO) and holy basil essential oil (HBO) at different concentrations and their effect on the sensory attributes in raw chicken sausages was studied. Blend (1:1 ratio) was added at 5X, 10X, 20X and 40X level (MIC-0.025%) in the product and the product without any essential oil served as control. Various physico-chemical parameters (cooking yield, pH and moisture content, TBARS), microbiological quality characteristics (total plate count, coliform count, yeast and mould count and psychrophillic count) and sensory attributes were evaluated under aerobically packaged conditions. Study was carried out at refrigeration temperature (4±10C), till the spoilage of product. It was found that cooking yield did not show any significant difference (P>0.05) between control and treatment products. pH and TBARS showed increasing trend with increase in storage period with significant difference (P<0.05) from 10th day onwards indicating secondary oxidative processes occurring in the product. The addition of CO and HBO blend led to reduction in total plate count. Significantly lower count (P<0.05) was observed for treatment products at 20X and 40X level than control. Coliform and psychrophiles also showed the same trend as total plate count. Higher total phenolic content in treatment products, the values being significantly higher (P<0.05) higher at 10X, 20X and 40X level. Blend incorporated products at 5X level received comparable sensory scores to control, followed by 10X level. Blend (1:1) of CO and HBO at 5X, 10X, 20X and 40X level in the product extended 3, 4,6 and 8 days shelf life in raw chicken sausages than control product, respectively.Blend of CO and HBO can be successfully incorporated in meat products at 5-10X levels (0.125-0.25%) with effictive anti-microbial and anti-oxidative activities, although some new methodologies need to be devised so that higher level of essential oils (0.5-1%) in products can be accepted in sensory evaluation.

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Intervention strategies against salmonellas

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Salmonella are the common naturally occurring bacteria in the intestinal tract of birds, animals including reptiles, turtles, insects, and farm animals, human. It can be transmitted both from animal to human and vice versa. Salmonella is a member of family enterobactereaicea, gram negative, motile, non-spore forming, facultative anaerobes that can grow at temperature range of 5-45 Cwith optimum temp .of 35-37 C. The organism enters to human food chain through egg or carcass contamination. 1st it goes to stomach to intestinal tract, interacts with intestinal mucosa. Salmonella attaches enterocytes that manifest a no. of effects including tissue necrosis, thickening, inflammatory response and fluid secretion leads to diarrhea. Routine antimicrobial therapy is not recommended for mild or moderate cases in healthy individuals as R-plasmid coding for multiple resistance are common in salmonella. Also antimicrobial may not completely eliminate the bacteria and may select for resistant strains which lead to the drug ineffective. The asymptomatic persistence of bacteria in the gut results from a suppression of gut micro flora that normally competes with nutrients for salmonella. Control is based on reducing of infection by implementing a closed herd policy, purchasing animals from a reliable source, wash hand properly before preparing food and after handling raw meat, cook meat and eggs thoroughly up to an internal temperature of 160ºF. Basic food hygienic measures such as "COOK THOROUGHLY" are recommended, isolation of clinically affected animals, restricting the movement of animal, vehicle, human, foot bath containing suitable disinfectant should be located at strategic location to limit the spread of the disease. Herd vaccination may be followed to limit the spread in case of outbreak. Besides this, national and international surveillance systems are important means to detect and respond to salmonellosis in early stages and thus to prevent them from further spread.

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Haematological alteration and antibiogram profile of *E. coli* isolates in dogs with gastroenteritis

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Gastroenteritis is a common disease of multiple etiologies seen in all breeds and age groups of dogs. Causative factors include viruses, bacterial infections, endoparasites, dietary errors, food allergy and irritant drugs etc. The present investigation was carried out to study the haematological changes and bacterial pathogens in dogs affected with gastroenteritis. A total of ten dogs of either sex aged between one month to eight years brought to Teaching Veterinary Clinical Complex, LUVAS with the history of sudden onset of vomiting, diarrhoea, dehydration, anorexia and depression were included in the study. The blood samples were collected in EDTA vials for haematological estimations. For complete blood count blood samples were analysed using automatic haematology counter. The mean haematological findings revealed haemoconcentration, leucocytosis with neutrophilia and lymphopenia. Faecal samples of all these dogs were also collected for bacterial culture and antibiotic sensitivity profile of isolates. Interestingly, cultural examination of all the faecal samples revealed presence of E. coli including Dipthroids in one sample. Though the E. coli is a normal inhabitant of gastrointestinal tract but it also plays an important role as secondary bacterial infections in dogs suffering from gastroenteritis. Antimicrobial sensitivity pattern of these E. coli isolates revealed chloramphenicol as most sensitive followed by ceftriaxone, ciprofloxacin, gentamicin, neomycin, cefotaxime, cephalexin, carbenicillin, enrofloxacin, norfloxacin, cefoperazone, streptomycin, tetracycline, cloxacillin, kanamycin, amikacin, tobramycin and oxytetracycline. All these affected dogs were treated with suitable antibiotics, NSAIDs, multivitamins and other supportive and fluid therapy depending on the haematological and antibiogram findings.

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Principles of viral disease management

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Biosecurity is the only sound method for viral disease management as virus mutate rapidly and can survive easily in the environment and are less likely to be treated due to the development of resistance against drugs. Biosecurity includes a set of preventive measures that reduce the risk of transmission of infectious disease in livestock. It takes into account the epidemiological triad for disease occurrence: the individual host, the disease, and the environment contributing to disease susceptibility. It aims to improve nonspecific immunity of the host to resist the introduction of an agent, or limit the risk that an agent will sustain in an environment at adequate levels. Practicing biosecurity is done by following general sanitary principles viz., washing and disinfecting hands, shoes, clothes etc. regularly. Farm hygiene and biosecurity practices can be implemented to reduce the risk of disease agents moving on to farms from outside sources as vectors and carriers. Feeding nutritionally balanced diet, maintaining a comfortable living environment and minimising fear and anxiety will help the animal's natural protective mechanisms to function optimally. Disease can be prevented by stimulating the animal's immune system in a way that enhances the immune response when exposed to a pathogen. Isolation, traffic control and sanitation will also help in disease control. Early detection and effectively being able to identify contagious disease outbreaks is very important in implementing harm minimisation action. Though biosecurity measures might appear to be functioning soundly, the imminent risk of disease does not diminish. Immediate action to further prevent the disease from spreading must be taken including quarantining visibly affected animal and contacting veterinarian.

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Genotoxicity evaluation of acute and chronic doses of ethion

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The present study was conducted on 40 healthy male mice aging 7-8 weeks to investigate the effects of acute (24 h) and chronic exposures (90 days) of ethion. The animals were divided into four groups viz. three treatments and one control for each experiment (n=10; each group). Among the treatments groups, Group 1 was administered 1/5th of LD50 of ethion orally for 24 hours. Group 2 and Group 3 were administered 1/10th and 1/20th of LD50 of ethion for 90 days, respectively. Control groups were administered corn oil. At the end of exposure period, half animals from each group were challenged with LPS @ 80µl/ animal and the remaining with NSS @ 80µl/ animal. Genotoxicity was assessed by comet assay in blood of mice. Acute and chronic treatment with 1/20th LD50 exposure to ethion alone did not show any significant increase in comet tail length, however there was a significant increase ($p<0.05$) in comet tail length in treated group challenged with LPS. Further, there was significant increase ($p<0.05$) in comet tail length in animals treated with 1/10th of LD50 as compared to control indicating genotoxic potential of ethion at 1/10th dose. Also, a significant increase ($p<0.05$) in comet tail length was observed in group treated with 1/10th dose followed by LPS compared to control and LPS treated groups, suggesting synergistic effect of interaction between ethion and LPS. It is the first data highlighting the genotoxicity due to interaction between ethion and LPS.

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Potential risk of brucellosis on animal and human beings

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Brucellosis is a zoonotic disease mostly causing abortion in human and animalsand other types of infection like malta fever. In the present investigation, 60 serum sample were collected. 20 from affected animals which shows symptoms of brucellosis, 20 from animal handler (owner of animals) and, 20 from field veterinarians. All the samples in the laboratory, Apex centre, RAJUVAS were treated byBrucellaabortus antigen. Out of 60, 22 (32.66%) samples were show clumping of coloured antigen which means presence of antibodies in the serum against Brucellaabortusand found positive for brucellosis. 12(60%) from animals, 7(35%) from animal handler and, 3(15%) from field veterianrians. This investigation revealed that brucella also affect the milk handler and field veterinarians.

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Studies on brucellosis in animals in Bikaner district and its public health significance

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Brucellosis is a zoonotic disease mostly causing abortion in human and animalsand other types of infection like malta fever. In the present investigation, 60 serum sample were collected, 20 from affected animals which shows symptoms of brucellosis, 20 from animal handler (owner of animals) and, 20 from field veterinarians. All the samples in the laboratory, Apex centre, RAJUVAS were treated byBrucellaabortus antigen. Out of 60, 22 (32.66%) samples were show clumping of coloured antigen which means presence of antibodies in the serum against Brucellaabortusand found positive for brucellosis. The result showing that 12(60%) from animals, 7(35%) from animal handler and, 3(15%) from field veterinarians were positive for the Brucellaabortus antigen against the antibody present in the serum sample. This investigation revealed that brucellosis also affect the milk handler and field veterinarians.

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Detection of antibiotic resistance patterns of Escherichia coli isolates among healthy and diseased camel

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In the present study Escherichia coli strains were isolated from nasal discharge of respiratory tract infected camels and healthy camels in Bikaner. From the total of 112 specimens of nasal swab (47 healthy and 65 diseased camels) collected for bacteriological examination, 55 (20 from healthy and 35 from diseased camels) were confirmed to be E. coli on the basis of phenotypic (Metallic sheen on EMB, IMViC patterns) and genotypic characterization (23S rRNA gene based Ribotyping). With the aim to reveal comparative multidrug resistance profile of the isolates, all were subjected to 24 antibiotics of various groups and generations and found marked difference in antibiotic resistance patterns. All the 35 isolates from diseased camels were 100 % sensitive to Faropenem, Gentamicin, Imepenem followed by Ampicillin + Sulbactam (94.28%), Cefepime and Norfloxacin (85.71%), and Trimethoprim (77.14%). Whereas in case of healthy camel all 20 isolates were 100 % sensitive to Ampicillin + Sulbactam, Cefepime, Cephotaxime, Ceftazidime, Ciprofloxacin, Faropenem, Gentamicin, Imepenem, Norfloxacin, and Trimethoprim. Isolates from diseased camels showed 100% resistance to Ampicillin, Bacitracin, Clinadmycin and Sulfadiazine followed by Rifampicin (94.28%), Vancomycin and Polymyxin-B (80%). Whereas isolates from healthy camel showed 100% resistance to Clinadmycin and Sulfadiazine followed by vancomycin and Rifampicin (90%) and Ampicillin (80%). The results revealed that all the 55 E. coli isolates from both diseased and healthy camels were susceptible Faropenem, Gentamicin and Imepenem and resistant to Clinadmycin and Sulfadiazine.

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Antibiogram studies of Klebsiella oxytoca from nasal discharge of respiratory tract infection in Dromedary camel

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One humped camel is native of Rajasthan and very less susceptible to many of the diseases. Pulmonary diseases are among emerging problems of camels that are causing considerable loss in production and leads to death. Klebsiella oxytocainfects animals and humans as normal flora or pathogens. The present study was intended to determine the prevalence of K. oxytoca in clinically infected pneumonic camels and to assess the antimicrobial resistance of K. oxytoca isolated from nasal discharge samples of respiratory tract infection from Dromedary camels. There were 68 samples of deep nasal discharge of respiratory tract infected camels were collected and Klebsiella oxytoca were foundin 33 samples (48.53%). The isolates were confirmed phenotypically and further genotypically by targeting species specific gene named as pehX with amplicon of 343bp.The isolated were screed for antibiotic resistance pattern with twenty four antibiotics and it was found that100 % isolates were resistant to seven antibiotics viz.amoxycillin, ampicillin, bacitracin, clindamycin, oxacillin, rifampicin, sulfadiazine and vancomycin, while sensitive to cefepime and imipenem were found effective against all the isolates.

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Recent advances in equine reproduction management

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Equine breeding management has made major progress towards understanding idiosyncrasies of equine reproduction. The purpose of this is to provide the horse industry with an overview of the type of technology equine scientists have developed as they relate to equine reproduction. Horses are seasonal estrus and failure to detect estrus or failure to breed the mare within 48 hrs prior to ovulation results in breeding failure. Mares are polyestrus. Two periods of reproductive status are seen: ovulatory and anovulatory season. Mare displays a definite estrus cycle accompanied by true estrus behaviour and ovulation during the ovulatory season. Anovulatory season characterized by cessation of estrus but normal ovarian function. During anoestrous, ovaries are usually small and hard. Number of estrus period is lowest in winter months. The process of reproduction is under neuroendocrine control. FSH and LH are pituitary hormones involve in the estrus cycle. Luteinizing hormone causes the ovarian follicle to mature and ovulate. If the mare is not pregnant, PGF2 alpha will be secreted by the uterus to lyse the corpus luteum and mare will return to estrus. Although just before the breeding season, special management practices are often employed to prepare mares & stallion for reproductive function. Uterine biopsies & cultures, proper nutrition, parasite control, proper vaccination schedule are all needed to making the breeding farm successful.

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Influence of seasonal variation on production in pigs: Physio-genomic perspectives

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With the rise in global mean temperature, it is virtually certain that there will be more frequent hot and fewer cold temperature extremes, increasing susceptibility of livestock to severe heat stress. Physiologically, pig may not be a versatile species to adapt very well to high ambient temperature and humidity. The present study was conducted to understand the physio-genomic basis of seasonal variation on (re)production in pigs. Blood samples of different genetic (native, crossbreed and exotic) and age (grower, finisher, sow and boar) groups were collected from the organized farm and farmers field round the year for studying the endocrine profile and relative expression of different genes influencing production. Weather data was collected round the year for construction of THI. Based on average THI, the seasons were classified as I (November - February), II (March - June) and III (July - October) and it was largely unfavourable during Season III followed by Season II. The hormones estrogen, progesterone, cortisol, insulin, T3 and T4 was found to be influenced by season ($P<0.01$), whereas cortisol, testosterone and T3 was found to be influenced by genetics of the animal ($P<0.05$, except T3 $P<0.01$), and estrogen, progesterone, cortisol, insulin, testosterone and T4 was found to be influenced by age of (re)productive life of the animals ($P<0.01$, except cortisol $P<0.05$). Level of GH, leptin and LDH was not influenced by season, genetics or stage of production. Relative expression of FSHβ, LHβ, PGR and ESRβ was found to be significantly influenced in exotic and crossbred pigs during season II and III compared to native pigs. Whereas relative expression of GH was higher in exotic and crossbred compared to native pigs across the season, expression of GHRH was downregulated in exotic and crossbred during season II and III. Expression of HSP70 was higher in native pigs compared to others across the season. Thus, seasonal variation was found to significantly influence endocrine profile and gene expression. It may be concluded that season in interplay with genetics and age of (re)productive life significantly influences production and productivity in pigs.

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Relationship of seminal plasma protein with semen characteristics of Murrah buffalo bulls

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Present study was carried out to assess the protein profile of Murrah buffalo seminal plasma by using SDS-PAGE and to correlate them with the semen characteristics. Semen samples were collected by a bovine artificial vagina from Murrah buffalo bulls maintained at College of Veterinary Science, N. D. University of Agriculture & Technology, Kumarganj, Faizabad. Semen characteristics were measured using standard procedures. Seminal plasma was separated after centrifugation of semen at 5000 rpm for 10 min. The supernatants were collected and precipitated using nine volumes of cold ethanol, and the proteins recovered. These seminal plasma proteins were separated on a 12% SDS-PAGE using standard procedure. A total of 22 protein bands could be identified by the fractionation of seminal plasma protein on the SDS-PAGE; ranging from 14.1 kDa to 88 kDa. Some of these bands were more prominent in some samples. A protein fraction of 25.8 kDa was significantly correlated with progressive motility of sperm in frozen samples. The semen samples that had greater sperm viability showed a prominent fraction of 58 kDa protein in both fresh and frozen samples.

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Identification of acetogens, methanotrophs and methanogens in the equine hindgut

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Identification of acetogens, methanotrophs and methanogens present in the hindgut of the horses was undertaken using universal primers. The partial nucleotide sequence encoding formyltetrahydrofolate synthetase (FTHFS) were amplified with primers 27F and 1529 R for acetogens. The second amplification (nested PCR) was performed by using type I and type II methanotrophs specific primer sets. Similarly 16S rRNA genes for methanogens were amplified using Met86 and F/1340R. Purified PCR products (FTHFS, 16S rDNA gene of typeI and type II methanotrophs and methanogens) were cloned in pDrive Cloning Vector and sequenced. The nucleotide sequence (969bp, 564bp, 528bp and 529bp) were obtained and deposited in the GenBank for FTHFS, type I, type II methanotrophs, and methanogens, respectively. The obtained FTHFS sequence was close and showed 75 to 78% similarity to the uncultured equine hindgut bacterium. The PCR products, amplified with the type I primer set was found to be = 100% sequence similarity with uncultured equine intestinal bacterium gene and uncultured archaeon gene for 16S rRNA. Similarly, the PCR products, amplified with the type II primer set was ≤ 99% sequence similarity with Uncultured bacterium clone horse, ≤ 97% sequence similarity with Uncultured bacterium clone calf. The obtained sequence for methanogens was close and showed ≤ 99% similarity with Uncultured bacterium clone ZEBRA and Uncultured bacterium clone ELAND; ≤ 98-99% similarity with Uncultured rumen bacterium.

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Antibiogram pattern against major pathogens responsible for diarrhoea in murrah buffaloes

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This study was conducted with the objective of isolation and biochemical characterisation of major pathogens associated with diarrhoea in buffaloes and to know the antibiotic sensitivity against the isolated pathogens. A total of eleven faecal samples were collected and processed for identification and further characterisation. Cultural examinationrevealed two major pathogens i.e. E.coli(seven samples) and Streptococcus spp. (two samples along with E.coli). Sixteen common antimicrobials associated with diarrhoea were selected for antibiotic sensitivity and it was found that E.coli was resistant to Ceftrioxone, Cefaperazone, Oxytetracycline and eight more commonly used antimicrobials whereas sensitive for Ciprofloxacin, Norfloxacin, Kanamycin, Chloramphenical and Amikacin. Isolates of Streptococcus spp. showed resistance against Chloramphenicol, Cefaperazone, Oxytetracycline, Chloramphenicol, Kanamycin and Cephalexin and sensitive against Ampicillin, Ampxycillin, Enrofloxacilin, Amikacin etc. It was concluded that E.coli and Streptococcusspp. were the two main pathogens causing diarrhoea in murrah buffaloes in this region of state with resistance to maximum number of antimicrobials used for therapy in field conditions which is a cause of concern

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Methionine sulphoxide reductase (MsrA) prevents neutrophill mediated killing of salmonella typhimurium

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Salmonella Typhimurium (ST) is the major cause of zoonotic gastroenteritis in human. ST survives and also replicates inside the phagocytic cells. Methionine (Met) is the primary targets of phagocyte generated oxidants. Oxidation of Met leads to the methionine sulfoxide (Met-SO) formation. Methionine sulfoxide reductaseA (MsrA) can repair protein bound Met-SO to Met thus maintain protein function. Here we develop an msrA gene deletion mutant of ST by the flip recombinase method. The gene deletion was confirmed by the PCR and western blotting. We have also checked the in vitro susceptibility of ST and mutant against different oxidants. The mutant was more susceptible to HOCl and azurophillic granules. Finally we checked the susceptibility against the neutrophil and it was found significant killing against the ST.

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Isolation and identification of platelet aggregating inhibitors from salivary glands of Hyalomma anatolicum anatolicum ticks

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Ticks being blood sucking ectoparasites are responsible for causing considerable production losses in animals. They produce a number of bioactive molecules in their saliva which help in blood feeding by overcoming normal hemostatic mechanism of the host. Various proteins /peptides in the salivary glands of ticks have been found to have the anti platelet-aggregating, anti-thrombotic, anti-inflammatory, fibrinogenolytic etc. activities. But, there is no report in Hyalomma anatolicum anatolicum which is a most prevalent tick in India. Platelet aggregating inhibitors present in salivary gland of blood sucking ectoparasites have been found to play a great role in prevention of blood coagulation. So, to isolate and identify the anti-platelet aggregating proteins/peptides from Hyalomma ticks salivary gland, ticks were collected from the animals kept in the villages nearby Hisar. Salivary glands were dissected out for preparation of extracts in HEPES saline buffer. Proteins/peptides present were fractionated using cationic ion exchange chromatography. Gel electrophoretic separation of cationic proteins showed bands having approximate molecular weight of 6,15,31,34,43,54,76,85,112 and 170kDa. The cationic proteins/peptides having molecular weight of approximate 26kDa supposed to have the antiplatelet aggregating activity as evidenced by their inhibitory effect on platelets isolated from platelet rich buffalo plasma. This study will help in providing different directions in therapeutics as well as in identifying the anti-tick vaccine candidate.

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Antibiogram of bacterial isolates from clinical cases of BRD complex in buffaloes

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Bovine respiratory disease (BRD) complex is a group of many diseases that have been shown to impact on the respiratory tract of bovine. It is a major health problem of respiratory system occurring worldwide in bovine and is a result of the complex interaction of bacterial and viral agents, environmental conditions, management factors and the animal health. New infection, recrudescence of pre-existing infection, misdiagnosis and irreparable damage caused by previous disease may also influence treatment response. The present study was carried out on 24 clinical cases of BRD complex in buffaloes brought to TVCC of LUVAS, Hisar. Nasal swab samples taken from affected animals used for cultural examination and identification of bacterial isolates. Different bacteria isolated in the order of frequency from these samples were Staphylococcus aureus (9), Streptococcus pyogenes (7), E. coli (7), Diplococcus spp. (2), Pasteurella multocida (1) and Klebsiella pneumoniae (1) including 3 cases of mixed infection in combination of Staphylococcus aureus + Streptococcus pyogenes, Staphylococcus aureus + Diplococcus spp. and Staphylococcus aureus + Klebsiella pneumoniae. Antimicrobial sensitivity pattern of bacterial isolates from nasal swabs revealed ceftiofur (100%) as most sensitive followed by levofloxacin (87.5%), amikacin (83.33%), moxifloxacin (79.6%), gentamicin (75%), neomycin (75%), chloramphenicol (70.83%), cephalexin (70.83%), enrofloxacin (66.6%), ciprofloxacin(62.5%), ceftriaxone(62.5%), clindamycin (62.5%), cefoperazone (58.33%), cloxacillin (58.33%), pencillin- G (58.33%), streptomycin(50%), tetracycline (50%), septran (45.83%), amoxyclav (41.66%), ampicillin (37.5%) and amoxicillin (33.33%). Antimicrobial agents used for the treatment of BRD complex are selected by the veterinarian on the basis of perceived efficacy, cost, convenience, availability, toxicity and residue profile. Thus, monitoring of the antimicrobial susceptibility trends of BRD complex pathogens is an important aid to veterinarians in selecting the most efficacious and cost-effective therapeutic agents.

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An outbreak of organophosphate (ethion 50%) in buffaloes

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Organophosphate (Ethion 50%) toxicity was reported in three buffaloes (2 adult, 1 calf) in TVCC, LUVAS, HISAR during the month of September, 2015. All the affected animals exhibited clinical signsof nicotinic & cholinergic overstimulation such as anorexia, tremors in large muscles, hypersalivation,typmanywith constipation. Respiratory distress with open mouth breathingwas also present. Haematologicalparameters didn't show any significant changes.All the affected animals were treated immediately with exclusive atropinisation therapy @ dose rate of 100 mg total dose/per day/adult animal. Supportive therapy included hypertonic saline, calcium borogluconate, glucocorticoids and vitamin suppliments. Out of three animals one (buffalo heifer) died during treatmentwhere as other adult buffalo died after one day of treatment. Recovered animal was treated as other animals except 1.3 % Soda-bicarbonate in addition. So the present study suggested that organophosphate poisoning is lethal and atropinisation with the judicious use of fluid therapy can be helpful in managing the organophosphate (Ethion 50%) toxicity.

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Cell mediated immune response against Newcastle disease virus in chickens

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Newcastle disease (ND) is one of the lethal, zoonotic disease causing colossal economic losses in poultry industry due to high morbidity and mortality. The disease is caused by a single stranded, enveloped, non-segmented RNA virus i.e. avian paramyxovirus serotype-1 classified under genus Avulavirus of family Paramyxoviridae. The present study was envisaged to study the cell mediated immune response elicited by the vaccine strains of NDV in chicken. F-1 and LaSota strains of NDV were propagated in laboratory using 10- day old embryonated hen eggs via allantoic cavity route. One group of thirty chicks was infected with F-1 strain and other with LaSota strain with 105 egg infective doses. Sequential blood samples at day 0, 3,7,14, 21 and 28 post infection were collected and Cell mediated immune response was determined by lymphocyte proliferation assay.

In both the group of chicks immunized with NDV, lymphocyte proliferation responses at different days post infection were significantly higher ($P<0.05$) compared to that of the control group on in vitro stimulation with antigen. On in vitro stimulation with Concanavalin A the mean stimulation index values in the group of birds immunized with F-1 or with LaSota strain of NDV were found to be significantly higher than that of control at most of the days. The findings of present study lead to the conclusion that infection with NDV induces cellular immune response in chickens. The F-1 strain of NDV appears to be better immunogenic than that of LaSota strain of NDV.

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Nanovaccinology: Dawn of Biomemetic Vaccine Carriers in Parasitology

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A wealth of genomic and proteomic information available on different parasites and recent advances in adjuvant and delivery systems are being harnessed to develop nanovaccines against parasitic infections. The use of nanoparticles in vaccine formulations allows not only improved antigen stability and immunogenicity but also targeted delivery and slow release. A prototypic malaria vaccine based on a highly versatile self-assembling polypeptide nanoparticle (SAPN) platform that can repetitively display a B cell immunodominant repeat epitope (DPPPPNPN)2D of the malaria parasite Plasmodium berghei circumsporozoite protein has been developed. SAPN platform not only functions to deliver an ordered repetitive array of B cell peptide epitopes but operates as a classical immunological carrier to provide cognate help to the P4c-Mal-specific B cells. The nanogel encapsulation of recombinant NcPDI (recNcPDI) following vaccination of mice by intranasal or intraperitoneal routes and challenge infection with Neospora caninum tachyzoites improved survival of intraperitoneally vaccinated mice. Cationic solid–lipid nanoparticle (cSLN) formulation has been used to administer a DNA vaccine harbouring the L. donovani A2 antigen along with Leishmania infantum cysteine proteinases [CPA and CPB without its unusual C-terminal extension (CPB_CTE)] which led to induction of specific Th1 and Th2 clones. A candidate malaria antigen, VMP001, conjugated to the lipid membrane of the nanoparticles and an immunostimulatory molecule, monophosphoryl lipid A (MPLA), was incorporated into the lipid membranes creating pathogen-mimicking nanoparticle vaccines (VMP001-NPs). Vaccination with VMP001-NPs promoted germinal center formation and elicited durable antigen-specific antibodies with significantly higher titers and more balanced Th1/Th2 responses in vivo as compared to vaccines composed of soluble protein mixed with MPLA. With such breakthroughs in use of nanoparticles in parasitology, the dream of researchers to provide 'stand alone' management of parasitic disease could be realized soon.

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B2L and IL-2 gene sequence based characterization of Orf viruses from North-India

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Orfor Contagious Pustular Dermatitis (CPD) is an economically important epithiliotropic viral disease affecting domesticated as well as wild ruminants as an acute, contagious and debilitatingdisease with public health significance. Full and partial length CPD virus specific B2L gene major envelope glycoprotein genes (1137 and 235 bp, respectively) and GIF/IL-2 gene (GM-CSF and interleukin-2 inhibitory factor, 408 bp) were amplified by PCR for detection of Orf virus, both in scabs and cell culture adapted field samples. The PCR amplicons were gel purified and sequenced using BigDye terminator chemistry fornucleotide sequence based confirmation.

Both B2L and GIF/IL-2 genes are highly conserved with >96 and 98% nucleotide (nt) sequence identities, respectively.The Orf virus isolates in the present study demonstrated lowest ntidentity (95.4%) with Chinese strain (Accession no: JN565694) while highest identity (99.1%) with other Chinese (Accession no: GU903501) and previously characterised Indian strains isolated in 2013 (CPDV/IND2013/01 and CPDV/IND2013/02) in their B2L gene. Further, the Indian CPDV strains (n=10) characterised in this study were distinct from other isolates andgrouped as a single separate clade. Additionally, these strains showed 97.7% nt identity with a most recent isolate from CIRG, Makhdoom, Mathura (Accession no. KC992325). The B2L gene based phylogenetic analyses revealed that four species of Parapoxvirus(Orf virus, Pseudocowpox virus, Bovine Papular Stomatitis virus, Parapox virus of reindeer in Newzealand)form separate clusters with 46–95%nt identity among themselves.

IL-2 gene of Indian isolates were very closely related to other Orf virus isolates from Norway with 98% nt sequence identity (Accession no. AY605992). Both of them share 90.8% nt sequence identity with PCPV and 96% with Seal parapoxvirus (Accession no. AY605975). These data showed that both B2L and GIF/IL-2 gene sequences can be reliably used to identity and characterise Orf viruses.

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