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SOUVENIR-CUM-COMPENDIUM

XX Annual Convention

of

Indian Society for Veterinary Immunology & Biotechnology

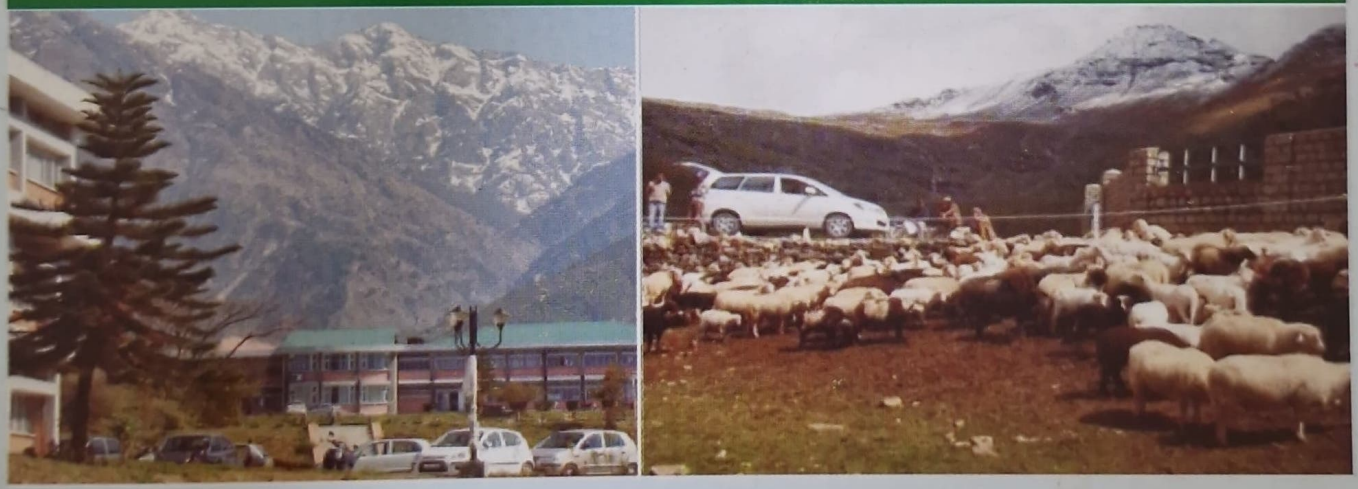
and

National Symposium

on

Emerging Challenges & Opportunities in Veterinary Immunology & Biotechnology for Improved Animal Health & Productivity

November 11-13, 2013



Department of Veterinary Microbiology

Dr. G.C. Negi

College of Veterinary & Animal Sciences

CSKHPKV, Palampur-176062, Himachal Pradesh, India

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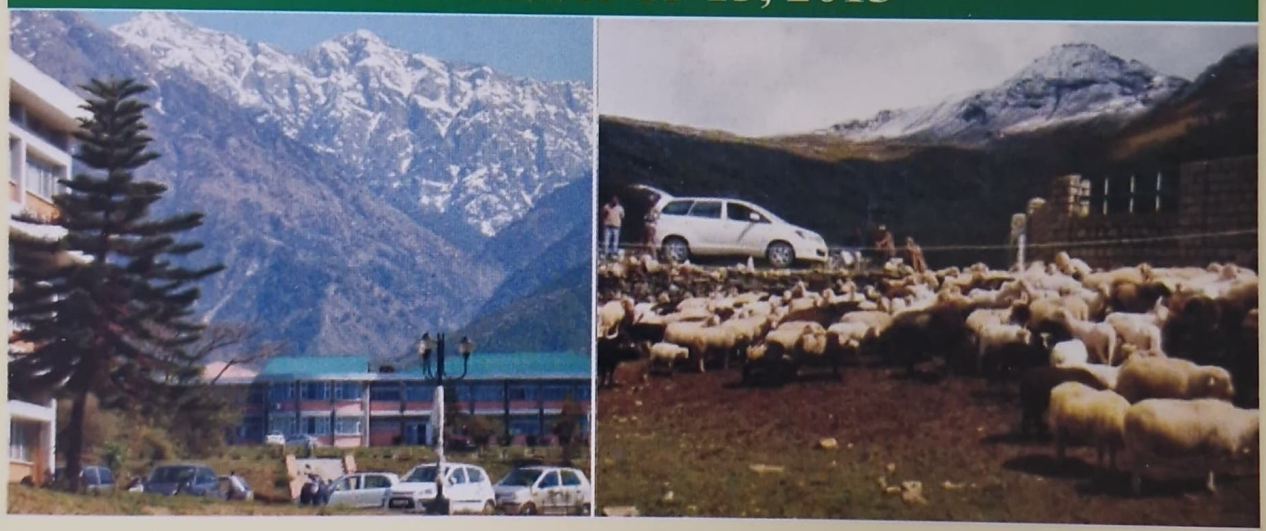

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Anil Sharma
Rural Development, Panchayati Raj
and Animal Husbandry Minister
Himachal Pradesh

MESSAGE

It gives me immense pleasure to learn that Indian Society for Veterinary Immunology & Biotechnology is organizing "XX Annual Convention & National Symposium on "Emerging Challenges & Opportunities in Veterinary Immunology & Biotechnology for Improved Animal Health & Productivity" at Department of Veterinary Microbiology, College of Veterinary & Animal Sciences in CSKHPKV, Palampur, Himachal Pradesh from 11th to 13 November, 2013.

The animal husbandry sector is an integral part of sustainable agriculture production system in our Country. Its importance in augmenting National Economy is well known. This sector is of paramount importance for our State where core agricultural activities cannot compete with agriculture intensive states of the country. It has also been seen that certain breeds or type of livestock is suited for particular region of the country. These breeds have attributes which make them suitable for optimal performance and disease resistance under certain settings. It, therefore, becomes important that these basic characteristics of these animals be studied so that this knowledge could be used for enhancing productivity of these animals.

I am sure several scientific attempts to understand the behavior of immune system of animals and how they respond to vaccines; biotechnological interventions to develop new vaccines & effective diagnostics against animal diseases must have been made which would be shared in this convention amongst the fraternity to provide some leads that would ultimately benefit the animals and the society.

I extend my best wishes to the organizers for the success of this convention and symposium and hope that participants will enjoy the salubrious nature and hospitality of the people of the State.

(Anil Sharma)

CSK-Himachal Pradesh Agricultural University
Palampur-176 062 (HP) India

Dr. K.K. Katoch
Vice-Chancellor

Message

I am extremely pleased to know that the Department of Veterinary Microbiology, Dr. G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agricultural University, Palampur is organizing the National Symposium and XX Annual Convention of Indian Society for Veterinary Immunology & Biotechnology on "Emerging Challenges & Opportunities in Veterinary Immunology & Biotechnology for Improved Animal Health & Productivity" from 11th to 13th November 2013 at Palampur, Himachal Pradesh.

Livestock sector is an integral part of viable agriculture and is playing a crucial role in the economic growth of the developing countries like India particularly hill states where land holdings are small. The advancement of research in Veterinary Immunology and biotechnology has been greatly contributing for the development of new production technologies, vaccines and diagnostics to fight against various diseases and for increasing the productivity of livestock. By using molecular markers to identify and select particular genes for desirable traits, it is possible to select superior germplasm and disseminate it through embryo transfer, transgenic and other assisted reproductive biotechnological technologies. In this context, the symposium is being organized at an appropriate time.

The need of the hour in the expanding animal husbandry sector is proper utilization of immunological and biotechnological innovations through development of new generation diagnostics and vaccines, nanobiotechnology for enhanced animal health and production, genomics and proteomics. I am sure that the advancements in the field of animal immunology and biotechnology will be deliberated in this symposium by the experts for the growing demand of the nation.

I extend best wishes for the success of the convention and symposium.

(K.K. Katoch)

CSK-Himachal Pradesh Agricultural University
Palampur-176 062 (HP) India

Dr. Sanjeet Katoch
Dean, COVAS

Message

I am very happy to learn that the Department of Veterinary Microbiology, Dr. G.C. Negi College of Veterinary & Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is organizing the National Symposium on "Emerging Challenges & Opportunities in Veterinary Immunology & Biotechnology for Improved Animal Health Productivity" and XX Annual Convention of Indian Society for Veterinary Immunology & Biotechnology from 11th to 13th November, 2013 at Palampur. Himachal Pradesh. The symposium certainly will provide an opportunity to the researchers working in the area of immunology and biotechnology to share their research findings and exchange new ideas.

I take great pride in emphasizing that the Department of Veterinary Microbiology, Dr. G.C. Negi College of Veterinary & Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur has grown as well-built research centre on microbiology from a modest beginning 27 years ago. Apart from its role in active UG & PG teaching and research, it renders animal disease investigation and consultancy services to the farmers of the state by attending animal disease outbreaks and suggesting prevention and control measures against infectious diseases.

I extend warm welcome to all the delegates to Palampur and wish the convention a great success.

(Sanjeet Katoch)

राष्ट्रीय अश्व अनुसंधान केन्द्र

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NATIONAL RESEARCH CENTRE ON EQUINES

SIRSA ROAD, HISAR-125 001 (HARYANA) INDIA

Dr R.K. Singh
Director, NRCE President
Indian Society for Veterinary
Immunology & Biotechnology

Message

It is a matter of delight for me that XX annual convention of the Indian Society for Veterinary Immunology and Biotechnology and National Symposium of "Emerging Challenges and Opportunities in Veterinary Biotechnology & Immunology is being organized during November 11-13 at Dr G.C. Negi College of Veterinary and Animal Sciences of CSKHPKV, Palampur.

The CSKHPKV is committed to create a knowledge society on hill and mountain agriculture and to provide access to knowledge and skills in hill and mountain agriculture. Accordingly, the College of Veterinary and Animal Sciences - an important component of CSKHPKV - has been following the ethos of the university and has been striving hard and contributing immensely in providing necessary back up for improved livestock health and thereby ensuring augmented livestock production and productivity in this region of Himalayas. The disciplines in animal health group have highly competent and committed faculty who have done remarkable in animal health group have highly competent and committed faculty who have done remarkable work as evidenced by technologies available, research publications, and improved productivity of livestock.

The topic chosen for the annual convention and the symposium, "Emerging Challenges and Opportunities in Veterinary Immunology and Biotechnology" is very apt not only to the country but also for this region of Himalayas as deliberations will lead to providing some plausible solutions for furtherance of welfare of animals, ecosystem, and human as well. To get desirable and palpable changes in our needs, a paradigm shift in research priorities by forging frontier technologies at suitable levels is essential to ensure animal and human welfare and accelerate animal productivity in the country to meet the ever growing demand for food of animal origin.

I am confident that this symposium will deliberate - besides cutting-edge Immunology and biotechnology - on some more issues which have direct relevance to Himalayan livestock productivity and livelihood of rural Himalayan peasants. I am also confident that the recommendations emerging out of this symposium will help the policy makers and the academic community alike to strategze the livestock-related activities so as to ensure the welfare and productivity of the libestock and wellbeing of rural livestock holding community.

I - on behalf of the ISVIB - thank the CSKHPKV administration for convening this convention and extend best wishes to the organizing committee for the grand success of this convention and symposium.

(R.K. Singh)

Dr. V. Purushothaman
Secretary

Former Director of Research & Centre
for Animal Health Studies,
Tamil Nadu Veterinary and Animal Sciences University,
Chennai, 600 051

Message

The Indian society of Veterinary Immunology and Biotechnology (ISVIB) has become one of the prestigious society in the country. It is the brainchild borne out of efforts of legends in the field of Veterinary Immunology and Biotechnology namely Dr. P. Richard Masillamony, Dr. V. D. Padmanaban, Dr. B. B. Mallick and Dr. Kesavamoorthy during 1990s.

The registered society motivates its members to research, analyse, and share their findings to improve animal health and productivity. Towards this objective annual conventions and symposium were organized in different parts of the nation. The society has grown in strength and has more than 800 members on its roll. The society confers fellowships, various awards, travel grants to the scientist at different levels.

The society has organized 19 conventions on various themes and came out with concrete recommendations for the policy planners. The 20th convention being held at College of Veterinary Science, Palampur, Himachal Pradesh on maximizing Animal Health and Productivity from Nov 11 -13, 2013.

I appreciate the keen interest shown by the organisiers at Palampur to make this event a great success. The modernisation of Agriculture and Animal husbandry in our country is ongoing at a rapid pace. The landscape of livestock product availability and the consumer awareness about livestock products is high. In addition the rays of globalization have started to impinge on the Indian livestock sector. This is an interesting time to work in the Veterinary Immunology and Biotechnology field. There are many challenges and much more opportunities ahead to improve our developing nation on par with the industrialised nations of the world. I am sure the 20th ISVIB conference will galvanize the scientists and professionals to take up the challenges and opportunities. I hope the convention motivates scientist at all levels and come out with concrete recommendations for the planners.

I wish the convention a great success.

(V. Purushothaman)

CSK-Himachal Pradesh Agricultural University
Palampur-176 062 (HP) India

Dr. Mandeep Sharma
Organizing Secretary

From the desk of Organizing Secretary

Dear Colleagues, Delegates, Guests and Friends,

I feel infinitely happy and contented while greeting you all on behalf of the organizing committee to the National Symposium and XX Annual Convention of Indian Society for Veterinary Immunology & Biotechnology being organized by the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agricultural University, H.P.

Arranging conferences and symposia is an execution of the duty that paves the way towards establishing a platform for research scientists to update their knowledge and to have a sharp focus on various researchable issues in the field of specialization. It is the result of your collaboration and support that we are able to put together a wonderful and interactive scientific programme, which I am sure, will give us value for our time. All efforts are being made to craft the symposium scientifically rewarding, and I am convinced that with your valuable participation and interaction, the symposium will be a resounding success.

I express my genuine thanks to the President, Secretary and Treasurer ISVIB for trusting us to host this convention at Palampur, HP. I show gratitude to all who have supported us by sparing their valuable time in associating with the conference and providing scientific input, our sponsors who have come forward to help the organizing committee putting up this event. I would like to acknowledge the services rendered by hard working faculty, office staff, technical staff, SRFs and PG students of the Department of Veterinary Microbiology; members and conveners of various committees, staff of various departments of Veterinary College who worked day and night to make the convention a success.

The go-ahead, blessings, and best wishes of honorable Vice Chancellor, CSKHPKV, Dr. K.K. Katoch and Dean, Veterinary College, Dr. Sanjeet Katoch can by no means be put in words.

Limitations with us particularly the time shortage is known to the fellow delegates. Despite these, assemblage in the convention will inspire us for future. I will remain apologetic for any inconvenience to the delegates during their stay.

Looking forward to meet you during the symposium!

(Mandeep Sharma)

Introduction - Dr. G.C. Negi College of Veterinary and Animal Sciences

It had been periodically felt that little efforts have been made to promote live stock production and control of Diseases, besides research and extension under Sub Mountain, temperate and sub-temperate conditions of Himachal Pradesh. There was also a general consensus that a tremendous scope for livestock development including education and research, besides conservation of natural germplasm of typical species like Yaks, Gaddi sheep, goats, hill cattle and a variety of wild species of fauna (animals ansd birds), which otherwise could not be better looked after by the veterinarians trained in other parts of the country. The gap was also very wide in livestock resources available in the state vis-à-vis the qualified human resources to provide efficient veterinary health cover. Thus the College of Veterinary and Animal Sciences (COVAS) was established by the State Government in 1986 and it was duly accredited by the ICAR and approved by the Board of Management of the University with the following six composite departments:

1. Animal Production
2. Anatomy and Histology
3. Veterinary Physiology and Pharmacology
4. Vety. Microbiology, Pathology and Parasitology
5. Vety. Surgery and Gynecology
6. Vety. Medicine

The livestock farm was proposed to be strengthened further for teaching of Veterinary Sciences subjects. Biology, Biochemistry, economics and statistics, extension education and forages were to be taught in collaboration with the College of Agriculture. Considering the significant importance of the fisheries in the State, the fishery section was also considered to continue to be the constituent section of the erstwhile department of Animal production.

The motto of the college is "Caring and curing animals, creating new knowledge, therapies and learning opportunities and communicating with students, farmers, veterinarians, scientists and the public".

The mission of the college is to enhance animal and public health and well-being through excellent professional veterinary medical education, undergraduate, resident, and postgraduate education in the veterinary sciences, animal biotechnological and clinical research, and public service through clinical care and diagnostic services, life-long education, and outreach. The College of Veterinary and Animal Science is unique within the state of Himachal Pradesh as the only college that is equipped for providing veterinary education.

Institutional growth

The college has grown as one of the best among the hill Veterinary colleges of the country and has been rated in 10 top veterinary colleges of the country. The first batch of 25 students was admitted on merit in July, 1986. With the development of teaching and research facilities and as per the recommendation of the Veterinary Council of India, the minimum standards of veterinary education (MSVE, 1993) 17 departments and 2 services units were created. With the adoption of MSVE, 2008 the college now has following departments.

1. Veterinary Anatomy & Histology
2. Veterinary Physiology and Biochemistry
3. Veterinary Pharmacology and Toxicology
4. Veterinary Parasitology
5. Veterinary Microbiology
6. Veterinary Pathology
7. Veterinary Public Health & Epidemiology
8. Animal Nutrition
9. Animal Genetics and Breeding
10. Livestock Production and Management
11. Veterinary Gynecology and Genetics
12. Veterinary Surgery and Radiology
13. Veterinary Medicine
14. Livestock Product Technology
15. Veterinary and Animal Husbandry Extension
16. Department of Fishery
17. Teaching Veterinary Clinical Complex
18. Instructional livestock Farms Complex

Recently an "Advanced Multidisciplinary Veterinary Services and Farmer's Capacity Building Centre" has been instituted in the college under RKVY.

Post Graduation has also been started in the following disciplines:

M.V.Sc.

1. Veterinary Anatomy
2. Veterinary Physiology
3. Veterinary Pharmacology and Toxicology
4. Veterinary Parasitology
5. Veterinary Microbiology
6. Veterinary Pathology

7. Animal Nutrition
8. Animal Breeding and Genetics
9. Veterinary Gynaecology and Genetics
10. Veterinary Surgery and Radiology
11. Veterinary Medicine
12. Veterinary Public Health

PhD

1. Animal Nutrition
2. Animal Breeding and Genetics including Biostatistics
3. Veterinary Surgery and Radiology
4. Veterinary Anatomy & Histology
5. Veterinary Microbiology & Immunology
6. Veterinary Gynecology and Obstetrics Veterinary Medicine

The College is duly accredited with Indian Council of Agricultural Research and Veterinary Council of India and follows academic standards prescribed by Veterinary Council of India vide minimum standard of Veterinary education for B.V.Sc. And AH programme w.e.f. session 1994-95, revised in 2008. From the session 2010-11, the intake capacity of the college for UG programme has been increased to 58 and is proposed to be increased in phased manner up to 100 by the year 2015. As on today there are 63 students registered for B.V.Sc. and AH, 46 for M.V.Sc. and 01 for PhD programmes, respectively.

MANDATE

The objective and the mandate of the college are as under:-

1. Human Resource Development to produce qualified veterinarians and postgraduates in different disciplines of Animal Health, Production and Products Technology besides updating the knowledge and skills of the field Veterinarians.
2. Enhancing Animal Production by genetic improvement of cattle, sheep and other livestock species from the health point of view, studies on fertility problems, development of viable and sustainable pastures/ grasslands and technology for improving feeds and herbages for optimum nutrition, development of hormonal and neuro-endocrinological and embryological and embryo transfer technology etc. for improvement of livestock production.
3. To provide animal health coverage to the livestock population of the State by giving specialized therapeutic service at the main campus, organizing animal health camps at the farmer's doors and attending disease outbreaks.
4. Strengthening Animal Products Technology for meat, milk, fur, wool and hair etc.
5. Transfer of technology to the farmers and to render referral health services for the state.

About Department of Veterinary Microbiology, Palampur

The department of veterinary microbiology has established molecular microbiology facilities in the areas of bacteriology, mycology, virology; and immunology & serology. Since 1992, the department achieved the status of a postgraduate (M.V.Sc.) department and from 2005, Ph.D. was started. Whereas, UG teaching is carried out strictly as per VCI curriculum, PG teaching as per ICAR and research is designed according to the priority research areas of the state & country. Till this year, 1 student has completed his Ph.D, 32 students have completed their M.V.Sc. in Veterinary Microbiology and 02 are pursuing their M.V.Sc. programme. Two scholars have been enrolled for Ph.D.

The animal disease investigation laboratory of the department attends to animal disease outbreaks of various infectious diseases/ conditions in field, establishes the etiology of the disease, determines the kind of effective drug(s) against infectious agents and suggests the treatment/ control measures to contain the disease(s).

The department has completed several research projects from reputed funding agencies like United State of Department of Agriculture, ICAR, DBT, DST, NATP, NAIP, RKVY and private companies. Currently nine research projects including two network projects (HS, VTCC) are in operation. These projects focus on immunology, molecular aspects of infectious agents, sero-surveillance of infectious diseases, and assessing immune response to vaccine candidates. The department is also involved in carrying out pioneer work on *Pasteurella* spp., cattle immunology, chlamydiae in India and fish diseases.

Apart from department's involvement in teaching and research, the department renders consultancy services to the farmers of the state by suggesting them prevention and control measures against infectious disease(s) for economical dairy, sheep/goat, fish, poultry and rabbit farming. The department also provides health coverage to various animal species of university livestock farms. This department has contributed significantly by attending to stubborn ailing cases and infectious disease outbreaks among animals of the state by provide spot and laboratory diagnosis. The faculty of this department routinely delivers scientific lectures to the vets, para-vets, ex-service men, progressive farmers and unemployed youth, on different aspects of infectious diseases from time to time and involves in different trainings /workshops organized by CSK H.P.K.V. Directorate of Extension Education / COVAS and other agencies. Active consultancy to farmers of the state is also being provided through TV/ radio talks.

The department has added to the existing knowledge of the discipline by undertaking research in frontier areas of veterinary microbiology, biotechnology & immunology and has state of the art laboratories housing modern equipments required to carry out modern molecular microbiology including real-time PCR, flow cytometer, cell cuture facilities, and department's animal house.

Research Projects completed

Sr. No.	Name of the research project(s)	Funding agency	Date of start	Year of comp-letion	Total budget
1.	"Epidemiology and Immunodiagnosis of *Chlamydia psittaci* infection in sheep and goats"	U.S.D.A.	1.4.1992	31.3.1997	39.45 Lac
2.	"Studies on the prevalence of various bacterial and mycotic diseases of fish with special emphasis on trout in H.P."	I.C.A.R.	11.5.1997	14.7.2001	11.35 Lac
3.	"Development of PCR based assay for molecular diagnosis of *Chlamydia psittaci*"	D.B.T.	20.3.2000	31.3.2004	24.99 Lac
4.	"Animal health information system through disease monitoring and surveillance"	NATP-ICAR	24.5.2000	31.3.2004	23.49 Lac
5.	"Weather based animal disease forecasts"	NATP-ICAR	24.5.2000	31.12.2004	13.87 Lac

Research Project(s) under operation

Sr. No.	Name of the research project(s)	Funding Agency
1.	"All India Network Programme on Haemorrhagic Septicaemia"	ICAR
2.	Establishment of Diseases Research Laboratory at Palampur, sero-prevalence of Brucellosis and establishment of Small laboratory Animal House.	State Research Scheme
3.	"*In-vitro* and *in-vivo* investigations on antimicrobial properties of seabuckthorn oil formulations and leaf extract against common bacteria and fungi associated with skin and wound infections of animals".	NAIP-ICAR

4.	"Serological diversity and molecular characterization of *Dichelobacter nodosus* and development of vaccine against virulent foot rot".	NAIP- ICAR
5.	"Development and characterization of indigenous vaccine and diagnosis for Johne's disease".	CSIR
6.	"Strengthening of animal disease investigation laboratory".	RKVY
7.	'Studies on Genetic Diversity of *Chlamydohila abortus* and *Chlamydophila pecorum* Species Infecting Domestic Animals Ruminants and Development of Recombinant Proteins Based Species – Specific Immunodiagnostic Assay"	DBT
8.	"Immunological & Immunogenetic Profiling of indigenous hilly (Pahari) cattle for their disease resistance potential)	RKVY
9.	"Establishment of Veterinary Type Cultures Centre (VTCC) cooperating centre"	ICAR

Faculty:

Department of Veterinary Microbiology

Dr. Mandeep Sharma (Professor & Head)

M.V.Sc, PhD. Post Doc (USA)

Research interests : Molecular microbial identification; Pathogenesis & pathogenicity of organisms like *P. multocida, Chlamydia ssp., Brucella* spp.; vacine development and food-borne pathogens.

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Dr. Rajesh Chahota (Associate Professor)

M.V.Sc, PhD. (Japan)

Research interests : Chlamydial and Rickettsial infections of animals, avian and humans; molecular diagnosis, bioinformatics and phylogenetic characterization and host-pathogen interactions.

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Dr. Subhash Verma (Associate Professor)

M.V.Sc, M.Sc. (UK) Ph.D (UK) Post Doc (UK)

Research interests : Molecular methods in microbial identification, vaccine development; virulence factors and regulation of their expression and phage display for scFv development.

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- web: http://hillagric.ernet.in/education/covas/vmicro/sv

Dr. Prasenjit Dhar (Assistant Professor)

M.V.Sc, PhD.

Research interests : Animal cell culture especially for viral disease diagnosis and fish disease diagnostic techniques.

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- web: http://hillagric.ernet.in/education/covas/vmicro/pd

Dr. Aneesh Thakur (Assistant Professor)

M.V.Sc, Ph.D (Denmark)

Research interests : Molecular diagnostic techniques related to bovine tuberculosis, paratuberculosis and vaccinology.

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About ISVIB

The Indian Society of Veterinary Immunology and Biotechnology, popularly called as "ISVIB" is a conglomerate organization of primarily veterinary scientists from microbiology, biotechnology, preventive medicine, parasitology, animal reproduction and fisheries disciplines with the objective of fostering the growth of veterinary immunology and biotechnology in the era of biological technology. The organization was started in the year 1990 by collective visionary zeal of Drs. P. Richard masillamony, B.B. Mallick and B.S. Keshavamoorthy, during an interactive session at Tirupati.

This registered society motivates the members to discuss, analyze and formulate suitable strategies for safeguarding animal health and thus to ensure improved animal production. To achieve this annual national conventions and symposiums are organized to critically analyse and update our knowledge and understand issues concerning with gene based technologies in animal production and health. The organization has grown in strength with a current membership of over 850 researchers both from Idnia and abroad.

The first green revolution of the country was possible by visionaries and classical genetic and microbiological technologies, with the second undoubtedly biotechnology based. This forum will maintain the tempo amongst its members and the profession by highlighting our role in the coming years for not only a hunger free world but also a world of balanced nutrition and freedom from zoonotic diseases using biotechnology in veterinary and fisheries sphere of agriculture.

National Symposium & XX Annual Convention of ISVIB –November 11-13, 2013

Organized by:

Department of Veterinary Microbiology
DGCNCOVAS, CSKHPKV, Palampur- H.P.

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Veterinary Vaccines - future prospective

Renukaprasad. C and Byregowda. S. M
KVAFSU, Karnataka

Vaccines with history of more than 200years have long been used against infectious diseases. After the invention of first successful vaccine, many milestones have been achieved in both human and veterinary vaccines. Several serious disease instances in the past of human and animals like small pox, anthrax, rinderpest, foot and mouth disease, rabies, etc, warranted development of different kinds of vaccines for animal and human use. These two medicines worked together and in some cases human vaccines are developed first and in other veterinary vaccines were developed first. Foot and mouth disease vaccine was first to be developed among veterinary vaccine during 19th century. The technology developed during the course of history served mankind and resulted in eradication of several diseases. With the knowledge of molecular biology, genetic engineering and immunology, the vaccine design and development has witnessed a revolution in the last decade. It is true that the vast knowledge in understanding the pathogens and response of host to these pathogens has opened newer avenues and approaches for the researchers in developing effective vaccines. Modern technologies such as genomics, proteomics, functional genomics, synthetic chemistry and computational capabilities are found to be handy tools in simplifying the task. Inspite of the breakthrough research in the field of vaccinology, pathogens and nature outsmarts the researchers and still it is not possible to develop effective vaccines to some of the disease such as HIV, tuberculosis and malaria etc,.

Beginning of vaccines:

In the beginning all the vaccines developed were with whole microbial pathogen to impart immunity. The causative agent was used either killed or inactivated or used as a live pathogen with less virulence. The inactivation procedures used were heat or chemicals like formalin, carbolic acid etc., which are still being used for some of the vaccines.

First live attenuated vaccine was accidently developed by Pasteur during 1881 when he injected the aged cultures of *Pasteurella multocida* to chickens. The term attenuation was coined and observed that exposure to environmental insults like high temperature, oxygen and chemicals could be used to attenuation.

The revolution in cell culture in the middle of 20th century was developed for growth of virus and found that it was also a means of attenuation. As a result during the period of 1950 and 1980 many human attenuated vaccines were developed like polio, measles, rubella etc. Cell culture technique was effectively used for growing viral agents and attenuating these by serial passaging. Cell culture was also used for development of live and attenuated vaccines of segmented viruses like Influenza and Rota virus by making use of genetic ressortment among

serotypes. A few of the Rota viral vaccines have developed using both human and animal RNA segments.

Identification of extracellular toxins of bacteria by Roux and others led to development of toxoids and are still being used successfully for diphtheria and tetanus. As the research continued, technology was also developed to separate the subunit fractions of organisms and used as vaccines such as typhoid Vi and Pneumococci. In the later part of the previous century, conjugation of proteins by polysaccharides became a powerful vaccine against encapsulated bacteria which was later found that it is necessary to stimulate T cell response along with B cells specifically in case of infants.

These traditional vaccines of inactivated and live attenuated vaccines have both disadvantages and advantages. Each type has its own limitations depending upon the pathogen type and methodology used to vaccine preparation. Some of these methods are being followed more than a century. There was need for improvement of existing vaccines at that time and to develop vaccines where organisms cannot be cultured in laboratory. Genetic engineering and knowledge of molecular biology led to the development of next generation of vaccines.

New generation vaccines:

The first generation vaccines with knowledge of biotechnology used for creation of temperature sensitive mutants developed either by random mutagenesis or by selection of phenotypically different mutants like Mycoplasma vaccines – which grow in upper but not in lower respiratory tract or by upregulation of certain protective antigens in nutrient restricted media like Pasteurella grown in iron restricted media or biofilm vaccines developed by KVAFSU. They are able to induce CTL and T_H cell response. But always there was a risk of reverting back to virulence specifically in immunocompromised animals.

The second generation biotechnology based vaccines used rationale and targeted attenuation like deletion of 'aro A' gene responsible for the synthesis of aromatic amino acids like aro A deleted *Salmonella typhimurium* and *Pasteurella multocida* vaccines. The subunit vaccines with immunogenic antigens or recombinant protein portions such as Hepatitis B surface antigen are the result of genetic engineering. Hepatitis B vaccine with 32 S protein expressed in yeast, E.coli, and Chinese hamster ovary cells (CHO) is a great success in the modern world.

DNA vaccines are the third generation vaccines consisting of a bacterial plasmid incorporating the genes of one or more antigens from an agent or multiple agents. These when delivered, produce the immunogenic proteins of the pathogen against which the antibodies are produced. In vitro expression in bulk of the gene of interest by different expression systems like E.coli, yeast or cell lines is yet another innovation using biotechnology.

Future vaccines and vaccine design strategies

Although considerable advancement has already been achieved in developing vaccines for both human and animal use there are still unanswered questions. There are no effective vaccines for some of the important diseases. Solutions have to be found with continued research for vaccination failures and for diseases where the agent can not be grown in the lab.

Knowledge of immunology is a must for development of any vaccine. For many years importance was given only for eliciting neutralizing antibodies with B cell stimulation and not much emphasis was given to elicit T cell response. All the present vaccines and future vaccines are to be designed to elicit CTL and T_H cell response (CD4 and CD8) in addition to B cell stimulation for proper protective immunity. The vaccine presentation such as oil in water emulsions, liposomes, Toll like receptors (TLR) agonists, cytokines and other substances will help in triggering required Th1 response. Considerations for T regulator cells have to be given in developing newer vaccines for pathogens that try to evade the immune system.

The role of adjuvants in future vaccines apart from the delivery systems is equally important. Adjuvanticity is measured in terms of levels of immunity, duration and localization of the antibody response. A good adjuvant should elicit stronger, longer and localized and genralised antibody response depending upon the antigen. It should be safe without any adverse reactions and should not interfere with vaccine or produce antibodies against itself. Many adjuvants for human and animal use are available commercially. Host inflammatory cytokines in particular IL-2 have been identified potential adjuvants against infectious diseases and cancer. In recombinant and vectored vaccines incorporation of gene coding for IL_2 along with the gene of interest is found to be beneficial.

New biotechnological tool helpful in developing future vaccines include finding of virulent genes by in vivo expression technology and signature tagged mutagenecity. Reverse genetics or reverse vaccinology is another tool in developing vaccines wherein, by knowing the sequence constructs are synthesized in vitro and expression system is optimized. The expressed proteins used for the vaccination checked for protection and there by identifying protective proteins. Reverse vaccinology has been successfully used in developing vaccines against meningococcal serotype B. Another important aspect considered in newer vaccine design strategy is stimulation of innate and adaptive immune responses. Adjuvants such as CpG oligonucleotide are known to stimulate both the responses.

Other future vaccine design strategy includes vector vaccines, marker vaccines for the serological identification, DIVA vaccines for differentiating the infected and vaccinated animals, micro arrays for expression of virulence genes, synthetic peptides, combinations of vaccines (hexavalent

or pentavalent), synthetic capsular polysaccharides and controlled expression vector vaccines. Researchers are exploring the RNAi based vaccine development specially for viral diseases to block the viral multiplication in the cell. Virus core like particles (VLPs) are found to be effective for some of the viral diseases like Bluetongue where in the empty capsids without viral DNA was able to elicit required immune response.

Apart from vaccine development vaccine delivery is also a challenge and innovative technologies are being developed to suit to the vaccine and ease of vaccination to larger population in remote areas. Most of the present vaccines are given by parental injections. The need for the present day is for non parental delivery route of vaccination because of many advantages. Intranasal, aerosol, oral and transcutaneous routes are alternatives to parentaral vaccinations practiced in humans. Eliciting mucosal immune response especially with respect to respiratory and enteric disease causing agents proves to be more effective than generalized response like in polio vaccine in humans. Such vaccines for veterinary applications are still under development stage. Vaccines which can be administered by aerosol route will be more beneficial for mass vaccination more so in veterinary vaccines. Vaccines developed for Oral route administration have greater advantage like ease of administration both in animal and human beings. Hepatitis B surface antigen has been successfully expressed in plants, is one such candidate for oral route of vaccination. Latest in vaccine delivery is transcutaneous/ intra dermal route which is already been approved for rabies vaccine in humans. Nanopatches with micro needles are being studied for vaccine delivery with multiple antigens. Advantage being it can be used by a non technical person even in remote areas and more than one disease can be addressed by a single injection. A similar silicon microprojection device has been found effective in eliciting protective immunity for acute lethal vaginal HSV-2 infection. Needleless delivery of vaccines are forth coming by making use disposable syringe jet injectors (DSJIs) of vaccine delivery units using compressed carbon dioxide or manual spring where liquid vaccines are delivered without use of needles. Gene gun is being used effectively for the delivery of DNA vaccines. Targeted drug or vaccine delivery using Liposomes tagged with specific antibodies (magic bullets) is fast gaining importance specially in cancer treatment and it can be used for specific vaccination to lympohoid organs like spleen where antigen processing and presentation will be effectively carried out. Intralymphatic vaccination is a strategy to maximize the immunogenicity and vaccine efficiency as they are rich in dendritic cells and antigen presenting cells.

There is a requirement of development of suitable vaccines with higher stability and presentability suitable for wild life population. Oral bite vaccines for rabies were success in controlling sylvatic rabies in some of the countries. The latest episode of FMD in herbivores in national Park, Bangalore and Canine Disptemer in cheeta in a national park is an

alarming situation. Free range animals have to be vaccinated with some means where researches can find a solution.

Apart from infectious diseases, Vaccines for non infectious diseases are under development such as autoimmune diseases like insulin dependent diabetes, coelic disease, cancer and allergies. Vaccines are also being developed for drug addictions including nicotine, methamphetamine and cocaine wherein the antibodies will rapidly remove the drug from the body. While developing newer vaccines, utmost importance should be given to regulatory issues and always the vaccine safety has to be considered on priority. Adverse reactions have to be addressed for vaccine to be acceptable by the public. In the recent episodes of F&M in Southern India most of the outbreaks were related to breakdown of herd immunity as farmers refused vaccination of their animals for the fear of drastic reduction in the milk yield and swelling at vaccination site in vaccinated animals. Research inputs to minimse these advrse reactions have to be given so that the vaccine is well accepted.

Lead Papers

Cytokines in Animal Health and Disease

Bhatia A.K and Goel Anjna

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Cytokines are the small soluble secretary protein or peptide messenger molecules mediating complex interactions among variety of cells involved in the development and regulation of innate and adaptive immune responses, inflammatory reactions, haematopoiesis, regulation of cellular proliferation and differentiation and tissue repair. Many cytokines are named as interleukin indicating that they are secreted by white blood cells mainly by T helper cells and act on other leucocytes. Some cytokines are referred to as chemokines that effect chemotaxis of leucocytes required for inflammatory response. Many cytokines are called growth factors which participate in cellular activities. There are cytokines that are named by common names such as tissue necrotic factors (alpha and beta) and interferons (alpha, beta and gamma). Most of cytokines elicit autocrine or paracrine action but some cytokines like hormones exhibit endocrine mechanism of action .Cytokines in addition have attributes of plieotropy, redundancy, synergism and antagonism and cascade induction. Moreover, they exert their influence on various biological activities at Pico level and are easily accessible in different body fluids and their up regulation and down regulation show wide variety of potential applications during infectious diseases, immunological disorders and inflammatory conditions.

Until recently study on cytokines has been comprehensively documented by the use of murine and human cytokine molecules. In the last 10 years work has been carried out in veterinary species and avian cytokines which include many proinflammatory cytokines, interferons , tissue necrosis factors and chemokines etc. Studies in relation to cytokines of veterinary importance includes the expression of an array of cytokines synthesized in response to infections caused by different pathogens, but it is essential to pinpoint cytokines involved in defense mechanism and which cause pathology or immunological disorders. Cytokines can be used as new immunologically based diagnostic tools and immunotherapeutic approaches including vaccine strategies. Further studies will also help to determine the genetic diversity of how animals belonging to different breeding lines respond to cytokines induction. Cytokines of veterinary species has opened the area of understanding of mechanism of both pathogenesis and immunity. We have been studying the under and over expression of interferon gamma, IL4, IL10 and TNF alfa and their impact using plant extracts of *Ocimum sanctum* and *A. maxicana*.

Mesenchymal stem cells derived from equine amniotic fluid: isolation, immunophenotypic characterization and tenogenic differentiation

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Tendon injuries are common in race horses and mesenchymal stem cells (MSCs) isolated from different adult and fetal tissues have been used for tendon repair. However, amniotic fluid (AF) derived MSCs have not been much exploited for tendon regeneration. In the present study, we evaluated equine AF as a source of MSCs and standardized methodology and markers for their characterization and *in vitro* tenogenic differentiation. The adherent colonies growing in a monolayer were observed in 12 out of 20 AF samples by day 6 post-seeding and 70-80% cell confluency was reached by day 17. These cells expressed mesenchymal surface markers (CD73, CD90 and CD105) by RT-PCR and immunocytochemistry, but did not express haematopoietic markers (CD34, CD45 and CD14). In flow cytometry, the expression of CD29, CD44, CD73 and CD90 was observed in 68.83±1.27%, 93.66±1.80%, 96.96±0.44% and 93.7±1.89% of AF-MSCs, respectively. Upon supplementation of MSC growth media with 50 ng/ml bone morphogenic protein-12 (BMP-12), AF-MSCs differentiated to tenocytes within 14 days. The differentiated cells were more slender, elongated, spindle shaped with thinner and longer cytoplasmic processes and showed the expression of tenomodulin and decorin by RT-PCR and immunocytochemistry. In flow cytometry, 96.7±1.90% and 80.9±6.4% of differentiated cells expressed tenomodulin and decorin in comparison to 1.6% and 3.1% in undifferentiated control cells, respectively. Our results suggest that AF is an easily accessible and effective source of MSCs. On BMP-12 supplementation, AF-MSCs can be differentiated to tenocytes, which could be exploited for regeneration of ruptured or damaged tendon in race horses.

Nanotechnology and its applications in Veterinary Immunology

Kumanan K

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Nanotechnology is the science of seeing, measuring and manipulating matter at atomic, molecular and supramolecular levels. Nanoscience originates from the Greek word meaning 'Dwarf' and a nanometer is one billionth (10^{-9}) of a meter. Nanotechnology, in simple terms can be defined as 'engineering at very, very small scale'. At this scale, the physical, chemical and biological properties of materials differ from those of the corresponding bulk materials.

In Veterinary science nanotechnology offers a wide range of possible applications. In animal production, nano nutrient delivery is one of the promising area. Nutrients and minerals can be coupled with biodegradable nanoparticles and used in nutrient deficient animals for improving their reproductive efficiencies. Sulphur containing amino acids, cystine and methionine are required for wool production in Sheep. Sulphate reducing bacteria, *Desulphovibrio* sp has been found to be present predominantly in the rumen of Sheep. By feeding nanosulphur to the sheep, wool production in sheep can be improved. Targeted drug delivery through biodegradable nano particles is another promising area.

In disease diagnosis, functionalized magnetic nanoparticles capable of entrapping bacteria have been synthesized and characterized. These nanoparticles can be used to entrap bacteria from clinical samples. Entrapped bacteria from clinical samples can be resuspended in bacterial culture medium and distributed in to glass vials containing antibiotic discs and an indicator dye. Following incubation, the bacteria that grow in the presence of antibiotic (resistant) turn the dye colourless, while the bacteria that are killed (sensitive) retain the blue colour of the dye. This approach can be used in assessing the antibiotic sensitivity of the causative organisms, like the mastitis causing ones.

Nanotechnology has been found to have more applications in vaccine delivery. Nanoshells are optically exitable nanocomplexes composed of dielectric core like silica, coated with ultra thin metallic gold layer. Specific drugs or antigens placed inside these nanoshells, when administered, can selectively reach the target cells. Nano particles are widely used to deliver vaccines which can induce potent immune responses. Poly (lactide-co-glycolide) which is commonly called as PLG is one of the primary candidate for the development of nano encapsulated vaccines. PLG is a biocompatible and biodegradable polymer. However, PLG is insoluble in water and soluble only in a selective range of organic solvents. Nevertheless, a variety of antigens have been efficiently entrapped in PLG nanoparticles without affecting the structural and immunological integrity of the proteins/ antigens. The efficacy of chitosan nanoparticles as a vaccine delivery system has also been assessed in ruminants. Chitosan nanoparticles have been coupled with whole cell and recombinant protein of *Mycobacterium avium* sub species *paratuberculosis*. Preliminary studies have indicated better immune response in nanoparticle coupled vaccines. Calcium phosphate and Carbon nano particles are also being used as vaccine delivery systems

Application of bacteriophages in the development of new vaccines and diagnostics

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Haemorrhagic Septicemia is a major disease of cattle and buffaloes occurring in many Asian and African countries resulting in high mortality and economic losses. Killed vaccines are the only preparations in use by most of the countries affected with the disease. Such vaccines confer immunity for a short period only. To provide sufficient immunity with bacterins, repeated vaccination is required. Bacteriophage lysates could be effective in eliciting protective immunity against a variety of bacterial strains. Phage lysates are superior to the preparation of whole bacteria for vaccination. Our studies on these lines have indicated the promise of this approach for Pasteurella infection.

Brucellosis causes great economic losses to the farmer by decreasing productivity and reproduction failure and is of public health significance. Accurate diagnosis of the disease in early stages is essential for correct and effective treatment to prevent losses due to the disease. Various advanced diagnostic tests e.g. ELISA, CFT and PCR are available but these are not commonly employed at field level because they are cumbersome, require very costly and sophisticated equipment and skilled personnel and can be performed in specialized labs only. Available kits based on ELISA and molecular assays are very costly. Bacteriophages which kill Brucella specifically can be employed along with suitable indicator systems to develop cost effective, simple and easy to use assays and kits for field use. We have been able to obtain encouraging results with phage based diagnostics for Brucella.

Past, present and future of veterinary vaccines

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Vaccinology has become a recognized science that combines disciplines of immunology, microbiology, protein chemistry, and molecular biology with practical considerations of production costs, regulatory affairs, and commercial returns. The ultimate aim of any new vaccine is to provide a product that will be used to protect animals and humans against disease. More recently, vaccines have also found applications in animal production and reproduction processes. Veterinary vaccines have already made enormous impacts not only on animal health, welfare, and production but also on human health. A continuous interchange between animal and human disease control agencies and scientists will be essential to be prepared for the ever-present threat of new, emerging diseases. This is exemplified most recently with the advent of avian influenza virus, where poultry and wildfowl are identified as the major carriers of the disease, but recent data have shown that both wild and domestic cats can also become infected and may present a source of disease for humans. Pigs are susceptible to both avian and human influenza viruses, and it is speculated that coinfection of pigs with highly pathogenic avian influenza virus and human influenza virus may create viral reassortant strains with the ability for human-to-human transmission. Increasing animal travel and wildlife-human interactions promoted by global climate changes will also require sustained surveillance for the spread of diseases in different parts of the world, with both domestic, production, and wild animals forming important reservoirs of many vector borne human diseases; e.g., the emergence of WNV in the United States and Europe requires continuous surveillance and control programs for the presence of the virus in birds and horses as well as humans. A novel addition to veterinary vaccines for human disease is a cattle vaccine against *Escherichia coli* O157:H7 that recently received a conditional license for distribution from the Canadian Food Inspection Agency. *E. coli* O157:H7 is a leading cause of food-borne disease in humans worldwide, and ruminant livestock are considered to be its major

reservoir. Much progress has been made in expanding the range of veterinary vaccines available as well as increasing efficacy and reducing side effects of existing vaccines. Many problems remain to be resolved, and there is ample scope to incorporate new knowledge and technologies into vaccine design. In particular, most vaccines are still based on live, attenuated pathogen strains. Apart from the obvious dangers involved with this type of immunization, this approach is not generally desirable for commercial companies, as it exposes them to risks of mitigation, and the short shelf life and strain/region specificity of many vaccines make them uneconomical to produce. While several variably defined subunit vaccines are available in market, they are generally much less protective than live organisms. A better understanding of molecular and immunological disease processes is likely to be required to improve effectiveness of killed or subunit vaccines. In particular, while it is well established that immune system has several effector mechanisms to deal with different pathogens depending on their individual life cycles and microenvironments, most killed and subunit vaccines still rely predominantly on the induction of neutralizing antibodies. An increased ability to target pathogens at different stages of their life cycle is likely to open up new avenues for antigen discovery and increase the effectiveness of killed or subunit vaccines. One way that this may be achieved is through novel delivery systems such as plasmid DNA, liposomes, nano- or microparticles, and live vectors that introduce the vaccine antigens into the intracellular compartment. Another major advance in immunology that will have an impact on an often neglected part of vaccine development is the increased awareness of central role that innate immunity plays in action of vaccine adjuvants. Recently discovered innate immune receptors are currently being screened for active novel adjuvant compounds, and their corresponding ligands (pathogen-associated molecular patterns) are being used to increase or modulate vaccine responses. Use of adjuvants in veterinary vaccinology is much less restricted than that in human vaccines, and a large number of different types and formulations of adjuvants are currently used in licensed veterinary vaccines, compared to only three adjuvants licensed for human vaccine use. In many cases, the details of the veterinary adjuvants are unfortunately with held as proprietary information.

Apart from scientific challenges that are addressed, development of a commercially successful veterinary vaccine also needs to meet regulatory hurdles that pave route to marketplace. For example, under current US law, vaccines that target noninfectious disease (e.g., production gains and reproduction) come under the more stringent jurisdiction of the FDA and are treated as pharmaceuticals, whereas most animal vaccines come under the USDA, with more rapid and lower-cost routes to registration. In European Union, regulatory matters are based on European Union legislation, and company dossiers are assessed and legalized by European Medicines Evaluation Agency. In principle, three different procedures can be used to register a vaccine. During the

centralized procedure, new and innovative vaccines are assessed and legalized in all member states in one procedure. During mutual recognition procedure, company selects a single reference country to evaluate vaccine dossier, which is followed by an application in relevant countries to have vaccine registered afterwards. Third possibility is to apply for recently introduced decentralized procedure, which can be chosen when a more expedient registration is desired. In this case, vaccine dossier is reviewed by all selected countries at same time to save first step in mutual recognition procedure. Veterinary International Committee for Harmonization brings together the regulatory authorities of the European Union, Japan, and the United States and representatives from the animal health industry in the three regions to harmonize technical requirements for the registration of veterinary products. Veterinary International Committee for Harmonization harmonizes guidelines that represent scientific consensus regarding regulatory requirements for three regions. Expert working groups, under supervision of Steering Committee, are created to draft and recommend harmonized guidelines. Research and development form basis for generation of new and improved veterinary vaccines. Animal scientists can borrow heavily from medical research, particularly in the areas of welfare and geriatric medicine for companion animals, which are becoming increasingly lucrative markets for animal health companies. On the other hand, animal research scientists can also significantly contribute to human vaccine development, as they are able to bridge the gap between results obtained in small-rodent models, which are often not directly translatable to humans. Due to their similar sizes and anatomies, large-animal models are particularly useful for the testing of different delivery systems and have been extensively used to optimize the uptake of plasmid DNA for effective DNA vaccination. New animal health vaccines are also likely to be therapeutic rather than prophylactic, with cancer and osteoarthritis in longer-lived companion animals being obvious targets. Expected reductions in the cost of recombinant antibodies should make the passive immunotherapy of dogs and cats feasible. Due to their less stringent regulatory requirements and quicker route to the market, veterinary vaccines are also at the forefront of the testing and commercialization of innovative technologies, as exemplified by the recent successful licensing of two DNA vaccines for horses and fish and the conditional license of a DNA vaccine against Canine malignant melanoma.

Current understanding of immunobiology of Shiga toxin–producing *E. coli* –a serious food borne pathogen

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Shiga toxin or verocytotoxin- producing *Escherichia coli* (STEC or VTEC) is recognized as an important food borne pathogen, responsible for sporadic cases to serious outbreaks worldwide. The morbidity and mortality associated with several outbreaks due to STEC has highlighted the threat this organism pose to public health. Since its discovery in 1982 the organism has been focus of extensive research on its epidemiology, pathogenesis, diagnosis etc. Though the most important virulent attribute of the STEC is verotoxin or shiga toxin, but other virulence factors like intimin, enterohaemolysin have also been found to contribute to the human or animal disease. The organism may cause serious human complications like bloody diarrhoea, hemolytic uraemic syndrome (HUS), hemorrhagic colitis (HC) and thrombocytopaenic perpura. Domestic animals especially cattle and sheep have been found the main reservoirs and sources of infection. Contaminated meat and meat products, dairy products, vegetables, drinking water; swimming pools have been recognized as main vehicles of spread of infection to humans. Recently air borne dispersion (contaminated buildings) has also been incriminated as another route of spread of this infection to humans. Epidemiological evidence indicates that O157:H7 accounts for a disproportionately large number of serious infections in humans. The other important serotypes include O26:H11, O111:H-, O145:H-, O45:H2 and O4:H- Distribution of STEC serogroups varies with geographical locations. In Canada, most HUS cases are associated with a single *E. coli* serotype O157:H7 although multiple other STEC serotypes also cause HUS. In Austria and Germany non-O157 are associated with 43% of STEC positive HUS patients. In Australia, O157:H7 is rare and O111 strains are the common STEC type. In contrast, in the US more than 80% of STEC infections are by O157 strains. In India, there is paucity of information on STEC. However, STEC strains belonging to the serotype O157 have been reportedly isolated in India from sporadic cases of diarrhoea, but the isolated strains have not been well characterized and the origin of these strains are uncertain. The morbidity and mortality associated with several recent outbreaks of gastrointestinal disease caused by STEC has highlighted the threat these organisms pose to public health. There have been many advances in rapid diagnosis of this infection using the current molecular biology, immunological and cytotoxic methods.

Prospects of Phage display technology to generate immunodiagnostics and immunotherapeutics

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Alternative to hybridoma technology would cut down the labour, cost, logistic, technical and inherent biologic limitations. Hybridoma technology necessitates the immortalization and propagation of antigen-specific B-cell clones and is therefore is very labour intensive & time-consuming process. For this, fusion of lymphocytes with a suitable myeloma cell line is created, seeded into multiple 96-well plates, and cultured for a 1- to 2-week period prior to screening. The screening process involves the evaluation of hundreds to thousands of antibody containing wells that must be completed rapidly as delays result in the loss of cell clones due to the overgrowth of other cells. This sequence of screening, preparing frozen stocks, and subcloning may take place number of times over many months until stable monoclonal cell lines producing the desired antibody specificity are obtained. Furthermore, the production of serologic reagents and therapeutic immunoglobulins in mice have biologic limitations for use in other animals and humans; particularly for therapeutic purposes, the efficacy of murine derived immunoglobulin preparations is limited by the induction of anti-mouse antibody responses in the recipient. Phage display technology on the other hand can overcome these problems by obviating the need for B-cell immortalization. Phage display immortalizes antibody genes rather than the cells from which the genes were derived. What this mean is that phage display technology for *in vitro* antibody production would be simpler, less time-consuming, and more efficient than conventional approaches because immortalization of the original antibody-producing cells is not required and the selected antibodies carry with them the genetic material necessary for their replication which would lead to the production of endless supplies of antigen-specific antibodies. If they are derived from the animal under investigation, passive transfer of MAbs should not provoke the antispecies responses triggered by delivery of murine monoclonals. This may enable rapid evaluation of *in vitro* observations in relevant animal infection models. This will speed up the development of MAb-based therapies for species of veterinary and economic importance and provide through transgenesis or other novel methods of immunoprophylaxis, a rational basis for enhanced disease resistance. Passive immunomodulation of a range of physiological processes and Ig-targeted drug or vaccine delivery would also become possible.

Thematic Area I

Basic Veterinary Immunology and Immunogenetics

ISVIB 2013/
TAI/1

In vitro functional characterization of prokaryotically expressed recombinant goat conglutinin

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Conglutinin, a high molecular weight C-type lectin belonging to collectin superfamily of proteins, act as soluble pattern recognition receptor (PRR) in vertebrate innate immune system. It recognizes various pathogen associated molecular patterns (PAMP) through its globular carbohydrate recognition domain (CRD) in presence of calcium ions and thus helps in opsonisation and elimination of invading microbes. In the present study goat conglutinin gene encoding the neck and CR domain was expressed in prokaryotic system for the first time and the purified recombinant protein (rGCGN) was renatured by extensive slow dialysis to retain its functionally active conformation. The renatured protein was found to be functionally active as assessed by sugar binding and LPS binding assay. The sugar binding activity was analyzed employing modified ELISA method with yeast mannan coated on to 96 well ELISA plate utilizing polyclonal antiserum raised in poultry against buffalo conglutinin. The assay revealed affinity of recombinant goat conglutinin towards coated yeast mannan in presence of calcium. Further inhibition of binding activity in presence of 20mM NAGA and 10mM EDTA suggested high affinity of towards NAGA and requirement of Ca2+ for activity, respectively. As mandatory requirement of calcium for sugar binding is the identifying characteristic of C-type lectin group of proteins, thus it confirms the identity of the purified recombinant protein. Similarly LPS binding assay was performed with coated *E coli* LPS and the binding activity was observed to be calcium independent indicating its potent antibacterial activity. The above performed assays characteristically reveal the protein identity and its *in vitro* physiological role as innate immune marker and further pave the pathway for exploration of other identifying features for its successful use as an immunomodulator.

ISVIB 2013/
TAI/2

Promoter variants at AP2 box region of Hsp70.1 affects thermal stress response and milk production traits among cross bred cattle

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Heat shock proteins (Hsp) are known to play major role in protection of cells from thermal stress. Nucleotide polymorphisms within the promoter of Hsp affect degree of expression and inducibility of Hsp mRNA. The present study aimed to investigate the effect of polymorphism within promoter region on the cellular expression of Hsp70 .1 mRNA and also to study the association of identified polymorphisms with the physiological parameters during summer stress and milk production traits in dairy cattle. Two hundred Frieswal cows were genotyped using double PCR-RFLP to identify deletion of cytosine within the Hsp70.1 promoter AP2 box at base position 895. Homozygous wild type genotypes (CC) were found in lower frequency (39.29, n=78) than heterozygous deletion mutants (C-) (60.71, n= 122). In the observed physiological parameters ($P<0.05$), cows that were homozygous wild types had better significant rectal temperature, respiration rate and heat Tolerance Coefficient than the heterozygous deletion genotypes. Cytosine deletion mutation in the promoter region negatively affected ($P<0.01$) the expression of Hsp70.1 mRNA in peripheral bovine mononuclear cells (PBMC) subjected to *in vitro* heat stress. Further association of observed polymorphism with the milk production traits was significant as the heterozygous cytosine deletion cows had lower Total Milk Yield, Peak Yield, Yield at 300 days, Protein% ($P<0.01$) and Fat% ($P<0.05$) than the native wild type promoter cows. The results from the present study suggest that the promoter region of bovine hsp70.1 gene is polymorphic and may be useful in selection of dairy cows for relatively better thermotolerance and higher milk production.

ISVIB 2013/ TAI/3

Immunophenotyping of peripheral blood lymphocytes from Hill and Jersey cross cattle of Himachal Pradesh

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Particularly found in remote areas of the state, the hill cattle is believed to be highly adaptogenic and have better innate and adaptive immune responses to fight infectious diseases. To study their disease resistance potential, immunophenotyping was carried out to look for the distribution of CD4 and CD8 cells in the peripheral blood lymphocytes. Six adult hill cattle and six adult jersey cross cattle maintained at livestock farm, Palampur were selected for investigation. Blood samples were collected from these animals in EDTA. Fluorescein isothiocyanate labelled monoclonal antibodies CD4 and CD8 were used to examine the peripheral blood lymphocytes in whole blood of these animals by single-parameter flow cytometry. The percentage of peripheral blood lymphocytes expressing CD4 and CD8 in hill cattle (CD4+ 24.46% and CD8+ 18.13%) was higher than in jersey cross cattle (CD4+ 14.63% and CD8+ 13.86%). The higher percentage of both CD4+ and CD8+ lymphocytes in the hill cattle could have implications on observed higher disease resistance potential compared to the jersey cross cattle.

ISVIB 2013/ TAI/4

Characterization of Umbilical Cord Blood-derived Equine Mesenchymal Stromal Cells by Immuno-staining and Their Trilineage Differentiation

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Mesenchymal stromal cells (MSCs) in equines offer promise as therapeutic aid in the repair of tendons and ligaments. Fetal adnexa, including umbilical cord, amniotic fluid and Wharton's jelly are considered ideal source of MSCs due to the non-invasive nature of their isolation and

being source of more primitive progenitor cells. However, MSCs isolated from these sources in equines have not been fully characterized. The objective of this study was to isolate and characterize MSCs from equine umbilical cord blood (UCB), with a view to select the markers to identify these cells and to optimize the conditions for their *in vitro* differentiation. We isolated colonies of plastic adherent cells showing fibroblast-like morphology from 13 out of 20 umbilical cord blood collected after full term foaling. These cells were able to proliferate till passage 20 with average cell doubling time of 46.40±2.86 h and plating efficiency of 2.57±0.16%. The cells showed expression of MSC surface markers (CD29, CD44, CD73, CD90 and CD105) by RT-PCR and but did not express haematopoietic / leucocytic markers (CD34, CD45 and CD14). On immunocytochemistry, the isolated cells showed expression of CD29, CD44, CD73 and CD90 proteins using cross-reactive mouse anti-human monoclonal antibodies, but tested negative for CD34 and CD45. The expression of CD29, CD44, CD73 and CD90 was shown by 96.36% (±1.28), 93.40% (±0.70), 73.23% (±1.29) and 46.75% (±3.95) cells, respectively in flow cytometry. Osteogenic induction of these UCB-MSCs was detected by Alizarin Red S and von Kossa staining and expression of *RUNX2* and *osteocalcin* by RT-PCR. When cultured in chondrogenic differentiation media, UCB-MSCs showed marked deposition of glycosaminoglycans as seen by Alcian blue staining and expression of *collagen 2αI* in RT-PCR. Optimum adipogenic differentiation was achieved using media supplemented with 15% rabbit serum after 10 days of induction detected by Oil red O staining. Differentiated adipocytes showed expression of *PPAR-γ* and *adiponectin* by RT-PCR. Our findings established the criteria and markers for characterization of equine MSCs and that UCB being a rich and safe source of MSCs in equines for therapeutic applications.

ISVIB 2013/
TAI/5

Candidate gene polymorphism for IL-2Rγ and ChB6 gene in indigenous chicken of North Western Himalayan state of Himachal Pradesh, India

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Immune system plays important role in protecting poultry from diseases. Interleukins, important group of cytokines, plays significant role in elicitation of immune response. Interleukin-2Rγ (IL-2Rγ) is shared by receptor complex of many interleukins and it induces proliferation and differentiation of T, B, and NK cells. The chicken B-cell marker (ChB6) gene has been proposed as a candidate gene in regulating B-cell

development. There is limited information on the genetic architecture of indigenous chicken population. Hardiness/resistance to disease is one of the unique features of these indigenous chickens. In the present investigation DNA polymorphism at Interleukin-2Rγ chain (IL-2Rγ) and chicken B-cell marker (ChB6) genes were studied by PCR-RFLP technique in 68 random bred indigenous chicken of Himachal Pradesh, North Western Himalayan state of, India. Amplification of IL-2Rγ and ChB6 yielded PCR products of 600 and 215bp, respectively. *Hph* I digestion of IL-2Rγ PCR product generated three pattern i.e. *Hph* I aa, *Hph* I a/b and *Hph* I bb with frequency 0.47, 0.23 and 0.30 respectively. *Hph* I aa and *Hph* I bb genotypes revealed only 465 bp and only 454 bp fragments, respectively, whereas, *Hph* I a/b genotype resolved both 465 and 454 bp fragments *Pvu* II digestion of ChB6 generated two patterns i.e. *Pvu* II aa and *Pvu* II ab with frequency 0.57 and 0.43 respectively. *Pvu* II aa generated 215 bp fragments while *Pvu* II bb generated 215, 147 and 68 bp respectively. Polymorphism observed at both the loci may be used for further exploration to improve humoral response.

ISVIB 2013/
TAI/6

Antioxidant capacity and free radical scavenging activity of cow and buffalo milk during different lactation stages

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Milk from dairy animals contains several enzymatic and non enzymatic antioxidants constituents which are crucial to prevent the production of reactive oxygen species and help to activate the body antioxidant defense mechanism. The aim of this study was to evaluate the comparative changes in total antioxidant capacity and free radical scavenging activity of milk during the course of lactation in different cattle types and buffaloes. Milk samples from a total of 55 healthy animals of Sahiwal cows (Indian native cattle), Karan Frisien cows (Cross-bred) and Murrah buffaloes (Riverine buffaloes) were collected at different lactation stages; early lactation (5-15 days), peak (30-60 days), mid (100-140 days) and late lactation (>215days). The total antioxidant capacity (TAC) of milk was measured by Ferric Reducing/Antioxidant Power assay (FRAP) and free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. TAC in milk was observed to be higher during early lactation in comparison to peak and mid lactation periods. Similar results were observed for DPPH radical scavenging activity of the samples. A correlation was observed between the results of FRAP and DPPH assays.

These data suggest that milk during the early lactation period of dairy cows had higher content of antioxidants in comparison to mid lactation; moreover, reduction in total TAC during the course of lactation may needs more attention about nutritional status.

ISVIB 2013/ TAI/7

Cloning and sequence analysis of *GPCR* gene encoded G-protein-coupled chemokine receptor homologue of Sheeppox virus

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Sheeppox and goatpox are highly contagious, transboundary and economically important diseases of goats and sheep, respectively. They are endemic in Indian sub-continent and caused by sheeppox virus (SPPV) and goatpox virus (GTPV) of the genus *Capripoxvirus* (CaPV), subfamily *Chordopoxvirinae* and family *Poxviridae*. Currently, capripox viruses are classified as per host species infected. However, recent studies showed that SPPV can infect goats and GTPV can infect sheep. Regular outbreaks of sheeppox and goatpox are reported from India in spite of using live attenuated vaccines. However, only limited number of studies was carried out on molecular characterization of vaccine strains, challenge viruses and field isolates for their host specificity. Therefore, the present study was aimed to characterize the Indian sheeppox virus - Srinagar vaccine strain (p 40) by *GPCR* gene sequencing. The complete ORF of *GPCR* genes was amplified from the extracted DNA and cloned into TA cloning vector and sequenced. Expected full length amplicons size of ~1684 bp was visualized in agarose gel electrophoresis. On multiple sequence alignment, the Srinagar vaccine strain possessed SPPV lineage specific amino acid signatures including 7-amino acid deletion near the N-terminal end. The present study confirms that SPPV Srinagar strain has sheep specific signatures.

ISVIB 2013/ TAI/8

Quantification of milk immunoglobulins in Pahari and Jersey cross cattle.

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The biological function of milk Immunoglobulins is to provide immunity to the neonates and to protect the mammary gland from infections. The indigenous cattle of Himachal Pradesh called as "Pahari Cattle" serve as an important source of income for the farmers and landless livestock owners of the state. Pahari cattle are known to be highly resistant to many infectious diseases including mastitis compared to exotic breeds. Amongst other factors, the concentration of igs could have a possible role to this effect. The present study was therefore focussed on quantifying milk Igs using indirect ELISA (iELISA) and to compare their levels in Jersey cross cattle. A total of 20 raw milk samples were processed which were collected from district Kangra and Kullu from both types of animals. Three different blocking reagents viz. BSA (Bovine Serum Albumin), skimmed bovine milk and dog serum were used for the optimisation of iELISA and later the experiment was performed with dog serum. Mean concentration of IgG was 0.245 mg/ ml and 0.234 mg/ ml, IgA was 0.1484 mg/ml and 0.1524 mg/ml and IgM was 0.0526 mg/ml and 0.0602 mg/ml in Pahari and Jersey cross cattle respectively. This study concludes that the mean milk immunoglobulin levels in animals of both breeds are similar under normal physiological health status. Similar studies performed under diseased conditions such as mastitis to look at the differential levels of milk igs would give insight into their breed-specific protective role.

ISVIB 2013/ TAI/9

Evaluation of Immunomodulatory activity of Seabuckthron fruit pulp and seed oil in male wistar rats

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Sea buckthorn (*Hippophae rhamnoides*) is a deciduous plant containing various bioactive substances such as vitamins, carotenoids, flavonoids, polyunsaturated fatty acids, free amino acids and elemental components. These components vary substantially amongst various species or sub- species. The clinical trials and experimental studies during the 20th have extensively confirmed medicinal and nutritional value of sea buckthorn (SBT). The present studies were planned to study immunomodulatory effects of ethanolic extract of the seabuckthorn pulp

and seabuckthorn seed oil in male Wistar rats. The animals were divided into eight groups *viz*. Group I control (no treatment), Group II vitamin C (500 mg.kg^{-1} orally), Group III Chromium (30 mg.kg^{-1} orally), Group IV Chromium + Pulp extract (100 mg.kg^{-1} orally), Group V Chromium + Pulp extract (200 mg.kg^{-1} orally), Group VI Chromium + seed oil (500 mg.kg^{-1} orally), Group VII Chromium + seed oil (1000 mg.kg^{-1} orally) and Group VIII Chromium + Vitamin C. Immuno-compromised status in rats was induced by oral administration of potassium-di-chromate. Immune status of rats against SRBC was evaluated by estimation of cytokine levels (IFN γ, IL-2 and IL-4) and delayed hypersensitivity reaction. Chromium significantly inhibited SRBC induced IL-2, IL-4 and IFN-γ production in rat serum besides significantly decreasing the delayed type hypersensitivity reaction. However, pulp extract (200mg.kg^{-1}) significantly ($P<0.05$) up-regulated the cytokine levels (IL-2 and IFN- γ) in chromium fed rats as compared to control besides significantly enhancing delayed type hypersensitivity reaction . However, no significant change ($P>0.05$) was observed in IL-4 count in any of the experimental groups. SBT seed oil did not show significant change ($P>0.05$) in the immune response compared to either pulp extract or control group. Thus, the present study on SBT pulp and seed oil indicated that concentration of 200 mg.kg^{-1} of SBT pulp extract can boost the immune system.

ISVIB 2013/
TAI/10

Genetic architecture studies of indigenous hill cattle of Himachal Pradesh based on microsatellite markers

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The genetic characterization of populations allows evaluation of genetic variability, a fundamental element in working out breeding strategies and genetic conservation plans. Microsatellites are presently among the most favored molecular markers, essentially owing to the option of blending their analysis with use of the polymerase chain reaction (PCR). Sixty eight unrelated animals were randomly chosen from the breeding tract for genotyping with 25 FAO recommended microsatellite markers. All the amplified microsatellite loci were polymorphic. A total number of 156 alleles were observed with a mean of 6.24 0.40 alleles per locus. These microsatellites exhibited a high polymorphism as revealed by a wide range of alleles which varied from 4to 12. The overall effective number of alleles was less than the observed values across all loci and ranged from 3.12 to 8.07 with a mean number of 4.29±0.24.The allele

size ranged between 86 bp to 304 bp. The overall means for observed and expected heterozygosities were 0.79±0.03and 0.74±0.01, respectively, with range of 0.5235 to 1.0000 and 0.6172 (MM8) to 0.8832 .The observed heterozygosity was higher to expected heterozygosity in 16 out of 25 loci studied. The genetic diversity in the breeds is expressed in terms of average heterozygosity. The average heterozygosity ranged from 0.6104to 0.8762. A higher degree of genetic diversity and excess heterozygosity suggests that hill cattle population of Himachal Pradesh is a large random mating population and there is absence of genetic isolation between the sub-populations of this breed from the sampling area. The study reveals an obvious need for more in-depth and objective information on wider samples of these cattle in order to access the future need for conservation and improvement programs to be undertaken.

ISVIB2013/ TAI/11

Preponderance of β-casein A2 allele in cattle population adapted to high altitude of Leh and Ladakh region

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Cattle genetic resources in Leh and Ladakh region are good source of primary nutritional requirement to the local as well as Indian army deployed in Ladakh (L- Sector). The cattle population native to Leh and Ladakh region has naturally been adapted over many generations region to high altitude (~3500–5500 m), and chronic hypoxic condition. The Leh cattle are considered to be genetically unique population which needs to be characterized for its optimal utilization and improvement. It would be prudent to look into the allelic profile of candidate genes related to milk quality production traits in Leh cattle. In the present study, an effort was made to delineate the allelic distribution of A1/A2 alleles of β-casein in Leh cattle. β-casein is second most abundant protein (25-35%) in cow's milk and has 13 known variants. However, A1 and A2 are reported to be the most abundant forms in dairy cattle. Variant A1 has been shown to have association with the production of beta-casomorphin 7 (BCM 7), a bioactive peptide 'opioid' produced during the gastrointestinal digestion of raw/processed milk. BCM-7 has been implicated as a risk factor to several health disorders such as type I diabetes mellitus, coronary heart disease, sudden infant death syndrome, arteriosclerosis, schizophrenia and autism. Although evidence for a clear link between A1 β-casein and a disease state has not been well demonstrated, it is necessary to monitor

the status of A1/A2 alleles in Leh cattle as a cautionary measure. Investigations in recent past have shown widespread presence of β-casein A1 allele in European cattle while Indian native cattle breeds have revealed the near fixation of A2 allele. In this study, the first systematic report on distribution of β-casein A1/A2 variant in Leh cattle adapted to high altitude was conducted. In 62 animals belonging to 10 different locations across Leh region, A2A2 genotype was found to be predominant (0.815), followed by A1A2 (0.185). None of the studied animal revealed A1A1 genotype. Allele frequency distribution indicated predominance of favourable A2 variant (0.908) in comparison to A1 (0.092). As A2 allele frequency pattern was on the higher side, it could be suggested that the scenario related to A1 β-casein associated health issues is on the safer side in Leh cattle. The present data also suggest that bulls being used under breeding programme should be screened in order to check dissemination of undesirable A1 allele among the Leh cattle.

Thematic Area II

Disease diagnosis (Conventional, Immunological, serological and molecular methods)

ISVIB 2013/ TAII/1

Detection of *Pasteurella multocida* by Loop mediated isothermal amplification (LAMP)

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Pasteurella multocida is an important pathogen affecting livestock and poultry causing significant economic losses. A loop-mediated isothermal amplification (LAMP) assay using four primers targeting a conserved region of the *kmt1* gene to diagnose *Pasteurella multocida*. The LAMP was carried out at 64°C for 45 min, the LAMP products could be visually confirmed using fluorescent dye SYBR Green I as detection reagent both with naked eye as well as under UV-illumination. The sensitivity of the LAMP assay was 10^4 fold higher than PCR. Furthermore, no cross-reactivity was founded with the other tested bacteria. The developed LAMP assay allows easy, rapid, accurate and sensitive detection of *Pasteurella multocida*.

Keywords: LAMP, *Pasteurella multocida*, *kmt1* gene

ISVIB 2013/ TAII/2

Isolation and antibiogram profile of pathogenic microbes from bovine and caprine mastitis

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Mastitis is very serious and most common infectious disease in dairy animals across the world. Mastitis is also economically damaging disease which not only affects the quality of milk, its products, its yield but also a great threat to dairy industries worldwide. The present study is a part of research work carried from February to October 2013 in the Dept. of

Veterinary Microbiology, COVAS Palampur. A total of 146 milk samples comprising of 123 and 23 mastitis infected bovine and caprine samples respectively. All these milk samples were subjected to microbiological analysis. 10 µl of inoculums of mastitic milk samples were inoculated on 5 % defibrinated sheep Blood Agar, MacConkey Agar plates and Sabaroud Dextrose Agar for bacterial and fungal isolation. Plates were incubated aerobically at 37°C for 24-48 hours for bacterial isolation and at 25°C for 3-4 days for fungal growth detection respectively. These plates were then examined for growth, hemolysis, colony morphology and cultural characteristics. Isolation was done by analysing colony morphology, cultural characteristics, staining affinity and biochemical characterization. On the basis of these tests microbes isolated from these mastitic milk samples were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus bovis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Enterobacter aerogenes*, *E.coli*, *Bacillus* spp., *Candida tropicalis* and *Aspergillus* spp. Antibiotic sensitivity test for isolated microbes was performed on Muller Hilton Agar (MHA) and 5 % defibrinated sheep blood agar for *Streptococcus* spp. using disc diffusion method. Antibiotic study revealed Gentamicin to be most effective drug followed by Ciprofloxacin, Ofloxacin, Enrofloxacin and Amikacin.

ISVIB 2013/
TAII/3

Occurrence of Marek's Disease in desi bird flock

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Marek's disease (MD) is a lymphoproliferative disease of chickens that causes significant economic losses in the poultry industry. Village or desi chickens are considered to be resistant to commonly encountered diseases in commercial layer flocks. However, now a days desi birds are reared in an intensive manner and more over proper vaccination is not carried out, hence it also susceptible to various diseases encountered in commercial layers. This report describes the occurrence and pathology of Marek's disease in desi chicken reared in a semi-intensive system. Two 85 days old desi birds from a flock of 210 were submitted for postmortem examination with history of dullness, depression and chronic mortality over a period of 3 weeks with cumulative mortality of 9 per cent. Grossly, the liver was diffusely enlarged and occupied the entire abdomen. Proventriculus was thickened with prominent mucosal glands. Spleen

was enlarged and appeared like a tennis ball. Both the kidneys were enlarged. Sciatic nerves were apparently normal. Histpathologically, diffuse accumulation of large lymphocytes and lymphoblasts, with distortion and atrophy of hepatic architecture were noticed in the liver. Spleen revealed massive proliferation of small and large lymphocytes in spleenic corpuscles. In kidneys, diffuse proliferation and infiltration of lymphoblastic cells were observed in the interstitial connective tissues with degenerative changes in tubules. Sciatic nerves showed extensive infiltration of mixed population of small and large lymphocytes. The PCR analysis of the liver, spleen and feather follicle samples confirmed the presence of Marek's disease virus serotype 1. Based on the gross, histopathology and PCR results the cause of death in the flock was diagnosed as Marek's Disease.

ISVIB 2013/
TAII/4

Predisposing Effect of *Mycoplasma gallisepticum* to *E.Coli* Infection in Layer Chicken

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Outbreaks of infectious disease are a constant risk for the poultry industry, among them respiratory tract pathogens are of major concern, which causes heavy economical losses both in terms of production and cost of treatment. The severity of the diseases was exacerbated as the result of mixed infections with other respiratory pathogens. Severe outbreaks with high morbidity and mortality observed in chickens were frequently due to concurrent infections. This report deals with the synergistic pathological effect of *Mycoplasma gallisepticum* (Mg) and *Escherichia coli* infection in layer chickens. *Mycoplasma gallisepticum* causes chronic respiratory disease (CRD) in chickens and other fowl. Predisposing pathological effect of *Mycoplasma gallisepticum* to *E.coli* in layer chicken was evaluated in 70 commercial layer chicken farms. Necropsy was carried out on recently died chickens and samples such as trachea, lungs, air sacs, and swabs of infraorbital sinus exudates and heart blood were collected. These samples were utilised for the confirmation of etiological agents. *E.coli* was confirmed by their growth characteristics on Eosin Methylene Blue (EMB) agar media. In polymerase chain reaction (PCR) Mg positive samples produced 530bp products corresponding to their 16S rRNA. Among the 70 farms 11 were positive for Mg alone and 27 were positive for both Mg and *E.coli*. The average mortality rates observed in the occurrence of CRD as an individual disease was 8 per cent, whereas *E.coli* combined outbreaks recorded 15 per cent. Severe airsacculitis characterised by appearance of large masses of caseous exudate in the air sacs and egg peritonitis were observed in

combined infection with *E.coli* , whereas uncomplicated CRD affected birds showed milder airsacculitis. Microscopically, air sacs revealed more intensity of epithelial hyperplasia, subepithelial infiltration of heterophils and macrophages, and thickening of connective tissue in complicated CRD with *E.coli*. The predisposing effect of *Mycoplasma gallisepticum* to *E.coli* is proved well by the gross and histopathological observations.

ISVIB 2013/ TAII/5

Current Status on Prevalence of Respiratory Diseases in Layer Chicken

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Prevalence of respiratory diseases among commercial layer chicken in and around Namakkal district were investigated. Respiratory infections due to single disease were observed only in 21 farms (30%), remaining 49 farms (70%) were affected with combined infections of different respiratory diseases. Colibacillosis, ND and CRD were the major respiratory diseases recorded either alone or in different combinations. Most of the respiratory disease outbreaks were recorded in the month of May followed by December. Newcastle disease dominated in hot months, whereas IB, IC and ORT infections occurred more frequently during colder months. The disease CRD occurred in both hot and colder months. Age related incidences were documented. IB and ORT affected the young birds of below 10 wk age. Though CRD was noticed in all age groups, most of the incidences were recorded in growers and early layers (11 – 30 weeks), but no occurrence was noticed in the age group of below 10 weeks. The occurrence of IC was more frequently noticed among the early and peak production layers (20 – 50 weeks). The incidence of ND was recorded in all ages (one day to 80 weeks). Severity of colibacillosis infection was higher in young birds Fowl cholera as a single and mixed infections was recorded in the age group of 15 – 40 weeks and not below 15 weeks age. Among the various respiratory ailments affecting the birds, CRD, IB and ND in different combinations caused high mortality of 17, 15 and 14 % respectively. Mortality due to other respiratory disease was negligible unless they combined with any one of the three major respiratory diseases mentioned above.

ISVIB 2013/ TAII/6

Molecular characterisation of infectious bronchitis virus isolated from broiler chicken with nephropathy

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Globally, infectious bronchitis (IB) is one of the important poultry diseases and vaccines containing Massachusetts strain 41 (M41) is being regularly used for prevention of IB in India. The variant strains of IB virus (IBV) that are antigenically different from vaccine strain complicate the control of IB in chickens. Different serotypes thought to be generated by nucleotide point mutations, insertions, deletions or recombination of S1 and other genes could be circulating among chickens in India. Thus, determining the genotype or serotype of field strains is empirical for selecting an appropriate candidate strain to be utilized for vaccination. There were reports pointing mutation in S1 gene of the virus especially hypervariable region 1 (HVR 1). Hence, the work is aimed at amplifying and sequencing of HVR 1 of one isolate obtained from broiler chicken affected with nephritis. The isolate formed a different cluster in the phylogenetic analysis. The virus showed more similarity with IBV isolated in Brazil (pigeon), China (poultry) and Sweden (Poultry) in phylogenetic analysis with regard to HVR 1. This indicated the variation and mutation in HVR 1 of S1 protein is quite common which may be attributed to selection pressure arising out of vaccination.

ISVIB 2013/ TAII/7

Comparison of haemagglutination test and polymerase chain reaction in detection of canine parvovirus in faecal samples

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Canine parvovirus (CPV) is the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, and being found world-wide, is one of the most common pathogenic viruses causing diarrhoea in dogs. High titre of the virus is shed in faeces for a few days post-infection and is transmitted to susceptible hosts by the oro-faecal route. A study was carried out to compare haemmagglutination (HA) test and PCR in detecting

canine parvovirus in faecal materials obtained from dogs suspected to be suffering from parvoviral gastroenteritis. The presence of CPV in the collected feces sample/rectal swabs was detected by demonstration of HA activity. Samples were homogenized (10% W/V) in PBS solution (pH 7.2) and subsequently clarified by centrifuging at 3000 rpm for 15 minutes. Freshly prepared one per cent porcine erythrocytes were used for performing HA test. Assay was performed in 4^0C. All the HA activity was neutralized by specific antiserum. Out of the 10 samples which were subjected to HA test, eight samples (80%) had the HA titre of 1:512 and above. The DNA extracts obtained were subjected for PCR by using H_{for} and H_{rev} primers for amplification of VP2 gene of capsid protein. All the 10 samples (100%) were positive in PCR and produced expected amplicons of 611 bp. This showed the presence of CPV 2a serotype in all the samples and PCR is more sensitive than HA test. We conclude that HA test can be effectively used for initial screening of CPV gastroenteritis owing to its simplicity and PCR can be used for screening HA-negative samples.

ISVIB 2013/
TAII/8

Assessment of the common methods for diagnosis of bovine subclinical mastitis revealed poor correlation

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In this study, a total of 69 lactating Jersey crossbred cows of an organised dairy farm were examined for the presence of subclinical mastitis. The diagnosis of subclinical mastitis was done on the basis of somatic cell counting (SCC) in the milk samples. The other methods of diagnosis of subclinical mastitis such as California mastitis test (CMT), bromothymol blue (BTB) test, microbial culture and milk yield evaluation were also performed. Different tests revealed different results. It was found that although these diagnotic methods correlated linearly with SCC but the correlation was not 100%. CMT and BTB failed to detect presence of subclinical mastitis in 10 and 24 percent cases, respectively; where somatic cell count was more than 0.4 million cells/ml. CMT was found to be more sensitive method than BTB testing, but specificity of BTB testing was greater than the CMT. Interestingly, twenty percent of cows were harboring pathogenic bacteria without any abnormal change in somatic cell count; whereas, in 41 percent cows, a high somatic cell count was observed in the absence of any bacteria. Furthermore, daily milk yield had a negative but weak correlation with SCC. No differences in the presence of proteins could be picked up by SDS-PAGE of milk samples

containing 0.2, 0.7, 1.3 million cells/ml respectively. It was concluded that the correlation between the different methods for testing subclinical mastitis is tenuous and as such none of the tests could be regarded as confirmatory for diagnosis of subclinical mastitis. This study warrants the development of alternative methods for achieving the confirmatory diagnosis of subclinical mastitis.

Subclinical mastitis; somatic cell counting; California mastitis test; bromothymol blue test; microbial culturing of milk; SDSPAGE

ISVIB 2013/ TAII/9

Rapid Polymerase Chain Reaction Protocol For Diagnosis Of Brucellosis Directly From Clinical Samples

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Clinical suspected material from 24 aborted Marathwadi buffaloes were screened for the Brucellosis by rapid PCR protocol directly using the clinical material i.e., abomasal content of the aborted fetus, uterine discharge after abortion and bacterial isolates. The Primers designed for the identification of Brucella isolates were subjected to PCR directly to amplify the *Omp* gene (Outer Membrane Protein). Simultaneously PCR was performed after extraction of DNA from the isolates and clinical material directly. Isolation and identification of the bacteria revealed the efficacy of direct PCR using the clinical material and bacterial isolates.

ISVIB 2013/ TAII/10

Animal Chlamydiosis in India: Current Challenges and Future Prospects

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Chlamydiae are obligate intracellular bacterial pathogens, responsible

for diverse disease conditions in animals, birds and humans. This genetically diverse group of organism, with unique biphasic lifecycle, belongs to order Chlamydiales and family Chlamydiaceae having two genera, *Chlamydia* (having 3 species) and *Chlamydophila* (6 species). In India animal chlamydiosis in known since many decades and has been reported from many livestock and wild animal species. But prevalence of different species and strains is not known and availability of chlamydial diagnostics for laboratory and field level applications are not available in India. Therefore, in last five years we studied the molecular epidemiology and genetic diversity of chlamydiosis in small and large ruminants particularly using *ompA* gene based nested PCR, which resulted in total 131(19.45%) positive samples out of 685 tested samples from various disease conditions. The overall involvement of chlamydiae in three major diseases was abortions (17.73%); endometritis/retained placenta/uterine tissues (21.03%); pneumonitis 15.42%; enteritis 27.27%. Livestock species wise chlamydial association was found predominantly in endometritis in bovine (21.02%); abortions in ovine (25.31%); pneumonitis (18.25%) and enteritis 44.4 in caprine (majority samples from kids). In this study we detected two chlamydial species- *C. psittaci* and *C. abortus* involved in various disease conditions and all strains were phylogenetically grouped into two genetic clusters. Recombinant proteins of three overlapping fragments of Chlamydial Major Outer Membrane protein (MOMP) were prepared by using plasmid vector pET22b(+) and expressed in Lemo21(DE3) strain of *E. coli* to develop immunodiagnostic. Besides this different outer membrane protein *ompA,* POMP and *omcA* and *omcB* were also studied by structural homology modeling to examine their role in chlamydial pathogenesis and adjudging their suitability as vaccine candidate. This work highlighted the importance of further extensive studies on animal chlamydiosis as well as appropriate control and prevention of chlamydial infections not only from the animal's health angle but also from the human health point of view owing to zoonotic potential of chlamydial species.

ISVIB 2013/
TAII/11

Detection of *S.pseudintermedius* from canine pyoderma by PCR

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Staphylococcus pseudintermedius is a coagulase positive species in the *S.intermedius* group (SIG) and it consists of *S. intermedius, S. pseudintermedius* and *Staphylococcus delphini*. It is difficult to

differentiate *S. intermedius* from *S. pseudintermedius* during routine diagnostic procedures. *S. intermedius* can be differentiated from *S. pseudintermedius* by a combination of biochemical tests. Commercial identification systems for the fast and correct identification of *S. pseudintermedius* are not available to date. *S. pseudintermedius* is a relatively new species and remains to be included in the databases of most systems. Hence, Molecular methods are required for correct identification and differentiation of members of SIG. In this study, we have isolated 50 staphylococcal isolates from canine pyoderma cases and detected *S.pseudintermedius* isolates by polymerase chain reaction. We have designed primers using online idt primerquest software of Integrated DNA Technologies, USA (www.idtdna.com) using *S.pseudintermedius* ED99 and HKU10-03 strains *nuc* gene sequences available in the NCBI database (Accession No. CP002478 & CP002439 respectively). Then, the selected primers were blast searched for the specificity using Blastn program of NCBI. *S.intermedius* (MTCC 6152) was used for testing the specificity of primers. The primers yielded specific 780bp product size only in *S.pseudintermedius* isolates and not in *S.intermedius* culture. Sequence analysis of one of the *S.pseudintermedius* isolate revealed that it was closely related to *S.pseudintermedius* HKU10-03 than to *S.pseudintermedius* ED99 strain. Out of 50 staphylococcal isolates, 37 (74%) were identified as *S.pseudintermedius* organisms. This study shows that the majority of canine pyoderma is caused by *S.pseudintermedius* organisms. Detection of *S.pseudintermedius* by PCR is a simple and an extremely useful method in veterinary diagnostic and clinical microbiology laboratories.

ISVIB 2013/ TAII/12

Antisperm antibodies detection in blood serum and cervical mucus of crossbred cows/heifers: Correlation between Immune Peroxidase Assay an Sperm Mart Test

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Anti sperm antibodies (ASA), developed / directed against the sperm can cause both male and female infertility. Therefore, blood serum and cervical mucus of 34 crossbred cows /heifers (that were 1-12 times in heat and 1-10 times inseminated by AI) were investigated for the status of ASA by immunoperoxidase assay (IPA) and sperm mart test (SPT). Blood serum (BS) and cervical mucus (CM) of all the animals tested

positive for ASA irrespective of number of inseminations. But ASA in BS and CM showed differential distribution of sperm surface antigens on acrosome (SAA), post acrosomal cap (PACSA), head (HSA), tail (TSA) and whole sperm (WSSA). This study revealed that: a) ASA detected by IPA were highest against SAA and PACSA in BS and CM of 0-10 % and 11-20 % of sampled animals respectively. b) ASA against HSA and SAA + PACSA + HSA were highest in the BS (11-20 %, 41-50 %) and CM (0-10 %, 11-20 %) of tested samples. c) IgG detected by SMT were maximum against HSA, and TSA in BS and CM of 11-20 % of tested samples, whereas, IgG against WSSA were highest in only 0-10% samples. d) However, 31-40 % and 41-60 % of samples were observed positive for IgG against HSA + TSA + WSSA to a maximum level in BS and CM. e) IgA detected by SMT were highest against HSA, TSA in BS and CM of 11-30% of samples investigated. f) IgA against WSSA were maximum in BS and CM of 0-10 % samples only, whereas, IgA against HSA + TSA + WSSA in BS and CM were maximum in 21-30% and 31-60 % of animals respectively.

Attempts to correlate between IPA/SMT and different parameters i.e. age of cow, difference between calving and conception (DCC), number of estrus /AIs and number of parity revealed: 1) a weak positive correlation between IPA and number of estrus /AI /parity. 2) SMT indicated a weak positive correlation between IgA in BS / CM & DCC; both IgA, IgG in BS and number of estrus/parity. 3) An average correlation between IPA and SMT indicated the authenticity of both the tests to detect ASA in BS and CM. This study highlights the detection of ASA development in BS and CM of variously inseminated cows/heifers showing sub fertility.

ISVIB 2013/
TAII/13

Pathogenicity of FAV-4 in chicken embryos and their detection by molecular methods

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Two field isolates of fowl adenovirus serotype -4 (FAV-4) along with a positive control isolate was propagated in embryonated chicken eggs to find out the effect of the virus in chicken embryos. Each isolate was passaged thrice in five numbers of embryonated chicken eggs of 6 to 8 days old via yolk sac route and observed for seven days. The pathogenicity of the isolates was determined by recording the growth rate, mortality and gross lesions of embryos. The growth rate was observed by recording

the length, width and weight of embryos that revealed smaller length, width and weight of the infected embryos as compared to control embryos. All the isolates produced almost identical pathology (petechial to extensive hemorrhages) in embryonated eggs with increase in intensity of pathogenicity at each passage level. Maximum mortality (20 embryos) was recorded at 96 hrs. post infection (p.i). Detection of the virus from infected parts of the eggs (livers, yolk, heart and amnioallantoic fluid) from each passage level was done with the help of hexon gene specific primers that amplifies a 0.7 kb product of the hexon gene of FAV-4. Livers, yolk and amnioallantoic fluid from embryonated chicks showed required amplification at all passage levels, while no detection of virus was possible from the heart. In conclusion, the FAV-4 isolates in the present study were found to be suitably pathogenic for chicken embryos with peak mortality at 96 hrs. p.i, but the virus may not be present in the heart during the course of infection.

ISVIB 2013/
TAII/14

Identification of avian leukosis virus subgroup A (ALV A) transmitter chickens on-Farm using combination of serological and molecular assays

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Retroviral avian leukosis/ sarcoma virus (AL/SV) infections are ubiquitous worldwide, with avian leukosis virus (ALV) as the most common infection under field conditions. Exogenous ALV has 5 subgroups, *viz.*, A, B, C, D and J; and endogenous ALV has one subgroup, *viz.*, J. Complex interaction of exogenous and endogenous viruses give rise to ALV shedders, which may also be ALV transmitters, congenitally/ vertically transmitting virions to their progeny chicks. ALV shedders or transmitters constitute permanent source of infection in a poultry farm causing immune tolerance among the commercial chickens that give rise to leukosis/ sarcoma (L/S) group of diseases responsible for significant economic losses each year in the commercial Poultry Industry, besides the infections being a public health threat through chicken- origin food products, chicken-origin vaccines, and chicken as reservoirs giving rise to newer virus isolates, eg., ALV J subgroup. To establish exogenous ALV free flock, the foremost preventive measure is to break the vertical transmission cycle

and also to prevent re-infection of the flock by congenitally/ vertically infected chicks. Hence, this study was carried out to identify the ALV transmitters *on- farm* using standard molecular and serological tools.

Sixteen exogenous ALV A positive CSML hens were chosen based on standard molecular and 'gold' standard major gsAg or p27 based serological assays. All the birds were artificially inseminated, fertile eggs were collected and kept for incubation for hatching. At day 21, the chicks did not hatch out on their own, hence during days 22-24 the unhatched chicks were sacrificed and their serum samples were collected and examined for presence of gsAg and anti-gsAg antibodies. Embryonic fibroblast tissues were also collected from each sacrificed un-hatched chick, and examined by PCR and RT-PCR assays using genomic DNA and tissue RNA, respectively. The results indicated that of 16 exogenous ALV A positive hens, only 12 were ALV transmitters. Shedding of gsAg was found variable among 21- days embryos. The results suggested that combination of molecular and serological assays were useful to identify the exogenous ALV transmitters.

ISVIB 2013/
TAII/15

Detection of Avian Metapneumovirus from Chikens with swollen head syndrome by reverse transcriptase-polymerase chain reaction (Rt-Pcr)

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Avian metapneumovirus (aMPV) causes upper respiratory tract infections in chickens and turkeys. Although the swollen head syndrome (SHS) associated with aMPV in chickens has been reported in many parts of the world, this is the first study investigated the prevalence of avian metapneumovirus (aMPV) from chickens in India. We examined 17 oropharyngeal swab or nasal turbinate samples collected from chicken flocks showing respiratory illness, swollen head syndrome and drop in egg production to investigate the presence of aMPV. Three samples were tested positive for aMPV by Reverse – Transcriptase PCR (RT-PCR) which detects the sequence of a 115 bp fragment of nucleoprotein (N) gene. Molecular subtyping of aMPV based on the G glycoprotein gene by multiplex reverse transcription polymerase chain reaction confirmed that the detected aMPVs belonged to subtypes B. This is the first report of avian metapneumovirus from chickens in India.

Key Words: Avian metapneumovirus, nucleoprotein gene, Genotype B, G protein.

ISVIB 2013/
TAII/16

Serological Detection of Avian Metapneumovirus Infection in commercial broiler Chicken Farms in Palladam, Tamil Nadu

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Avian metapneumovirus (aMPV), the primary causative agent of severe rhinotracheitis in turkeys, is associated with the swollen head syndrome in chickens and it is a source of significant economic losses during commercial chicken production. This study was conducted to detect the presence of aMPV antibodies in commercial broiler chicken farms using Enzyme-linked immunosorbent assay (IDEXX APV, Liebefeld-Bern, Switzerland). Four hundred and sixty five chicken serum samples were collected from several commercial broiler farms in Palladam, Tamil Nadu during 2011-2013. The serum samples were collected from birds of various ages (from 2 to 5 weeks of age). All the flocks had not been vaccinated against avian metapneumovirus. Antibodies to APV were detected in 60% (9 out of 15) of the farms by Idexx ELISA. The total positive samples were 126/465 (27 %) of all examined samples. In conclusion, this study indicated the presence of antibodies to APV among 2-5 weeks old commercial broiler chicken at farms in Palladam, Tamil Nadu.

Keywords: avian metapneumovirus; seroprevalence; ELISA; swollen head syndrome; broilers.

ISVIB 2013/
TAII/17

Serological survey on avian metapneumovirus infection in broiler breeder chicken farms in Tamil Nadu

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Avian metapneumovirus causes an acute highly contagious upper respiratory tract infection pri-marily of turkeys and chickens. The disease can cause significant economic losses in turkey and chicken flocks, particularly when exacerbated by secondary pathogens. The purpose of

this study was to determine the prevalence of avian metapneumovirus antibodies in broiler breeder flocks in Tamil Nadu, India. All the flocks had not been vaccinated against avian metapneumovirus. The blood samples were taken randomly regardless of the presence of any signs of respiratory or any other clinical disease in the flocks. A total of 485 blood samples were collected from 20 broiler breeder chicken flocks (aged between 18 and 72 weeks). The presence of antibodies against avian metapneumovirus in each serum sample was tested by enzyme-linked immunosorbent assay using a commercial kit (IDEXX APV, Liebefeld-Bern, Switzerland) which was able to determine antibodies against A, B and C subtypes of avian metapneumovirus. Out of 485 serum samples obtained from broiler breeder chickens, 165 (34%) were positive to avian metapneumovirus antibodies, which represented 14 of 20 (70 %) examined broiler breeder flocks. The results of this study may indicate the possible involvement of avian metapneumovirus in the respiratory disease in India. Its prevalence has to be investigated in other parts of India. Future work may and should include the use of molecular methods and isolation of the virus. Isolation of avian metapneumovirus will allow the possibility of making autogenous vaccines.

Keywords: avian metapneumovirus; seroprevalence; ELISA; broiler breeder; chicken

ISVIB 2013/ TAII/18

Isolation and Characterization of *Mannheimia haemolytica* from Japanese Quail

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Mannheimia haemolytica is the causative agent of several economically significant veterinary diseases occurring in ruminants and, much more rarely, in other animal species. A Japanese quail flock was suffering with 30% mortality and drop in egg production. On post mortem examination the liver showed small necrotic foci. The liver impression smear revealed bipolar organisms on Leishmans staining. Bacteriological examination of heart blood and liver specimens yielded pure colonies. The organism are β-hemolytic, Gram-negative coccobacilli, oxidase-positive, catalase-positive, nitrate-positive, indole-negative and MacConkey-positive. They are arabinose- negative and trehalose-negative. Based on these findings the isolated organisms are identified as

Mannheimia haemolytica. An autogenous formalin inactivated vaccine prepared from the isolated *M. haemoltyica* protected the birds from mortality and drop in egg production. *M. haemolytica* isolate showed susceptibility to enrofloxacin, gentamicin,amikacin,ceftiofur, trimethoprim and ampicillin.

Keywords: *Mannheimia haemolytica*; pathogenicity; Japanese quail;

ISVIB 2013/ TAII/19

Bacteriological status of milk and milk products procured from various outlets at Palampur, Himachal Pradesh.

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Milk and milk products are ideally suited for growth of pathogenic as well as and spoilage microorganisms particularly bacteria, many of them have public health significance. The present investigation was conducted in Palam Valley, Himachal Pradesh, with the objective of assessing the bacteriological quality & safety of milk and milk products. A total of 28 samples comprising of raw milk from indigenous pahari cows (04) & cross bred cows (08), organic milk (01), pasteurized milk of different brands (03) and ready to eat milk products (12) were collected, randomly. The bacteriological quality of these samples was evaluated based on Total Psychrotropic Count (TPC), Total Mesophillic Count (TMC), Total Thermophillic Count (TTC) and Total Coliform Count (TCC). Out of 28 samples, 16 (57.14 %) samples comprising of 04 raw, 03 pasteurized & 09 ready to eat milk products were found to be unsatisfactory for human consumption as per Bureau of Indian Standards. However, all the samples of raw milk from indigenous pahari cows & cows reared under organic environment were found to be satisfactory. The mean mesophillic counts in poor quality raw, pasteurized & ready to eat milk products were 6.20 $\log_{10}$ cfu/ml, 5.49 $\log_{10}$ cfu/ml and 6.02 $\log_{10}$ cfu/gm, respectively, while coliform count in the respective samples were 2.60 $\log_{10}$ cfu/ml, 2.39 $\log_{10}$ cfu/ml and 2.96 $\log_{10}$ cfu/gm, respectively. The results of present study revealed that majority of milk products and pasteurized milk exceeds the permissible limits of different microbial count so consciousness and care need to be adopted to ensure contamination free milk for the good health of all consumers. Therefore, further detailed study along with biotechnological tools is required to determine the entry of contaminants during various stages of milk handling to avoid public health hazard.

ISVIB 2013/
TAII/20

Infectious lameness among migratory sheep & goats with particular focus on anaerobes

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Livestock sector in India contributes about 27% of the GDP from agriculture making it an important component of the economy. Sheep and goat rearing although is a profitable activity amongst the farmers, but these animals remain highly vulnerable to foot infections due to migratory husbandry practices. Lameness as a squeal to affection of digits and caused by microbes is a great concern for poor welfare in small ruminants. Contagious digital dermatitis, foot abscess, scald, virulent footrot, shelly hoof and toe granuloma are some important conditions that may lead to lameness. Various bacterial species particularly *Dichelobacter nodosus*, *Fusobacterium necrophorum*, *Treuperella pyogenes* (Previously *Arcanobacterium pyogenes*) and *Treponema* spp. have been implicated in some of these conditions. The current study establishes the causes of infectious lameness in north-western Himalayan region particularly Himachal Pradesh (H.P.) where no such study has been conducted in the past. Amongst, a total surveyed population of 27,586 comprising of 15,006 sheep and 12,580 goats at risk, 216 samples were collected from affected sheep and goats suffering from foot lesions. A total of 6.48 % (14/216) samples were positive for *D. nodosus*, 20.83% (45/216) for *F. necrophorum* and 20.37% (44/216) *T. pyogenes*. In three instances, all the three bacteria mentioned were found to be present in conjunction. Most of the positive samples arose from bordering areas of H.P. adjoining to Jammu and Kashmir where the association of these bacteria had been shown in the past and where footrot is endemic. The confirmation of footrot in the H.P and the fact that *F. necrophorum* and *T. pyogenes* were detected from cases of foot/hoof infection in high numbers confirms an important role for these organisms in inducing morbidity in migratory sheep and goats. The present investigation also confirms the detection of *D. nodosus* from cases of emerging footrot for the first time from H.P. and hints towards potential of *D. nodosus* establishing a niche in the region threatening infections of endemic nature. The study also concludes that there is a need for formulation of a combined vaccine against these organisms which would prove to be beneficial for the development of economic stature of people.

ISVIB 2013/ TAII/21

Combinatorial polymerase chain reaction for detection of *Brucella abortus, B. melitensis* and *B. suis*

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Combinatorial polymerase chain reaction (PCR) procedure to identify three major species of the genus *Brucella* (*B. abortus, B. melitensis, B. suis*) was standardised. Four pairs of primers targeting the genes encoding a cell surface protein (*bcsp*31) and outer membrane proteins (*omp2b*, *omp2a*, and *omp 31*) were used. PCR using these primers gave rise to specific patterns of amplification for each *Brucella* species examined in this study. *B. abortus* could be identified when fragments of *bcsp31* and *omp2b/2a* were amplified by *B. abortus* specific primers(224bp & 186bp). *B. melitensis* could be identified by the amplification of fragments of *BCSP31*, *omp2b/2a* and *omp31* using pair of primers B4/B5, JPF/JPR-ab and *omp31* (224bp, 249bp & 186bp). *B. suis* could be identified by amplification of *BCSP31*, *omp2b/2a* and *omp31* genes using all pair of primers B4/B5, JPF/JPR-ab, JPF/JPR-ca and *omp31* (224bp, 249bp, 186bp & 187bp).

ISVIB 2013/ TAII/22

Outbreak of goat pox in an organized goat farm in polur, Tiruvannamalai district of Tamil Nadu

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Goat pox is an economically important viral disease of goats caused by Goatpox virus belonging to the genus Capripox, family Poxviridae. The goat pox outbreak occurred during July 2013 in an organized goat farm in Polur, Tiruvannamalai district which had flock strength of 300 goats of different breeds. Initially the symptom observed was similar to an allergic reaction. Scab formation was noticed 20days after the onset

of cutaneous eruptions. Only Sirohi and Tellicherry breeds of goats were affected and mortality was observed in Tellicherry breed alone. Scab specimens (n=2) were collected for viral detection and histopathological examination. The DNA extracted from the scab specimens were amplified by p32 attachment gene primer and yielded 192 bp product. RFLP analysis of field samples and sheep pox control with EcoRI enzyme yielded 192 bp product for the field scab specimens (n=2) and two bands with a size of 129 bp and 63 bp for sheep pox control. Histopathological examination of scab specimens revealed extensive vacuolar degeneration and eosinophilic intracytoplasmic inclusions in the epidermal cells. This study highlights the outbreak of a different form of goat pox disease in an organized goat farm in Polur, Tiruvannamalai district of Tamil Nadu and susceptibility of Tellicherry breed of goats to goatpox virus than other breeds. To counter protect the outbreak vaccination is warranted.

ISVIB 2013/ TAII/23

Incidence and management of parvoviral enteritis in dogs in Palam Valley of Himachal Pradesh

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A total of 65 dogs of different breeds, aged between 1-8 months presented in the Department of Veterinary Medicine, Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Palampur (H.P) with the history of anorexia, anaemia and haemorrhagic enteritis/ gastroenteritis during the year 2012-13 were screened for parvoviral infection using ScanVet parvovirus antigen detection kits. 32 dogs were found to be positive with these kits. Faecal swabs of all these positive dogs were also sent to Department of Veterinary Microbiology which confirmed infection in 30 dogs by PCR indicating incidence as 46.15%. The maximum incidence was found during the months of March – August and in dogs aged between 2.5 – 5 months. 2 dogs which were found to be weakly positive with these kits were found to be negative by PCR. All these dogs were positive for antigenic variant CPV 2b. Treatment was done with broad spectrum antibiotics, fluid therapy, oral rehydration and multivitamins which resulted in recovery of 28 out of 30 dogs.

ISVIB 2013/
TAII/24

Preparation of different antigens of *Pasteurella multocida*

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Pasteurella multocida is a causative agent of a number of economically important diseases in livestock. The major diseases of economic significance include haemorrhagic septicaemia in cattle and buffalo; enzootic pneumonia in cattle, sheep, goat; atrophic rhinitis in pigs; fowl cholera in poultry and snuffles in rabbits. Due to the fatal nature of the disease, there is urgent need for rapid diagnosis so that appropriate therapeutic and preventive measures could be undertaken. A study was designed to extract different antigens of *P. multocida* type A and B which included whole bacterium, capsular antigen, Outer membrane proteins (OMPs). Bacteria were grown on brain heart infusion broth and capsular antigen was separated by fractional precipitation with addition of polar organic solvents which yielded high molecular weight capsular polysaccharide. OMPs were extracted by ultracentrifugation of the supernatant obtained by addition of HEPES Buffer containing Sodium Lauryl Sarcosinate detergent and the detergent insoluble OMP enriched fractions were obtained. Whole cell antigen was obtained by centrifugation of sonicated *P. multocida* suspended in HEPES buffer. The Total protein concentration of antigens was estimated and found to be 0.5mg/ml and 0.7mg/ml of OMP type A & OMP type B respectively, while whole cell protein concentration was 2.7mg/ml & 7.9mg/ml of type A & type B. OMPs & whole cell lysate extracted were subjected to discontinuous SDS-PAGE. Seven polypeptides of MW ranging from 15-90 kDa from OMPs preparation & fourteen polypeptides of MW ranging from 21-97kDa from whole cell lysate were visualised.

ISVIB 2013/
TAII/25

Genetic Analysis of Enteric Viruses and Development of Innovative Diagnostic Approaches

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Viral gastroenteritis is one of the utmost common diseases distressing young animals globally. Gastrointestinal infections leading to diarrhea in animals and humans are of great concern globally due to devastating economic losses. The diarrheal diseases accounts for more than half (52%) of the neonates mortality in animals (National Animal Health Monitoring System; NAHMS). Since, the first connotation of rotaviruses as the cause of gastroenteritis, information on other viral causes linked with diarrhoeal diseases in animals increased gradually, including astroviruses, caliciviruses, coronaviruses and picobirnaviruses. We are working as a collaborating research team specifically concerned with the timely and accurate detection of enteric diseases of animals that are viral in origin. Veterinarians are bound to encounter enteric infections in their practice and this problem is unavoidable, because of numerous viruses' involvement and associated factors. We have developed diagnostic methods that a veterinarian can use in the diagnostic laboratory to solve the diagnostic puzzle.

ISVIB 2013/
TAII/26

An outbreak of sheep pox in an organised farm of Tamilnadu

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India has vast resource of sheep which play a vital role in improving the socio-economic conditions of small and marginal farmers and landless laborers in rural areas. The factor which hampers the growth of goat industry is infectious diseases. Among the infectious diseases Sheep pox poses main threat to the small ruminant industry. Sheep pox is an acute highly contagious viral disease of sheep. An outbreak of sheep pox was

noticed in Kilakaraisal breed of sheep in an organized government farm of Tirunelveli district. Total population in the farm was 180 sheep. The mortality rate observed was 4.17% and morbidity was 80% in sheep. Both the sexes of sheep between 6 months to 2 years old were affected. The clinical symptoms observed were cough, sneezing, bilateral mucopurulent nasal discharge with high body temperature, hair loss, generalized raised skin nodules on body and anorexia. Nasal swabs, conjunctival swabs and skin nodule biopsy were collected from live animals and samples of skin, heart, spleen, mesenteric lymph node and lung were collected from dead animals in 10% formalin. DNA extracted from the scab specimens (n=5) were amplified using Capri pox specific p32 gene primers and yielded 192 bp product. In order to differentiate between sheep pox and goat pox PCR – RFLP was carried out. Digestion of PCR product with EcoRI enzyme yielded two fragments with a size of 123bp and 63bp which confirmed the presence of sheep pox virus. Histopathological examination of tissues collected from sheep revealed extensive hydropic degeneration of proliferating epidermal cells with presence of large number of intracytoplasmic eosinophilic inclusion bodies in the keratinocytes of epidermal cells in skin. Lung revealed large infiltration of lymphocyte and macrophages with presence of intracytoplasmic eosinophilic inclusions in the macrophages, proliferation of type II pneumocytes were prominent along with bronchiolia hyperplasia.The outbreak was controlled by effective preventive measures like ring vaccination of susceptible animals, affected animals were treated with a course of antibiotic for 7 days, contaminated areas were disinfectant with virucidal solution and restricted the movement of animals from unaffected areas to infected areas.

ISVIB 2013/ TAII/27

Molecular Detection and Characterization of Mycoplasmas of Avian Origin

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Avian mycoplasmosis is one of the major respiratory problems in poultry and is creating havoc in the poultry industry. A total of 73 lungs tissue samples from birds suspected to be died of avian mycoplasmosis infection were collected from 20 different outbreaks. Out of total samples, 71 samples (97.26%) were detected PCR-positive through a broad range genus specific PCR using GPO3 and MGSO primers. There were forty two (42) numbers of samples collected from 15 different outbreaks detected positive through MG-PCR (59.15%) for CRD caused by *Mycoplasma gallisepticum.* A 16S rRNA gene targeting PCR using MG13F and MG14R

primers was used for identification of *M. gallisepticum* infection. Similarly, a total of twenty four (24) cases collected from eight (8) different outbreaks were detected positive through MS-PCR (33.8%) for *Mycoplasma synoviae* infection using MS-1/MS-F and MS-2/MS-R primers targeting 16S rRNA gene. Multiple sequence alignment of partial sequence data obtained from the *M. gallisepticum* and/or *M. synoviae* positive samples revealed that all the 16S rRNA partial gene sequences of this study were about 99% - 100% homologous. On phylogenetic analysis all of the *M. gallisepticum* 16S rRNA partial sequences showed similar source of origin to that of the *M. gallisepticum* and similarly, in case of *M. synoviae*. The sequences of the study were submitted to NCBI gene bank database. There was also occurrence of various *Mycoplasma* spp. other than *M. gallisepticum* and *M. synoviae* as a rare cause of avian mycoplasmosis found in this study. A proper panel of rapid diagnostic procedure is to be developed for detection of various other *Mycoplasma* pathogens associated with respiratory tract infection in poultry and the phylogeny is to be investigated properly.

Keywords: *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, avian mycoplasmosis, PCR.

ISVIB 2013/ TAII/28

Epidemiology of Recent Outbreaks of Infectious Laryngotracheitis Virus in Namakkal

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Infectious laryngotracheitis (ILT) is an important respiratory disease of chickens and annually causes significant economic losses in the poultry industry worldwide. Over the past 3 years (2010-2013), numerous outbreaks of infectious laryngotracheitis (ILT) have occurred in poultry in Namakkal region of Tamilnadu. A study was conducted to identify the viral strains involved in the recent outbreaks and to determine possible epidemiological links between these outbreaks. A combination of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses of several genes (ICP4 and Tk)of the ILT virus was used to identify genetic differences in field/vaccine ILT virus

isolates. Analysis of 34 field ILT viruses demonstrated four new classes: I,II, III and IV. Class I was responsible for four outbreaks in Western region of Namakkal and demonstrated to be distinct from other Namakkal strains of ILT. Class II was the Merial ILT vaccine (USA). Class III was responsible for the majority of the outbreaks in Northern regions of Namakkal and was phylogenetically close to class II. On one occasion, classes I and II were identified in an outbreak on a Thiruchengode region farm that had used the Merial ILT vaccine. Class IV, also phylogenetically close to classes I and II, was found only in Sendamangalam region of Namakkal. The previously identified class II was also found to be responsible for a large number of outbreaks, mainly in Western region of Namakkal.The results demonstrate that, epidemiologically, most outbreaks of ILT in Namakkal are related to Merial ILT vaccine.(Class II).

ISVIB 2013/
TAII/29

Concurrent outbreak of infectious bursal disease (IBD) and aflatoxicosis in a commercial integrated broiler farm

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A deadly outbreak of infectious bursal disease (IBD) along with aflatoxicosis in two adjoining organized poultry farms of farm size of 40,000 nos each of Madurai District, further complicated by secondary microbial invasion, was investigated. Morbidity and mortality was 80-90% among broiler chicks in the 4 to 6 week age group. Samples of vital organs comprising liver, spleen, kidney, bursa of Fabricius, lungs, heart blood, bile and intestines were processed microbiologically. Morbid materials yielded *Escherichia coli,Streptococcus faecalis and Proteus spp*. Aspergillus flavus was isolated from feed samples collected randomly from feed stocks. Representative specimens of liver, spleen, bursa and kidney collected at 10% formal saline were subjected to histopathological investigations. Agar gel precipitation test conducted on the bursal tissue homogenates of 22 birds discerned strong precipitation lines with known positive IBD hyperimmune serum. The aflatoxin level in feed offered to the affected birds varied from 100 ppb to 500 ppb. Conspicuous histomorphological alterations corresponding to aflatoxicosis, infectious bursal disease, as well as other secondary bacterial infections, were evident in these visceral organs

ISVIB 2013/
TAII/30

Quantitative analysis of biofilm formed by *Salmonella* Enteritidis by using different media and substrates

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In this study, *Salmonella* Enteritidis biofilm was formed successfully in vitro by using five different media and substrate combination. The total biomass of the biofilm formed using different media and substrate varied considerably. The best biofilm was observed when incubated using TSB in combination with 1% Chitin (4.824±0.288), while the least biofilm was formed in LB in combination with 1% Glass wool (1.539±0.02). The average of all the media irrespective of substrates revealed that in RPV maximum biofilm (4.068±0.144) was formed whereas, the least was formed in LB.

Key words: *S.* Enteritidis, Biofilm, LB, TSB

ISVIB 2013/
TAII/31

Development and evaluation of recombinant protein based indirect ELISA for Classical Swine Fever Virus antibody assessment

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Classical Swine Fever (CSF) is a contagious viral disease of pigs and the laboratory diagnosis includes the detection of live virus, viral antigen/ viral nucleic acid or detection of specific antibodies. The envelope E2 glycoprotein is responsible for eliciting neutralizing antibodies which gives protection to animals and hence become target for the CSFV antibody assay. In this study, CSFV E2 recombinant protein was produced by cloning of CSFV E2 gene (1171 bp) into vector – pTriEx 1.1 Neo and expressing in prokaryotic host cell – *E. coli* BL21 (DE3) pLysE cells. The cloning was confirmed by restriction enzyme digestion to check the insert release and further the expression of the CSFV E2 gene was confirmed by SDS-PAGE and western blotting method using CSFV positive serum. Bulk

production of the recombinant CSFV E2 envelope protein (~52 kDa) was done with LB broth having Neomycin and Chloramphenicol. The recombinant protein was purified using His tag fusion protein in Ni-NTA agarose affinity column and the protein concentration was found using Bradford method as 6 mg/ml. For optimizing indirect ELISA, the coating antigen was found to be 700 ng per well using checker board titration. The optimized test was compared with a commercial competitive ELISA (Prionics) only with 40 vaccine trial serum samples and the statistical analysis showed a specificity of 85.7%, sensitivity of 80% and accuracy of 83.3%.

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TAII/32

Detection of Brucellosis in humans by serological tests

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Brucellosis, an important but neglected zoonotic disease in India, is present in all livestock. Human brucellosis is a severely debilitating disease, often leaves permanent and disabling sequelae, and results in considerable medical expenses in addition to loss of income due to loss of working hours. An increased demand for dairy products and intensified farming practices led to the increased spread of this infection to the humans. The disease causes more than 5,00,000 infections per year, globally and still, the true incidence of human brucellosis is unknown for India and it may be 25 times higher than the reported incidence due to misdiagnosis and underreporting. A total of 750 serum samples from patients manifesting pyrexia of unknown origin (PUO), headache and myalgia were received from private hospitals in Namakkal. About 0.66 per cent cases were positive by Rose Bengal Plate Test (RBPT) and 0.4 per cent by Standard Tube Agglutination test (STAT) to *Brucella abortus* with a highest positive titre of 1:320. Females (1.7%) were found to be at risk than males. Hence, routine serological surveillance in human should be practiced as a part of laboratory testing to understand the zoonotic potential.

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TAII/33

Antibiotic sensitivity in milk samples of household cows and buffaloes

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Milk is a complete and nutritious food for animals and human beings, however, considered as a good medium for many microbial agents. Raw milk is still used by a large number of farm workers and general population and hence, utilization of both raw milk and its products has frequently been associated with foodborne illness due to many zoonotic agents viz., *Campylobacter jejuni*, *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Yersinia enterocolitica*. A total of 84 milk samples were collected randomly from cows and buffaloes during a rural survey at Namakkal and screened for their sensitivity to the antibiotics namely, gentamicin, cephotaxime, cefixime, ampicillin, amoxicillin and enrofloxacin in Muller-Hinton agar. Highest sensitivity in milk samples was observed to gentamicin (78.5%) followed by enrofloxacin (73.8%) and cephataxime (69.0%), whereas, the highest resistance was observed to ampicillin (88.1%) and amoxicillin (76.2%). Nineteen per cent of the collected milk samples were from cows showing clinical mastitis and 81.0% from cows which were apparently healthy.

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TAII/34

Isolation of E. coli from diarrhoeic calves of cattle and buffaloes

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Colibacillosis is one of the serious diseases in newborn calves causing neonatal mortality due to diarrhea and septicaemia and is caused by pathogenic serotypes of *E. coli* in the first week of life. About 54 faecal samples were collected in sterile vials from the calves of buffaloes at different age groups showing diarrhea in local villages at Namakkal. Samples were cultured in eosin methylene blue agar (EMB) agar and isolates of *E. coli* were identified as bluish green colonies with metallic sheen in seventy four per cent of the diarrhoiec calves. The faecal samples

were also examined for the parasitic ova by centrifugal sedimentation technique and revealed no significant helminthic infection as they had a history of either periodical or occasionally deworming. Strict hygiene in the calf barns and surroundings therefore be enforced for prevention of this agent.

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Comparative efficacy of iELISA as a diagnostic tool for Brucellosis

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As both RBPT and iELISA are the prescribed tests for international trade in livestock. STAT shows higher false positive non-specific reactions, as a consequence the use of STAT has been discouraged by OIE in international trade. RBPT is an oversensitive test for diagnosis in individual animal especially those vaccinated. Further, in field conditions the sensitivity is known to vary from antigen to antigen preparation. So comparative serological study was conducted in and around Jammu region to determine the sensitivity and specificity of iELISA over other classical serological tests *i.e.* RBPT and STAT. Cut-off values of iELISA were determined in percent positivity (PP), serum samples having PP value more than 65.5 were taken as positive. Out of 40 bovine serum samples 10 samples were exclusively positive in iELISA while negative in RBPT and STAT; simultaneously no samples were found positive in all the three tests. Sensitivity and specificity of 98% and 100% was observed by iELISA against classical serology tests. Sensitivity to RBPT and STAT was 98% and 86% respectively and a specificity of 88% in RBPT and 100% in STAT. The sensitivity and specificity of iELISA showed a statistically significant superiority in comparison with classical serological tests. The high seropositivity exclusively to iELISA is due to its nature being a primary binding assay which can detect 1/100th of the antibodies. So, iELISA was observed to be more sensitive test over RBPT and STAT and should be applied on large scale to evaluate it for screening purpose for diagnosis of brucellosis in the country.

ISVIB 2013/
TAII/36

Detection of canine parvovirus from diarrheic dogs in Himachal Pradesh using serological and molecular methods

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Canine parvovirus (CPV) is a highly contagious and fatal disease of dogs, causing acute hemorrhagic gastroenteritis and myocarditis. The aim of the study was to isolate and molecularly characterize CPV from cases of diarrhea among dogs in the state of Himachal Pradesh. A total of 102 faecal samples from clinical cases of diarrhea/ hemorrhagic gastroenteritis were collected from different parts of the state with complete history of the affected animals. HA-HI and dot ELISA were used to detect the virus from faecal samples with the help of anti CPV hyper immune serum raised in rabbits which revealed 40 per cent (41/102) and 48 per cent (49/102) positivity, respectively. CPV specific PCR targeting VP2 gene of CPV revealed presence of only CPV-2b antigenic variant to a tune of 50 per cent (52/102). Comparative evaluation of various serological tests revealed that PCR and dot ELISA were comparatively more sensitive for the detection of CPV than either HA or HI. In conclusion, canine parvo virus (antigenic variant CPV-2b) was found widespread in canine diarrhea affecting susceptible dog populations of Himachal Pradesh, while PCR was found to be most sensitive method for detection of CPV.

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TAII/37

Replacing conventional methods of bovine subclinical mastitis with biomarker assay

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Subclinical bovine mastitis is a highly prevalent disease in cows which remains undiagnosed because of the absence of marked clinical signs. In high yielders, if it is not diagnosed at an early stage, it may lead to the onset of clinical mastitis, which once sets in is not only difficult to treat but also adversely affects the patency of udder and subsequent milk yields. Therefore, the subclinical mastitis should be diagnosed and

eliminated at the earliest. The traditional methods of diagnosing subclinical mastitis are by somatic cell counting and other tests which are based on pH change and gel formation due to increased cell count. However, in various studies it has been shown that these methods are conflicting and do not correlate fully with each other and thus liable to misdiagnose subclinical mastitis. These methods need to be replaced by quick and reliable molecular methods of diagnosis. In this study, it was found that that expression of an acute phase protein in milk co-occurred with the presence of subclinical mastitis. The presence of subclinical mastitis was ascertained firstly with the use of combination of conventional methods such as somatic cell counting, bromo thymol blue Test (BTB), California mastitis test (CMT) and presence of the pathogenic bacteria. Somatic cell counting was taken as the gold standard for subclinical mastitis. It was found that CMT and BTB tests as well as presence of pathogenic bacteria were not sufficient to access subclinical mastitis. However, presence of serum amyloid A (SAA) protein correlated with an increase in somatic cell count and onset of subclinical mastitis. The detection of SAA transcript was also done by RT PCR. The presence of SAA in milk was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) two dimensional PAGE, enzyme linked immmunosorbant assay (ELISA) and matrix-assisted laser desorption/ionization time of flight (MALDITOF). Thus detection of SAA protein can form the basis of detecting subclinical mastitis. The SAA was cloned in expression vector for the production of recombinant protein for developing lateral flow assay.

ISVIB 2013/
TAII/38

Prevalence of group A bovine rotavirus in neonatal calves in Punjab

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Group A rotaviruses play an important role in causing gastroenteritis and mortality in neonatal calves. To investigate the epidemical characteristics and prevalence of bovine rotavirus (Group A rotavirus) in Punjab, 120 fecal samples were collected from buffalo calves (n=93) and cow calves (n=27) up to 3 months age group calves exhibiting diarrhea during the period from July 2012 to March 2013 and screened for the presence of rotavirus using RNA-PAGE. Twenty-three samples (19.16%) were positive by RNA-PAGE having characteristic electrophoretic pattern that corresponded to mammalian group A rotaviruses. Male diarrheic calves (22.2%) were found to be more susceptible to rotavirus infection than female diarrheic calves (12.8%). Calves below 20 days of age and

buffalo calves were found to be more susceptible to rotavirus infection. High incidence was seen in winter months at low relative humidity.

ISVIB 2013/ TAII/39

Concurrent occurrence of cutaneous and diptheretic pox in a commercial layer chicken

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Avian pox is an infectious and contagious viral disease of numerous domestic and wild birds, which occurs particularly in tropical and subtropical countries in spite of the regular vaccination. The present study reports concurrent occurrence of cutaneous and diptheretic pox in a vaccinated commercial layer flock. Four (two alive and two dead) chicken of 13 wk from a flock of 4500 commercial white leghorn laying hens were submitted for diagnosis to the Poultry disease diagnosis and surveillance laboratory, Veterinary College and Research Institute Campus, Namakkal, Tamil Nadu with the history of chronic (cumulative) mortality (1.6 per cent) over a period of seven days. On external examination firm nodules of 0.5 to 1 cm in diameter were noticed on the comb, eyelids and legs. The lesions mostly appeared in the form of single occasionally as multiple nodular growths. Larynx and the upper trachea revealed yellowish diptheretic membrane which firmly adhered to the underlying mucosa and on removal bleeding was observed. Histopathologic examination of lesions in all necropsied chickens revealed similar pathology, mainly characterized by the presence of epidermal hypertrophy and hyperplasia with ballooning degeneration of stratified squamous epithelium. Epithelial cells were commonly swollen, rounded, and separated from each other in the stratum spinosum layer. Cytoplasm of the hyperplastic epithelial cells contained characteristic large eosinophilic inclusions identified as Bollinger bodies with vacuoles. The clusters of epithelial cells found in some areas resembled a papilloma, probably formed by basal cell proliferation in the epidermis. In some chickens, moderate heterophilic infiltrations in the dermis could also be observed. In addition to skin lesions, severe epithelial hyperplasia was found in the mucosa of the trachea and bronchi, causing partial obstruction of the airways, and numerous intracytoplasmic pale-eosinophilic inclusion bodies were noticed within the hyperplastic cells. Underlying lamina propria and muscularis showed severe accumulation of mononuclear cells, composed mainly of macrophages, lymphocytes, plasma cells, and a few heterophils. Cutaneous and tracheals samples inoculated onto the chorioallontoic membrane of 10 days old embryonated

eggs revealed proliferative pock like lesions on the chorioallontoic membrane on 5 day after inoculation. On the basis of clinical findings, histopathological leasions and laboratory tests the cause for the present mortality was diagnosed as avian pox.

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TAII/40

Occurrence of fowl cholera in a commercial layer flock

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Fowl cholera is an acute septicaemic disease of poultry caused by Gram negative *Pasturella multocida* and is characterized by high mortality and loss to the poultry industry. Six dead birds of 16 week of age from a commercial layer farm with the flock capacity of 20,000 birds were submitted for postmortem examination with a history of one per cent mortality within a period of 5 days. The birds were treated with Enrofloxacin @ 10mg per kg body weight, even than the mortality was continued. The birds died without premonitory signs. On postmortem examination, liver and spleen showed mottling and congestion, splenomegaly and occasional necrotic foci on the liver surface. Pericardium contained serous fluid and the epicardium showed petechial and ecchymotic haemorrhages. Lungs and trachea were congested. Leishman staining of liver impression smear revealed bipolar organisms. Histopathological examination revealed generalized vasculitis, hyperemia, fibrinous throbmi and presence of bacterial colonies in the liver and spleen. In the heart myocardial degeneration, necrosis and leukocytic infiltration were noticed. Heart blood swab and liver samples collected from the dead birds showed growth in brain heart infusion agar (BHI) and blood agar plates. The isolates revealed typical morphological and cultural properties i.e., the colonies were small about 1 to 2 mm in diameter, entire, convex and opaque on BHI agar suggestive of *P.multocida*. None of the strains produced hemolysis on blood agar and no growth was observed on MacConkey's agar plates. Gram's staining of the smears from culture materials revealed Gram negative coccobacilli. The isolates were non motile. Biochemical tests revealed that all strains were oxidase and catalase positive, urease negative, fermented glucose, mannitol and sucrose while no fermentation with lactose and maltose. Based on the cultural characteristics and biochemical reactions the isolates were identified as *P.multocida*. The *P.multocida* isolates were tested for its *in vitro* antimicrobial sensitivity test against 12 commonly used antimicrobial agents by disc diffusion on

Muller – Hinton agar. Accordingly the birds were treated successfully with Sulphamethaxazole and Trimethoprim at the dose rate of 25 mg/kg body weight for five days through drinking water.

ISVIB 2013/ TAII/41

Spontaneous cases of gangrenous dermatitis in commercial layer chickens

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Gangrenous dermatitis is a bacterial disease affecting poultry characterized by areas of necrosis in the skin and underlying tissues usually resulting in death. Gangrenous dermatitis principally occur in younger birds around 4 to 8 weeks of age, however in recent past its incidence was observed even up to 20 weeks in cage reared layer pullets. In the present study 10 out breaks of gangrenous dermatitis were investigated in the age group of 10 to 18 weeks of age with mortality ranging from 4.6 to 7.5 per cent from January 2012 to December 2012. Dead birds from the above said outbreaks were subjected to detailed postmortem examination and materials collected from subcutaneous tissue, muscle and liver aseptically were subjected to aerobic and anaerobic cultural examination. Giemasa and gram staining of impression smear from subcutaneous tissues, cut thigh muscle and liver surfaces showed heavy populations of large gram positive rods and gram negative cocobaccilli. Oval subterminal spores were observed in the baciili on grams staining. On cultural examinations, among the ten outbreaks six revealed mixed infection of *Clostridium sp*, *Satphylococcus sp*., and *E,coli* and the remaining four revealed concurrent infection of *Clostridium sp*, and *Satphylococcus sp*. The isolates were subjected to antibiotic sensitivity test by Kirby Bauer Disc Diffusion method. Based on the antimicrobial sensitivity test results the flocks were successfully treated with Amoxycillin at the dose rate of 30 mg/kg wt as pulse dose through drinking water for five days.

ISVIB 2013/
TAII/42

Epidemiological and pathological studies of infectious bursal disease in vaccinated layer chicken

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A ten year retrospective study (January 2000 to December 2009) of the prevalence of infectious bursal disease (IBD) and its concurrent occurrence with other diseases in Namakkal poultry belt of Tamil Nadu was conducted. During the study period 300 layer chicken flocks revealed IBD, among them IBD alone (230) and its concurrence with Newcastle disease (22), hepatitis (9), malnutrition (9), gout and nephritis (11), wing rot (3), GIT affections (4), tracheitis (3), chronic respiratory disease (6) and colisepticaemia (3) were noticed. The cumulative morbidity and mortality observed in flocks vaccinated both with live and killed IBD vaccines simultaneously were comparatively less (28 and 15.72 per cent) against flocks vaccinated with live vaccines alone (80 and 39.10 per cent). The disease occurred throughout the year but it was more common during the rainy season (65 per cent). The concurrent affections in number of flocks in rainy, winter and summer seasons were 37 (53 %), 15 (21 %) and 18 (26 %) respectively. The clinical disease mostly affected immature chickens of 4 to 6 wks age (154 flocks), but those up to 24 wks were also affected. The concurrent affection in different age groups of chickens viz., 0 – 3, 4– 6, 7 – 9, 10 – 12, 13 – 15, 16 – 18 and 19 – 24 wks were 3 (4 %), 28 (40 %), 14 (20 %), 7 (10 %), 8 (12 %), 5 (7 %) and 5 (7 %) respectively. In almost all the affected flocks, the birds showed clinical signs of dullness, depression, anorexia, ruffled feathers, diarrohea and inability to move. At necropsy, the gross lesions were observed mainly in bursa of Fabricius followed by changes in the thigh and pectoral muscles and kidneys. Bursal homogenates from affected birds produced clear precipitation lines in AGPT.

ISVIB 2013/
TAII/43

Concurrent Occurrence of newcastle disease, Infectious Bursal disease and caecal coccidiosis in desi birds

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Rural poultry production constitutes important component of agricultural economy in India. Small poultry holders are practically capable of contributing more significantly to alleviate malnutrition, poverty and unemployment. Village chickens are generally considered resistant to many of the diseases commonly noticed in commercial poultry flocks. The present report describes the rare occurrence of viral and coccidial infections in a desi bird flock. Two dead birds of thirty days old from a flock size of 200 birds were submitted for the postmortem examination with the history of sudden increase in mortality for the past two days and cumulative mortality of 12 per cent. The birds revealed the clinical signs of ruffled feathers, depression and whitish watery diarrhea. On postmortem examination, the carcasses were dehydrated. Liver showed paleness. Gizzard proventriculus junction revealed ecchymotic haemorrhages and proventricular papillae showed petechial haemorrhages on its tip. Caecal tonsil showed haemorrhages. Caeca was distended with haemorrhagic exudate. Kidneys were pale in colour and the ureter was distended with urates. Bursa of fabricious was moderately enlarged and the mucosal folds were congested. Lungs and trachea were congested. Microscopic examination of caecal contents revealed the presence of Eimeria oocyts. Bursal homogenates revealed positive reaction for IBDV in agar gel precipitation test with specific antiserum. Spleen, proventriculus, caecal tonsil and lungs revealed haemagglutination titer of 64 to 128 and it was netralised by the specific antiserum against Newcastle disease virus. Based on the gross lesions and laboratory test results cause for the mortality in the desi bird flock was diagnosed as combined infection of Infectious bursal disease virus, Newcastle disease virus and Eimeria oocyst infection.

ISVIB 2013/ TAII/44

Serological survey on the prevalence of chicken anaemia virus infection in Namakkal, Tamil Nadu layer flocks

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Chicken anemia virus (CAV) is an important pathogen of poultry and has been found in broilers, breeders and specific-pathogen-free flocks. Virus isolation is difficult and time-consuming. Screening for the presence of antibody will indicate exposure to the virus. The enzyme-linked immunosorbent assay (ELISA) has been used to detect antibody against CAV. This method is quite useful in large-scale testing of flocks for exposure to CAV. A total of 420 serum samples were collected from 14 commercial layer flocks (30 samples/ flock) in Namakkal were tested for the presence of chicken infectious anemia virus (CIAV) antibodies using a commercial ELISA kit (IDEXX). The results showed that all the 420 samples (100%) were positive for CIAV. All the flocks had not been vaccinated against CIAV. It indicated that the seroprevalence of CIAV in the commercial layer flocks of the Namakkal of Tamil Nadu is very high. This high rate of prevalence might be due to vertical and naturally occurring horizontal CAV infection in commercial layers.

ISVIB 2013/ TAII/45

Seroprevalence of Reovirus in commercial layers

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Avian reoviruses (REO) are ubiquitous among poultry populations and have been reported to be responsible for viral arthritis (tenosynovitis), runting/stunting, malabsorbtion syndrome and feed passage in birds 4–16 weeks of age. The incidence of reovirus infection in older birds is high, but clinical symptoms are not seen in most birds. An assessment of immune status, as well as serologic identification of avian reovirus, requires

a measurement of antibody to reovirus in serum. Enzyme-linked immunosorbent assays (ELISAs) have proven efficacious in the quantification of antibody levels to other diseases, and facilitate the monitoring of immune status in large flocks. This survey was carried out to determine the seroprevalence of REO infections in commercial layer farms in Namakkal of Tamil Nadu. A total of 552 serum samples were collected from commercial layer flocks. Sera tested by ELISA method using commercial ELISA Kit supplied by IDEXX. The results showed that 538 of 552 (97.46%) flocks were REO positive. All the flocks had not been vaccinated against REO. It seems that the seroprevalence of REO in the commercial layer flocks of the Namakkal of Tamil Nadu is high.

ISVIB 2013/
TAII/46

Seroprevalence of avian encephalomyelitis virus in commercial layers

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Avian encephalomyelitis (AE) is a viral infection primarily affecting young birds. The disease is characterized by a variety of neurological signs, including incoordination, ataxia and tremors of the head and neck. An assessment of immune status, as well as serologic identification of AE, requires a measurement of antibody to AE in serum. Enzyme-linked immunosorbent assays (ELISAs) have proven efficacious in the quantification of antibody levels to AE, and facilitate the monitoring of immune status in large flocks. This survey was carried out to determine the seroprevalence of AE in commercial layer farms in Namakkal of Tamil Nadu. A total of 552 serum samples were collected from commercial layer flocks. Sera tested by ELISA method using commercial ELISA Kit supplied by IDEXX. The results showed that 467 of 552 (84.6%) flocks were AE positive. All the flocks had not been vaccinated against AE. It seems that the seroprevalence of AE in the commercial layer flocks of the Namakkal of Tamil Nadu is high.

ISVIB 2013/ TAII/47

Seroprevalence of inclusion body hepatitis in commercial layers

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Adenoviruses are widespread throughout all avian species. Studies have demonstrated the presence of antibodies in healthy poultry, and viruses have been isolated from normal birds. Despite their widespread distribution, the majority of adenoviruses cause no or only mild disease; however, some are associated with specific clinical conditions. Inclusion body hepatitis (IBH) is an acute infectious disease of young chickens of 2 to 7 weeks of age caused by several serotypes of group 1 avian adenovirus. This survey was carried out to determine the seroprevalence of IBH infections in commercial layer farms in Namakkal of Tamil Nadu. A total of 626 serum samples were collected from commercial layer flocks. Sera tested by ELISA method using commercial ELISA Kit supplied by TropBio. The results showed that 505 of 626 (92.86%) flocks were IBH positive. All the flocks had not been vaccinated against IBH. It seems that the seroprevalence of IBH in the commercial layer flocks of the Namakkal of Tamil Nadu is quite high.

ISVIB 2013/ TAII/48

Seroprevalence of ALC J virus in commercial layers

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Lymphoid leukosis, the most common manifestation of the avian leukosis/sarcoma group of viruses, produces a variety of neoplastic diseases, including erythroblastosis, myelocytomatosis, myeloblastosis and others. Not all infected birds will develop tumors. Infection can occur horizontally from bird to bird by direct or indirect contact, or vertically from an infected hen to her eggs as virus is shed into the albumin of the egg. In addition, vertical transmission may occur from virus incorporated in the DNA of a germ cell. Viremia in the hen is strongly associated with

the transmission of virus congenitally. Enzyme immunoassays have proven efficacious in the detection of both leukosis antibody and antigen. This survey was carried out to determine the seroprevalence of ALC J viral infections in commercial layer farms in Namakkal of Tamil Nadu. A total of 200 serum samples were collected from commercial layer flocks. Sera tested by ELISA method using commercial ELISA Kit supplied by IDEXX. The results showed that none of the samples were positive for ALC J. It denoted that the seroprevalence of ALC J virus in the commercial layer flocks of the Namakkal of Tamil Nadu is nil.

ISVIB 2013/
TAII/49

Seroprevalence of *mycoplasma synoviae* in commercial layers

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Infection with *Mycoplasma synoviae* (MS) may be seen in chickens and turkeys in association with synovitis and/or airsacculitis. It occurs in most poultry-producing countries, especially in commercial layer flocks. Infection rates may be very high. Spread is generally rapid within and between houses on a farm, whilst illness is variable and mortality less than 10%. The study was carried out to determine the seroprevalence of MS infections in commercial layer farms in Namakkal of Tamil Nadu. A total of 420 sera samples were collected from 14 commercial layer flocks (30 samples/flock). Sera tested by enzyme-linked immunosorbent assay (ELISA) method using commercial Kit (IDEXX). The results showed that 390 of 420 (92.86%) flocks were MS positive. All the flocks had not been vaccinated against MS. It indicated that the seroprevalence of MS in the commercial layer flocks of the Namakkal of Tamil Nadu is very high.

ISVIB 2013/
TAII/50

Development of a multiplex PCR for detection of mareks disease virus, egg drop syndrome virus, chicken infectious anemia virus, and avian mycoplasmas

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Mareks disease virus (MDV), egg drop syndrome virus (EDSV), chicken anemia virus (CAV), and avian mycoplasmas (AM) have been recognized as the most important pathogens of poultry and may also be present as adventitious agents in the live poultry vaccines. The testing of these adventitious agents in veterinary vaccines is prerequisite as per the Indian Pharmacopoeia. In order to screen these agents, a multiplex polymerase chain reaction (mPCR) was developed and optimized in the present study. Four sets of specific primers for MDV, EDSV, CAV and AM were used in this test. The mPCR DNA products were visualized by gel electrophoresis and consisted of fragments of 680bp, 230bp, 419bp and 163bp for MDV, EDSV, CAV and AM respectively. The detection limit (DL) of this assay was determined from the serially diluted recombinant plasmids containing gene of insert of each agent. DL was found to be 10-100 copies of each agent. The mPCR also demonstrated high sensitivity in the DNA isolated from the samples spiked with MDV, EDS, CIAV and AM. No product amplification was obtained with DNA of other agents using these primer sets. Thus, mPCR developed in the present study can be used as a rapid diagnostic method for the simultaneous detection of these agents in the clinical samples and can also be used for screening of these agents in live poultry viral vaccines as contaminants.

ISVIB 2013/
TAII/51

Development of duplex PCR for detection of Mycoplama and porcine circovirus-1 in cell lines

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Mycoplasmas are one of the most frequent contaminants in cell cultures and use of porcine trypsin also increases the possibility of porcine circovirus -1(PCV-1) contamination. Therefore, regular screening of these agents is very essential in the cell lines used for research, diagnostic and vaccine production. In this context, a duplex PCR was developed for detection of mycoplasma and PCV-1 DNA in a single PCR reaction. Two sets of specific primers, one based on 16s rRNA of mycoplasma and other on ORF-1 region of PCV-1, were used in present study. The duplex PCR products were 450bp and 349bp for mycoplasma and PCV-1 respectively. Recombinant plasmids containing gene insert of both agents were constructed and used as positive controls for sensitivity determination. The detection limit of this assay was found to be 10-100 copies for both agents. No product amplification was obtained with DNA of other agents using these primer sets. The method could be successfully employed on commonly used cell lines for production of veterinary viral vaccines. The duplex PCR standardized in the present study can be used for regular and rapid screening of these extraneous agents in cell lines and attenuated cell culture viral vaccines.

ISVIB 2013/
TAII/52

Development of SYBR Green based real-time RT-PCR for detection and quantification of Bovine Viral Diarrhea virus and its comparison with conventional RT-PCR

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Bovine viral diarrhoea virus *(BVDV)* is one of the most common contaminants in fetal calf serum and cell lines used in vaccines production. The use of vaccines contaminated with BVDV can result in an outbreak of BVD in cattle and pigs. Hence, detection and qualification of BVDV RNA in the cell line and fetal calf serum is very essential before its use in vaccine production. For this, a SYBR-Green based real-time RT-PCR was developed and optimized for detection and quantification of bovine viral diarrhea virus (BVDV). Specific primers were designed based on highly conserved 5' untranslated region (5'UTR) for amplification of both BVDV-1 and BVDV-2. Initially, conventional RT-PCR was standardized to amplify 105bp PCR product from the BVDV infected cell line. The amplified product was gel purified, cloned into pTZ57R cloning vector, screened for the presence of specific gene fragment, isolated and quantified. The recombinant plasmid was 10-fold serially diluted and used as a template for both conventional and real-time PCR. Conventional PCR could detect upto 10 copies/µl while real-time PCR could detect upto 1 copy/µl. The amplification efficiency and linearity range of the real time PCR assays were demonstrated by amplifying triplicate amounts of serially diluted plasmid DNA (10^6 to 1 copies) containing the insert. Melting curve analysis was performed for each sample to verify the specificity of each product. The intra-assay and inter-assay variability were determined for each dilution as Mean ± S.D and C.V. (in %) of the Ct values. A high regression coefficient of 0.99 was observed with 98% amplification efficiency with Tm value on melting curve analysis showed 80.3±0.1°C in each sample. Thus, the optimized real-time PCR is 10 fold more sensitive than conventional PCR and can be routinely used for the detection in clinical samples or screening for the BVDV contamination in the animal derived products particularly in cell line, fetal calf serum and live attenuated cell line vaccines.

ISVIB 2013/
TAII/53

Multiplex PCR for detection and differentiation of Capripox and Orf viruses: A tool for identification of mixed/co-infections

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Multiplex polymerase chain reaction (mPCR) is a cost-effective technique to detect more than one target for each of a microbial agent in single reaction. In this study, mPCR assay in the form of gel-and real-time TaqMan probe based formats was developed and evaluated for the detection of Capripox and Orf viruses simultaneously either in single or mixed/co-infection from clinical samples of sheep and goats. Specific primers for sheeppox virus (SPPV), goat pox virus (GTPV) and orf virus (ORFV) targeting DNA binding phosphoprotein (I3L) of Capripox virus (CaPV) and DNA polymerase (E9L) of parapox virus for gel based mPCR whereas, the multiplex real time PCR targeted highly conserved DNA Pol gene for designing primers and probes specific for CaPV and ORFV for unequivocal identification of these viruses. Both the conventional and real time mPCR assays were found specific for targeted viruses and did not cross react with other related pox viruses. Also, they found highly specific in detecting one or more of the same viruses in various combinations. The gel based PCR was sensitive enough to clearly differentiate these species as SPPV, GTPV and ORFV in cell culture isolates whereas the real time format was highly sensitive to detect, quantify and differentiate them as CaPV and ORFV. A total of two hundred and thirty (n=235) clinical samples suspected of capripox and orf infections was collected from sheep and goats from different geographical regions of the country were analyzed by mPCR assays. This study revealed that gel based mPCR could simultaneously differentiate all three species whereas, the real time format could detect, differentiate and quantify the targets and both were found to identify mixed form of infections and be handful for clinical and differential diagnosis of these pox infections in sheep and goats.

ISVIB 2013/
TAII/54

Development and application of the novel loop mediated isothermal amplification (LAMP) of IS711 sequence for rapid detection of *Brucella* species

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Brucella IS711 based novel LAMP was standardised in the laboratory for rapid and specific detection of the *Brucella* species using newly designed set of primers. This assay imparted high amplification efficiency and specificity to detect the reactivity of the results. Four specific LAMP primers were designed online by targeting IS711 which is highly conserved element in the genus *Brucella*. After the amplification SYBR Green dye was added in reaction tube to analyse the results either through unaided eye under day light and UV light or by electrophoresis on 2% agarose gel. The assay could correctly amplified *Brucella abortus* S19, *B. abortus* S99, *B. abortus*, *B. melitensis* and eight clinical isolates of *B. abortus* but did not show any cross reaction with non-*Brucella* organisms. Detection limit of LAMP assay was 75 fg/µl.

Thematic Area III

Genomics/Proteomics

ISVIB 2013/ TAIII/1

Restriction site patterns of nephropathogenic infectious bronchitis virus isolates with regard to conserved regions

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In India, infectious bronchitis (IB) is one of the important poultry diseases and vaccines prepared only from Massachusetts strain 41 (M41) is applied for prevention of IB among poultry. From the year 2002 onwards, visceral gout and nephritis was observed in broiler chickens less than 2 weeks of age. Thereafter, several nephropathogenic and novel strains of IBV were identified in India. Therefore, determining the genotype or serotype of field strains is empirical for selecting an appropriate candidate to act as vaccine strain. Different serotypes or variants thought to be generated by nucleotide point mutations, insertions, deletions or recombination of S1 and other genes could be circulating among chickens in India. The present work is aimed at the restriction site mapping of nephropathogenic IBV (NIBV) isolates obtained from two different geographical locations of south India namely Ind/KA/07/1 and Ind/TN/07/2, with respect to highly conserved partial M protein and 5b glycoprotein genes to determine variations in the regions, if any based on prevalence of various restriction enzyme (RE) sensitive sites. The genes were amplified by reverse transcriptase PCR (1020 bp) and direct-sequenced. Restriction enzyme map of both Ind/KA/07/1 and Ind/TN/07/2 NIBV isolates revealed variations between two isolates and M41 strain. Similarly, the comparative RE map drawn for the isolates versus M41 indicated that the isolate Ind/TN/07/2 is more close to the commonly used vaccine strain M41 in India. In this comparative RE map, isolate Ind/KA/07/1 showed variations from M41 strain at six different sites whereas isolate Ind/TN/07/1 showed only three variations. Though single nucleotide variations either due to mutation or due to sequence error may also add or eliminate RE sites, lesser the addition of such sites the more will be the homogeneity. Presence of single nucleotide variations even in the highly conserved regions among NIBV isolates indicates that there will be continuous emergence of variant IBV.

ISVIB 2013/
TAIII/2

Analysis of VP2 gene from Canine Parvovirus denotes single nucleotide polymorphism

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Canine parvovirus (CPV) is the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, and being found world-wide and is one of the most common pathogenic viruses causing diarrhoea in dogs. The study involved detection of CPV in faecal materials obtained from dogs suspected to be suffering from parvoviral gastroenteritis by PCR for VP2 gene using H_{for} and H_{rev} primers. Only two of the amplified products (611 bp) obtained from samples namely CPV A- Nkl and CPV F- Nkl were direct-sequenced, based on the fact that these two faecal materials were obtained in two different climatic seasons. The phylogenetic analysis of the sequences of VP2 genes of the samples were analyzed with 22 other sequences which also included the CPV prototype gene sequence, using MEGA software version 5.0 and was aligned using CLUSTALW software. The sequences of VP2 genes of the two samples showed 100 % similarity between themselves and shows 96% similarity with isolate (accession number JX660690) reported from south China. The variations of nucleotides are at the positions 3617, 3756 and 4104 with sequences of isolates reported elsewhere. This finding proves that the CPV undergoes mutation often which can be attributed for the failure of vaccination. It is important that the prevalence of CPV genotype is analyzed for selecting the appropriate vaccine strain in order to provide effective prevention of parvoviral gastroenteritis in dogs.

ISVIB 2013/
TAIII/3

Rapid Identification of Bacteria Isolated from Respiratory tract infection in Bovines by MALDI-TOF MS

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Matrix-Assisted Laser Desorption Ionization–Time Of Flight Mass Spectrometry (MALDI-TOF MS) is recognized as one of the rapid method for identification of bacteria causing respiratory tract infection in major pneumonia using bacterial culture grown on media. To reduce the time required for identification of bacteria by overnight culture, the clinical samples from pneumonic cases were incubated for 6 hours using enriched serum broth culture at 38°C and identification of bacteria was done using conventional method (Identification based on biochemical characteristics) and MALDI TOF MS. In the present study 32 clinical samples were subjected for proteome analysis. Broth culture incubated for short period was centrifuged at high speed (15000xg) and pellet was collected and used for MALDI TOF MS analysis. 28 clinical bacterial isolates were identified as *Klebsiella pneumoniae*, *Pasteurella multocid*a, Streptococci sp. and *Acinetobacter*. 04 samples failed to grow within short incubation period, were subjected to 14 hours incubation revealed as Streptococci. 02 samples could not develop peaks were not identified using MALDI TOF MS within short incubation period. Result shows rapid identification of bacteria causing respiratory tract infection in major pneumonia such as *Klebsiella pneumoniae*, *Pasteurella multocid*a and *Acinetobacter* is possible in a short time, whereas identification of Streptococci requires high bacterial count for MALDI TOF analysis with high accuracy.

ISVIB 2013/ TAIII/4

Identification of exfoliative and panton-valentine like toxin in *Staphylococcal pseudintermedius* isolates

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Staphylococcus pseudintermedius, first described in 2005, is the most prevalent coagulase positive staphylococcal inhabitant of the skin and mucosa of dogs and cats and is a common opportunisitic pathogen causing skin and soft tissue infections of these animals. Exfoliative toxins are serine proteases which act as a molecular scissors and hydrolyses desmoglein 1 (Dsg1) – a desmosomal cell-cell addesion molecule resulted in separation of skin layer. Panton-Valentine like toxin (PVL) is a bicomponent leucocidin which is cytotoxin to neutrophils, monocytes and macrophages. PVL toxin of *S.pseudintermedius* is known as LukI which is encoded as a *LukI* operon with co-transcribed genes, *LukS* and *LukF* encoding LukS and LukF. In this study, we have attempted to identify the

ISVIB 2013/
TAIII/7

Sequence characterization of major facilitative glucose transporter gene in Sahiwal cows

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During milk production, glucose is the most important nutrient and its availability to mammary gland directly impacts the rate of milk lactose, protein and fat synthesis. The glucose uptake in mammary gland is mediated by a special class of facilitative transporters, known as glucose (GLUT) transporters. The facilitative diffusion of glucose across plasma membrane is mediated by a family of glucose transporters. A number of glucose transporter genes (*GLUT1*, *GLUT3*, *GLUT4*, *GLUT5*, *GLUT8*, and *GLUT12*) responsible for basal glucose uptake were found to be expressed in the lactating mammary gland at different levels. However, no systematic study has been conducted to characterize these transporters in Indian cattle. In the present study, an attempt was made to sequence characterize and identify SNPs/variations across promoter, coding (CDS) and untranslated regions (UTRs) in one of the major glucose transporter gene (*GLUT8*) in Indian cattle. For identification of SNPs, random animals representing five diverse cattle breeds *viz*., Amritmahal, Gir, Hariana, Rathi and Red Sindhi were selected. Region of~3.5kb was amplified and sequenced to cover 5′ regulatory region, CDS and 5′ and 3′ UTRs of *GLUT8* gene in 5 Sahiwal cows. Sequence analysis revealed the transcript length of 1995 bp long with 10 exons representing ORF of 478 amino acids, with a molecular weight of approximately 51 kDa. The 5′ and 3′ UTRs were 95 and 463 bp long, respectively. The structural organization and structural features for Sahiwal *GLUT8* was similar to *GLUT8* from other mammalian species. Similar number of exons (10) was observed across human, pig, poultry, mouse while the number of amino acids differed across the species. Cattle (*B taurus* and *B. indicus*) and pig *GLUT8* had 478 amino acids while poultry, human and mouse *GLUT8* showed 482, 477 and 477 amino acids, respectively. Search for different repeat elements indicated the absence of LINE (long interspersed nucleotide repetitive elements), SINE (short interspersed nucleotide repetitive elements) and microsatellite repeats across the Sahiwal *GLUT 8* gene. The hydropathy plot analysis by ProtScale predicted a structure comprised of 12 long hydrophobic and presumed membrane-spanning regions, consistent with the presumed tertiary structure of a facilitative transporters characterized by a larger and presumably glycosylated extracellular loop 9. Several conserved sugar transporter signatures that are characteristic

of the facilitative glucose transporter family were observed including; Sugar transport protein signature 1 located between amino acids 87 and 104 (GGwlLDrAGRKlslvlcA) in loop 2 and between 310 and 327 (AAliMDrAGRRllltlsG) in loop 8. The sugar transport protein signature 2 was found to be located between amino acids 129 and 154 (LtGLacGiaslvapvYisEiaypevR) in TM 4 and loop 4. Different analysis revealed the length of the promoter to be 851 bp. The promoter was compact with a rich array of sequence elements known to be involved in transcription regulation. Annotation of regulatory regions for search of putative transcription factors binding sites (TFBSs) revealed a total of 9 different putative TFBSs with few overlapping binding sites. No TATA box was observed in the Sahiwal *GLUT8* promoter. Homology of regulatory domains among different mammalian species revealed divergence at several TFBSs. Differences at such important regulatory domains could possibly reflect the species-wise distinctness at functional level. The deduced amino acid sequence *of* Sahiwal *GLUT8* was most identical to *B taurus* (99.4%) and most divergent with Chicken (60.1%). Comparative sequence analysis of GLUT8 across Indian 5 cattle breeds revealed 21 SNPs in approximately 3.0 Kb region comprising promoter, CDS and UTR region of GLUT8 gene. In the coding region, 9 SNPs were found of which 5 were nonsynonymous. Five of the identified SNPs were novel for Indian cattle breeds. In promoter region, 10 SNPs were obtained of which 7 were located within the TFBSs. 5′ UTR was monomorphic whereas 2 SNPs were observed in the 3′UTR, of which one was located within the TFBS. The present endeavor thus contributes to the knowledge of structural feature and existing variation in *GLUT8* gene of Sahiwal and other Indian native cattle.

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TAIII/8

Identification of potential internal control genes in milk derived mammary epithelial cells of Sahiwal cows for normalization of transcriptional data

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Gene expression analysis is becoming increasingly important in many fields of biological research. Understanding expressed genes pattern is critical to provide insights into complex regulatory networks and identification of genes relevant to new biological processes. Use of internal control genes (ICG) or housekeeping genes (HKG) that have constant

expression in response to experimental treatment or physiological state is an effective mean for normalization of expression data to account for the experimental variations. The quantitative PCR (qPCR) technique requires normalization of expression data as the technique is prone to analytical variations. To the best of our knowledge, no report is available till date on identification of suitable ICG for normalization of transcriptional data in milk derived mammary epithelial cells of Indian cattle. The objective ofthe study was to evaluate known reference genes from different functional categories that could serve as suitable appropriate ICG in milk derived MEC during different lactation stages of Sahiwal cows. A total of 52 MECs were isolated from milk of Sahiwal cows across different stages of lactation: Early (5-15 days), Peak (30-60 days), Mid (100-140 days) and Late ($\geq$ 240 days). Epithelial cells were isolated from somatic cells by using Dynabeads (Invitrogen) coated with Cytokeratin 18. The cells were trizolated and stored at -80ÚC till further processing. A total of 10 candidate reference genes *viz*., *GAPDH*, *ACTB*, *RPS15A*, *RPL4*, *RPS9*, *RPS23*, *HMBS*, *HPRT1*, *EEF1A1* and *UBI* from different functional categories were selected for the present study. Three different statistical algorithms: geNorm, Normfinder and BestKeeper were used for identification of best stable reference genes in the Sahiwal MEC samples collected during different lactation stages. In geNorm analysis, all the candidate genes exhibited expression stability (M) values below 0.5. On the basis of relative gene expression stability and stepwise exclusion of the gene with the highest M value, genes were arranged in descending order of stability: *EEF1A1=RPL4> RPS9> GAPDH> ACTB > RPS23> HPRT1> UBI> RPS15> HMBS*. Similar to geNorm, Normfinder also identified *EEF1A1* and *RPL4* as most stable and *HMBS* as least stably expressed genes. There was good agreement between geNorm and Normfinder outcome, albeit slight variation was observed in the ranking of other genes. The Bestkeeper algorithm was used to calculate gene expression variation based on Ct values. Each of the 10 candidate reference showed consistent expression levels. *EEF1A1, RPL4, GAPDH* and *ACTB* exhibited higher coefficient of correlation (r) to the bestkeeper index, lower coefficient of variance (CV) and standard deviation (SD), pointing towards their expression stability. In the present investigation, all three algorithmic methods gerNorm,Normfinder and BestKeeper have demonstrated that *RPL4*, *EEF1A1*, *GAPDH*, and *ACTB* are the most stable internal control genes and gometric means of these 4 ICG could be used for the normalization of expression data in MEC of Sahiwal cows.

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TAIII/9

Genomic changes at 3′-untranslated region (UTR) of classical swine fever virus (CSFV) genome during process of adaptation in cell culture

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Classical swine fever (CSF) is highly contagious and a lethal disease of pigs and wild boars causing severe economic losses worldwide. The causative agent, CSF virus (CSFV), belongs to the genus *Pestivirus* within the family *Flaviviridae*. CSFV genome is single positive-stranded RNA of approximately 12.3 kb containing about 11.7 kb long single open reading frame (ORF) flanked by the 5′- and 3′-untranslated regions (UTRs). The UTRs contain signals for viral replication, transcription, and translation. The 3'-UTR is usually the recognition site of the viral replicase to initiate minus-strand RNA synthesis and have possible role in viral pathogenesis. To analyze the significance of UTR at 3′-end of the viral genome, in this study, a field isolate of CSFV was propagated *in vitro* in PK-15 cell line and analyzed for changes that occurred during multiplication. The virus was isolated from the spleen collected from a clinical case of disease and propagated in PK-15 cells till 70 passages. To amplify the 3′-UTR, the total RNA was isolated from spleen tissue (passage 0) and from passage 1, 10, 20, 30, 40, 60 and 70. Approximately 240 nt at 3′-UTR of the viral genome from the each passage was amplified by PCR and cloned in pJET1.2 cloning vector. The recombinant plasmids carrying 3′-UTR from each passage were sequenced and analyzed using different bioinformatics tools for nucleotide deletions, insertions, substitutions and secondary structure formation. The sequence analysis revealed that the CSFV had undergone genomic changes during different passages in cells *in vitro*. There was nucleotide deletions observed at passage 1 compared to CSFV at passage 0 (spleen tissue). The nucleotide deletions were increased at passage 10 and become fixed in further passages till passage 70. The results from the present study indicated that there were genomic changes at 3′-UTR of CSFV genome and these changes might have role in adaptation of CSFV in cell culture or in disease pathogenesis.

ISVIB 2013/
TAIII/10

Isolation and phylogenetic analysis of an ORF virus from sheep in Makhdoom, India

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ORF (contagious ecthyma) is an exanthematic disease caused by a parapoxvirus and occurs primarily in sheep and goats with zoonotic implications. In the present investigation, an orf outbreak in the Muzzaffarnagari sheep flock at the Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh, India was investigated. Primary goat testes cell culture was used for isolation of the orf virus (ORFV) for the first time. The identity of the virus was confirmed by amplification and sequence analysis of the major envelope glycoprotein (B2L) gene and named ORFV/sheep/India/2012/CIRG. On phylogenetic analysis of B2L protein gene, it clustered with the ORFV strains from China suggesting distinct ORFV strains are circulating in India. On comparison of nucleotide and deduced amino acid sequence analysis (n=63), a unique 126S residue was observed in ORFV/sheep/India/2012/CIRG. Because only sheep flock but not any goatherd of the Institute was affected in the current outbreak, we speculated the role of the B2L protein in determining host specificity. A host specific conserved residue in B2L protein at position 249 was identified when analyzing the ORFV strains (n=49) originated from sheep (249S) and goats (249G). The findings provide evidence of genetic variation in circulating ORFV strains in India which may contribute to an improved understanding of the ORFV epidemiology to develop a proper vaccine. The findings also make a primary insight into the critical residues which may contribute in determining host specificity of the ORFV.

ISVIB 2013/
TAIII/11

Leptin Receptor (LEPR) and Luteinizing Hormone Receptor (LHR) genes in Murrah buffaloes express polymorphism without any association with post partum anestrus (PPA) condition

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The Leptin receptor (LEPR) and luteinizing hormone receptor (LHR) play a major role in reproduction through involvement in energy metabolism and adaptation. In Murrah buffaloes, to study polymorphism in LEPR and LHR and to establish any association of these genes with post partum anestrus (PPA) condition, blood samples from post partum anestrous (>120 days/ gestation, n=20)and normal cyclic (<60 days/ gestation, n=20) were taken from organized herds. LEPR (exon 20, 413bp) and LHR (exon 11, 275bp) partial fragments were amplified using bovine published primers. PCR amplicons of all samples were sequenced for both genes. Multiple sequence alignments were done to detect any sequence variation. LEPR gene showed polymorphism at A231G, C247A, G347A and LHR gene showed one nucleotide deletion polymorphism at 243 base pair (bp) in aligned sequence. Statistical analysis between the groups revealed no significant association of polymorphism with post partum anestrous condition in these animals. Hence, role of LEPR (exon 20) and LHR (exon 11) in PPA cannot be established in this study.

ISVIB 2013/
TAIII/12

Quantification of *Mx* gene expression in Equine Influenza resistant and susceptible Marwari horses

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Differences in innate immune mechanisms have been shown to be critical in host susceptibility to infection. The *Mx* protein confers resistance to *Orthomyxo* virus infection by modifying cellular functions needed along the viral replication pathway. It has been observed that variations exist in the susceptibility to Equine Influenza within the same geographical area and even in same organized farm. In the present study, the quantization of *Mx* gene expression in EI resistant and susceptible Marwari horses was performed. Blood samples were collected from Marwari horses declared positive for equine influenza and in contact animals with history of no clinical signs. To assess the expression of *Mx* gene, the blood samples, were treated with selected doses of IFN α/β. The samples induced with

2080 IU of IFN alpha exhibited marked increase in *Mx* gene expression. *Mx* gene expression from EI resistant and susceptible animals was measured by relative quantification, which compared the threshold cycle (Ct) of *Mx* gene to the Ct generated by a reference i.e. â actin gene using Taqman chemistry. The probes for *Mx* gene and housekeeping gene labeled at the 5′-end with the reporter dye 6 FAM (6-carboxyfluorescein) and at 3′-end with the quencher dye TAMRA (6-carboxytetramethyl-rhodamine) were used. The expression of Mx gene in samples from different phenotypes in response to stimulation with interferon α/β and its association with disease susceptibility will be discussed.

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TAIII/13

Sequence Analysis of Meq oncogene among Indian isolates of Marek's Disease Virus

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Marek's disease (MD), caused by Marek's disease virus (MDV), is a highly contagious neoplastic disease of chicken. Vaccination seems to be the only method to prevent occurrence of viral neoplastia. However, in recent years many cases of vaccine failure have been reported worldwide where chickens develop symptoms of MD in spite of proper vaccination. Distinct polymorphism and point mutations in *Meq* gene of MDV have been reported to be associated with virulence and oncogenicity. The present study was carried out with the objective to isolate and characterize field isolates of MDV (named LDH isolates) on the basis of *Meq* gene. Twenty five samples of suspected cases of MD were collected and processed for virus isolation in duck embryo fibroblast (DEF) primary culture. The samples were given 4 serial passages in the DEF where 28% (7 of 25) samples showed characteristic cytopathogenic effects of MDV in the form of plaques and syncytia. Additional evidence of presence of MDV in these samples was confirmed by PCR amplification of ~434 bp product. To analyze diversity in all seven isolates of MDV, a polymorphism study was carried out by cloning and sequencing of full length of *Meq* gene (~1040 bp). Sequence homology of 7 isolates with 23 reference strains (publically available from database) showed 98.10 – 99.40 % similarity in nucleotide and 95.90 – 98.50 % similarity in amino acid sequences. Among six isolates revealed 5 repeat sequences of 4 prolines (PPPP) whereas, one isolate revealed only 4 repeats. In phylogenetic analysis, these isolates formed a separate cluster showing closely related to the Chinese isolates. The study indicates a high mutation rate in field isolates of MDV that probably is causing vaccination failure.

ISVIB 2013/
TAIII/14

Comparison of DNA Extraction Protocols From Tracheal Tissues Of Infectious Laryngotracheitis Virus Affected Cases In Layers

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Four protocols were attempted for DNA extraction from tracheal tissues of Infectious laryngotracheitis (ILT) affected layers in Namakkal District. Twenty tracheal tissues homogenised and filtered were subjected to DNA extraction and quantified by Nanodrop method (Nano drop., USA) and checked for purity. The four protocols viz., Phenol-chloroform method, One Tube Tissue DNA Extraction kit (Bio-Basic Inc, Canada) ,high salt method and Medox-Easy Ultrapure Genomic Spin Minipreps kit .The maximum yield obtained was 545 ng/µl(in DNA Extraction Kit-Bio-Basic Inc,.,Canada), mean yield being 249.4± 4.2 ng/µl in all the other three methods and an acceptable purity of 1.86 was obtained using the DNA purification kit and minimum yield was obtained Medox-Easy Ultrapure Genomic Spin Minipreps kit with mean yield of 23.8± 4.2 ng/µl. The minimum purity of genomic DNA was 1.02± 0.41 obtained using Medox-Easy Ultrapure Genomic Spin Minipreps kit and in high salt method the DNA was obtained only with minimum yield of 21.03±3.3 ng/µl .Phenol chloroform method was cumbersome and the yield was very low when compared to other three methods. (144.4± 3.3 ng/µl).

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TAIII/15

Differentiation of modified-live vaccine virus and field isolates of infectious laryngotracheitis virus by restriction endonuclease analysis pattern in layers of Namakkal region

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Infectious laryngotracheitis virus (ILTV) causes mild to moderate forms of ILT in commercial layer flocks with high mortality and drop in egg production. During the year 2011, there was a severe outbreak of infectious laryngotracheitis (ILT) in an intensive production area of commercial layer farms in Namakkal, Tamilnadu. Twenty field isolates, one modified-live (ML) infectious laryngotracheitis (ILT) vaccine virus and two reference strains were compared by restriction endonuclease analysis of their DNA. Viral DNA digestion patterns were standardized for vaccine virus using restriction endonucleases viz., *Hae III, Sau96I and NciI.* Using these enzymes, vaccine virus had identical restriction endonuclease cleavage patterns. Vaccine virus had distinct patterns compared with ILT virus reference strains viz., Brazil USP-06 and USP-09. Restriction endonuclease cleavage patterns of 20 field isolates of ILT virus obtained from ILT outbreaks in Namakkal were indistinguishable from vaccine virus. These results suggest a possible role of vaccine or vaccine-like viruses in recent ILT outbreaks in Namakkal region.

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Alignment analysis of nucleotide sequences of ICP4 gene of field isolate of Infectious Laryngotracheitis virus (ILTV) in layers of Namakkal region

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Twenty field isolates isolates of ILTV of Namakkal region were identified by Polymerase chain reaction for ICP4 gene of 635 bp and were submitted to NCBI and blasted for homology analysis with that of *Gallid Herpesvirus* strain ILTV/Brazil/2003/USP-06 and USP-O9/Bastos isolate of ICP4 gene. The two reference strains ID is FJ477356.1 and FJ 477357.1. The homology analysis of ICP4 gene with Brazil strains showed that the

homology of the nucleotide sequences within the twenty isolates and vaccine strain was in the range between 96.5 - 100.0 per cent and the divergence was in the between 0.1 to 1.4. The homology analysis of Tk gene with UK strain of the nucleotide sequences within the twenty isolates was in the range between 99.2 - 100.0 per cent and the divergence was in the range between 0.2 to 0.6. Alignment analysis of the nucleotide sequences with that of vaccine strain of ILTV showed that nucleotide mutation at two different positions in ICP4 gene.

ISVIB 2013/
TAIII/17

Alignment analysis of nucleotide sequences of Tk gene of field isolate of Infectious Laryngotracheitis virus (ILTV) in layers of Namakkal region

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Twenty field isolates isolates of ILTV of Namakkal region were identified by Polymerase chain reaction for Tk gene of 649 bp were submitted to NCBI and blasted for homology analysis with that of *Gallid Herpes virus* strain ILTV/Brazil /2003/USP-41 and USP-45/Bastos isolate of Brazil and Thorne strain of United Kingdom of Tk gene. The three reference strains ID were FJ 444842.1, FJ 444844.1 and D 000565.1. The homology analysis of Tk gene with Brazil strains showed that the homology of the nucleotide sequences within the twenty isolates and vaccine strain was in the range between 96.5 - 100.0 per cent and the divergence was in the between 0.1 to 1.4. The homology analysis of Tk gene with UK strain of the nucleotide sequences within the twenty isolates was in the range between 96.1 - 100.0 per cent and the divergence was in the range between 0.2 to 1.9. Alignment analysis of the nucleotide sequences with that of vaccine strain of ILTV showed that nucleotide mutation at four different positions in Tk gene.

ISVIB 2013/
TAIII/18

Real-Time PCR Assay for studying cytokine profile (IL-6, TNF-α and IFN-γ) in an experimental mice model using brucellaphage as therapeutic agent

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Brucellosis is an economically important, contagious, zoonotic disease of animals and humans. Recently there has been a renewed interest in the applied use of bacteriophages in a diverse range of fields, including their use as anti-bacterial agents (phage therapy). In the present study, mice (n=6) were experimentally infected with a field isolate of *Brucella abortus* and were later treated with either a whole brucellaphage isolated against *B. abortus* S19 or a brucellaphage adsorbed *B. abortus* S19. As a control, 6 mice in each group were administered *Brucella abortus* only, brucellaphage only and PBS. The mice were sacrificed after 2 days of phage treatment and mRNA extracted from tissues like spleen, liver, testicles / ovaries was subjected to cDNA synthesis using available kits. IL-6, TNF-α and IFN-γ gene expression profile was studied using TaqMan gene expression chemistry. Measurement of target genes in all the samples was normalized to housekeeping gene, GAPDH. TNF-α is an important mediator of body's response to infection. It plays a central role in initial host response to infection. There was an upregulation of TNF-α in livers and testicles / ovaries of group of mice administered with phage adsorbed *B. abortus* S19. But no change in livers and downregulation in testicles / ovaries of group of mice administered with whole brucellaphage as therapy. IL-6 gene expression was downregulated in testicles/ovaries and upregulated in spleen and livers of group of mice administered with whole brucellaphage as therapy, however, upregulation of IL-6 was observed in testicles, ovaries and spleen in group of mice administered with phage adsorbed *B. abortus* S19. Down regulation of TNF-α and IL-6 as observed in testicles / ovaries of mice administered with whole brucellaphage as therapy is an indication of less inflammatory signs. IFN-γ was upregulated in liver of group of mice administered with whole brucellaphage as therapy and no change was observed in any of the organs of different groups. This study indicates that the host control of infection requires the moitoring of a set of factors which promote a complex response against *Brucella* infection and Real-Time PCR can be employed to study the cytokine profiling.

ISVIB 2013/ TAIII/19

Study of Expression patterns of Toll-like receptors (TLRs) in natural caprine brucellosis by using real time PCR

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The aim of this study was to investigate the role of Toll-like receptors (TLR 2, TLR 4 & TLR 9) during natural *Brucella melitenis* infection in goats. In present study, we took recently aborted goats (5) and normal goats (5) which were screened for *B.melitensis* and grouped into infected and control on the basis of results obtained fromserum agglutination test (SAT), isolation of organisms, Stamp's modified Ziehl-Neelsen staining and the confirmatory identification of *Brucella*by PCR using 16S rRNA and Omp 31 genes as target.The tissue samples were collected from mammary gland, supra mammary lymph nodes and uterus from both groups of goats after humane sacrifice. Total RNA was extracted from collected tissues and quantified byNanoDrop ND-1000 Spectrophotometer. From the total RNA,cDNA was synthesized and amplified by quantitative SYBR Green Real Time PCR by using specific primers. Analysis of Real Time-PCR results were done by comparative Cq method (Livak method) and GraphPad Prism V 3.02 Software. The mRNA expression pattern of TLR 2 was found3.5 fold, 2.5 fold and 2 fold higher in supramammary lymph nodes (SMLN), mammary glands and uterus respectively while the TLR 4 expression was observed 5 times higher in SMLN, 3 times in mammary gland and1.5 times in uterus of *Brucella* infected goats.The TLR 9 was observed 4 foldhigherexpressions in SMLN, 8 fold in mammary gland and 2 foldhigher in uterus of *Brucella* infected goats as compared to control goats.In this study we found that the role of TLR 4 and TLR 9 was more as compared to TLR 2 in caprine brucellosis and the Supramammary lymph node and mammary gland were found more affected tissues as compared to uterusin terms of TLRs mRNA expression patterns.

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TAIII/20

Insight into Diversity and Evolution of TLR Gene Family in Tropically Adapted Indian Native Cattle Breeds

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Toll-like receptors (TLRs) comprise a family of evolutionary conserved pattern recognition receptors playing a crucial role in the innate immune system. Attempts were made to characterize naturally occurring variation and haplotype structure in Indian native cattle breeds (*Bos indicus*) to identify novel SNPs, responsible for their unique immune adaptability. The comparative sequence analysis revealed substantial diversity in the studied TLRs and 28% of the observed SNPs were unique to Indian native cattle. Overall, observed polymorphic nucleotide sites were biallelic, unbiased and distributed across all the domains. Further, haplotype structure, haplotype sharing, median joining networks as putative representations of haplotype evolution among taurine and indicine breeds was also carried out. On comparison across different species, level of haplotype sharing was low and only 1-4 predominant haplotypes were observed for the majority of the investigated species. Using maximum likelihood approaches, positively selected codons (PSC) were found in all the TLRs studied. The number of PSC in TLR7, 8, 9 and 10 were 2, 4, 4 and 5 respectively. The diploid genotypes obtained for TLRs were resolved using the Baysian PHASE platform. Calculations were carried out over 1000 iterations, 10 thinning intervals and 1000 burn in iterations. Examination of the intragenic pattern of LD via 95% confidence intervals constructed for D', application of the four gamete rule and estimates of recombination revealed 5 haploblocks in TLR6; 4 in TLR1, TLR10; 3 in TLR7, 8 and 2 each in TLR2,3 and TLR9 with varying LD. On evaluation of putative functional effects of AA substitutions encoded by TLR SNPs using PolyPhen and SIFT, 19% (16/85) were predicted to impact protein function. Overall, the study suggests the existence of sufficient diversity within Indian cattle and divergence from the taurine counterpart. This information would be useful in genome assisted selection and develop cattle populations with increased resistance to infectious diseases.

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Alignment analysis of *Meq* gene nucleotide sequences of Serotype 1 Marek's Disease virus isolates from field outbreaks against reference strains

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Eighty six blood, 54 organ and 42 feather follicle samples were collected from 15 commercial layer farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines. Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered using Duck Embryo Fibroblast (DEF) and adapted them in Chicken Embryo Fibroblast Culture (CEF). Isolates were named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/03. Isolates were subjected to direct sequencing using the forward and reverse primers for the *Meq* gene. Alignment analysis of *Meq* complete nucleotide sequences of the three field isolates and 10 published MDVs were performed. Nucleotide mutations, insertions, and/or deletions were observed when RB1B strain was used as reference strain. The nucleotide mutation in the *Meq* gene of MDVs displayed regularity at five positions, including 251, 260, 284, 300 and 437 in the entire field MDV isolates of this study. The mutation at positions 251, 260 and 437 were unique and coincides with very virulence strains of China GX0101, GXY2 and Hungarian strain ATE. The mutation at positions 283 and 300 were unique and coincides only with very virulence strain ATE of Hungary. There is a single nucleotide mutation at position 155 (A to T), 369 (A to C), 462 (C to A) and 548 (C to T) in the isolate Ind/ TN/12/03. There is a single nucleotide mutation at position 625 (C to T) and 930 (C to A) in the isolate Ind/KA/12/02.There is a single nucleotide deletion mutation at positions 676, 728, 802 and 1043 in the isolates Ind/ TN/11/01 and Ind/KA/12/ 02. There are two nucleotide deletion mutations at positions 845 and 846, 935 and 936 in the isolate Ind/TN/12/03. There is a single nucleotide addition mutation (G) at position 873 in the isolates Ind/TN/11/01 and Ind/KA/12/02. The isolate Ind/TN/12/03 shows single nucleotide mutation at position 251(A to G), 260 (G to T), 300 (A to G), 284(G to A), 369 (A to C) and 451 (C to A) with the reference isolate TNN2 of this area.

ISVIB 2013/
TAIII/22

Alignment analysis of *pp38* gene nucleotide sequences of Serotype 1 Marek's Disease virus isolates from field outbreaks against reference strains

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Eighty six blood, 54 organ and 42 feather follicle samples were collected from 15 commercial layer farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines. Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered using Duck Embryo Fibroblast (DEF) and adapted them in Chicken Embryo Fibroblast Culture (CEF). Isolates were named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/03. Isolates were subjected to direct sequencing using the forward and reverse primers for the *pp38* gene. Alignment analysis of *pp38* complete nucleotide sequences of the three field isolates and 10 published MDVs were performed. Nucleotide mutations were observed when MS 53 strain of China was used as reference strain. The nucleotide mutation in the *pp38* gene of MDVs displayed regularity at two positions, including 477 and 640 in the entire field MDV isolates of this study. The mutation at position 477 was unique and coincides with very virulence strains of China and USA including XJ 03, TQ20 and Md5. At position 96 all the isolates are matching with US strains and local isolates of this area reported by earlier workers and defers from MS 53 (G to C). There is a single nucleotide mutation at position 172 (G to C) in the isolate Ind/KA/12/02. There is a single nucleotide mutation at position 548 (G to A) in the isolate Ind/TN/11/01

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TAIII/23

Alignment analysis of *vIL8* gene nucleotide sequences of Serotype 1 Marek's Disease virus isolates from field outbreaks against reference strains

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Eighty six blood, 54 organ and 42 feather follicle samples were collected from 15 commercial layer farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines. Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered using Duck Embryo Fibroblast (DEF) and adapted them in Chicken Embryo Fibroblast Culture (CEF). Isolates were named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/03. Isolates were subjected to direct sequencing using the forward and reverse primers for the *vIL8* gene. Alignment analysis of *vIL8* complete nucleotide sequences of the three field isolates and six published MDVs were performed. Nucleotide mutations were observed when RB1B strain was used as reference strain. The nucleotide mutation in the *vIL8* gene of MDVs displayed regularity at three positions, including 112 (T to C), 329 (T to C) and 368 (A to G) occurred in entire field MDV isolates of this study. At position 265 the very virulent plus strains including 584A, 648 A and very virulent strain Md5 possess a mutation G to C which is not present in isolates of this study as well as in RB1B. This is the first sequencing data for vIL8 gene of serotype 1 Marek's Disease Virus in India.

ISVIB 2013/
TAIII/24

Phylogenetic analysis of *Meq* gene nucleotide sequences of Serotype 1 Marek's Disease virus isolates from field outbreaks

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Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered from samples collected from 15 commercial layer and breeder farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines and named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/03. Oncogene *Meq* was amplified from each one of the CEF adapted isolates by PCR. Amplified product size was 1081 bp and subjected to direct sequencing using the forward and reverse primers. Phylogenetic analysis on the *Meq* sequence of three isolates and other 10 reference strains showed that the analyzed 13 MDVs could be separated two groups (cluster 1 and cluster 2). Phylogenetic tree, based on the *Meq* nucleotide sequences, revealed that the field MDVs of this study formed an independent cluster (Cluster 1), while virulent MDVs from USA and China formed another cluster (Cluster 2). Even though the Chinese strains are closely similar to isolates of this study, they are placed as a branch of the cluster comprising the very virulent strains of USA (Cluster 2). The isolate Ind/KA/12/02 also branches from the cluster of USA which also includes one very virulent isolate of this area reported earlier workers. In addition, cluster 1 comprises the field isolates of this study also includes very virulent Hungary strain ATE. These results implied that field MDVs from of this study might have evolved independently but have more similarities with very virulent strain ATE of Hungary.

ISVIB 2013/
TAIII/25

Phylogenetic analysis of pp38 gene nucleotide sequences of Serotype 1 Marek's Disease virus isolates from field outbreaks

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[4]The Registrar, TANUVAS, Chennai.
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Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered from samples collected from 15 commercial layer and breeder farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines and named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/03.Oncogene *pp38* was amplified from each one of the CEF adapted isolates by PCR. Amplified product size was 1006 bp and subjected to direct sequencing using the forward and reverse primers. Phylogenetic analysis on the *pp38* sequence of three isolates and other 10 reference strains showed that the analyzed 13 MDVs could be separated three groups (cluster 1, cluster 2 and cluster 3). In the cluster 1 the entire local isolates of this study together with very virulent strains from USA including Md5 and RB1B are present along with other isolates of this area reported by earlier workers. Cluster 2 contains very virulent isolates of China including XJ03, MS 53 and TQ20. Cluster 2 also contains virulent strain JM along with other isolates of this area reported by earlier workers.

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TAIII/26

Phylogenetic analysis of *vIL8* gene nucleotide sequences of Serotype 1 Marek's Disease virus isolates from field outbreaks

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Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered from samples collected from 15 commercial layer and breeder farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines and named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/ 03. Oncogene *vIL8* was amplified from each one of the CEF adapted isolates by PCR. Amplified product size was 887 bp and subjected to direct sequencing using the forward and reverse primers. Phylogenetic analysis on the *vIL8* sequence of three isolates and other six reference strains showed that the analyzed nine MDVs could be separated two groups (cluster 1 and cluster 2). Cluster 1 contains very virulent plus strain 584 A, 648A, very virulent strains Md5, RB1B and TQ2. Cluster 2 contains all the field isolates of this study. This is suggestive of independent evolution of the local isolates.

ISVIB 2013/ TAIII/27

Pathotyping of Serotype 1 Marek's Disease virus isolates from field outbreaks

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Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered from samples collected from 15 commercial layer and breeder farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines and named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/ 03. One hundred and fifty numbers of one day old unvaccinated layer chicks were randomly divided into two major groups (A&B) to assess the pathotypes of the isolates. The chicks in major group (A) were inoculated with field isolates approximately 1000 PFU/bird at one day old by intra

abdominal route to assess depression in body weight and relative bursal weight. The chicks in major group (B) were inoculated with field isolates approximately 1000 PFU/bird at six days old by intra venous route to compare the Lymphoma incidence among the isolates. Early mortality syndrome (EMS), incidence of lymphoma and overall mortality were recorded. Birds inoculated with field isolate Ind/TN/12/03 were highly depressive in body weight (75.34±3.04 g 15 dpi) and relative bursal weight (1.64±0.06 at 15 dpi) when compared to those inoculated with the other two isolates (Ind/TN/11/01 and Ind/KA/12/02) and uninoculated control (bwt 111.33±1.30 g & b/b 4.33±0.1115 dpi). Incidence of Early Mortality syndrome (53%) and Lymphoma (86%) induced by Ind/TN/12/03 was comparable with very virulent strains published elsewhere in pathotyping assay in experimental birds. Based on the above findings in pathotyping experimental trials isolate Ind/TN/12/03 was designated as very virulent MDV and other two isolates (Ind/ TN/11/01 & Ind/ KA/12/02) were considered as virulent MDVs.

ISVIB 2013/ TAIII/28

Protectotyping of Serotype 1 Marek's Disease virus isolates from field outbreaks

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[4]The Registrar, TANUVAS, Chennai.
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Three serotype 1 Marek's Disease Virus (MDV) isolates were recovered from samples collected from 15 commercial layer and breeder farms throughout Tamilnadu and parts of Karnataka in which Marek's Disease outbreak occurred even after vaccination with monovalent and bivalent vaccines and named as Ind/ TN/11/01, Ind/ KA/12/02 and Ind/ TN/12/03. To assess the protective efficacy of monovalent and bivalent vaccines against isolate Ind/TN/12/03 designated as very virulent in pathotyping assay, one hundred 1-day-old layer chickens were divided into three groups. Groups of 40 chickens were vaccinated with one dose of HVT, HVT plus SB1, respectively on day old, which were obtained from the commercial vaccine market and remaining 20 birds were maintained as unvaccinated control. Six days later, all the birds from each group, including an unvaccinated control, were challenged with approximately 1000 PFU of Ind/ TN/12/03 by intra venous route. The infection was confirmed by

PCR with DNA extracted from the five new growing feather tips for serotype 1 specific MDV at 20 and 30 days postchallenge. Chickens were observed for 16 weeks after challenge. The percentage of MD incidence induced by Ind/TN/12/03 was 57.5 and 25 per cent in monovalent vaccine and bivalent vaccine inoculated birds respectively compared to uninoculated control (100%). Based on the above findings in protectotyping experimental trials isolate Ind/TN/12/03 was designated as very virulent MDV and other two isolates (Ind/ TN/11/01 & Ind/ KA/12/02) were considered as virulent MDVs.

Thematic Area IV

Veterinary Biotechnology/ Nanobiotechnology as applicable to Animal health, reproduction and production

ISVIB/2013/ TAIV/1

Open pulled straw (OPS) vitrification and slow freezing of parthenogenetic sheep embryos using different cryoprotectants

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The aim of the present study was to evaluate the post thaw survival and hatching rates of sheep blastocysts using different cryoprotectants by slow freezing and Open pulled straw (OPS) vitrification. *In vitro* matured sheep oocytes were parthenogenetically activated and blastocysts on day six were subjected to different experiments. In Experiment 1, Day 6 sheep embryos were cryopreserved by a slow freezing protocol using 10% concentration of ethylene glycol (EG), 10% dimethyl sulphoxide (DMSO) or 5% EG and 5% DMSO mixture. Embryos were warmed and cultured for two days. Although there was no difference in re-expansion rates, hatching rates were higher in 10% EG group than 10% DMSO or mixture groups (30% vs. 18% vs. 20%, respectively). In Experiment2, embryos were cryopreserved by OPS vitrification using either 33% EG, 33% DMSO or 16.5% EG and 16.5% DMSO mixture. Re-expansion and hatching rates in mixture group were higher than those in EG group, while the outcomes from the DMSO group were the lowest (79.16% and 52.74% vs. 64.28% and 30.02% vs. 45.18% and 8.6%, respectively). In Experiment3, embryos were cryopreserved by OPS vitrification using either 40% EG, 40% DMSO or 20% EG and 20% DMSO mixture. Both re-expansion and hatching rates were the highest in the EG group, followed by the mixture group, while parameters in the DMSO group were the lowest (92.16% vs. 76.30% vs. 55.84% and 65.78% vs. 45.55% vs. 14.46%, respectively). In conclusion, OPS vitrification was found more efficient for sheep embryo cryopreservation than traditional freezing; and change of concentration and composition of cryoprotectants within the commonly accepted limits may have a significant effect on blastocyst survival and development *in vitro*.

ISVIB 2013/ TAIV/2

Antioxidant activity of *Curcuma longa* and *Berberis aristata*

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A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana proposed for their interesting antioxidant activities. Among the medicinal plants used in Ayurvedic Rasayana for their therapeutic action, two of these have been investigated in the present study. In the present investigation two medicinal plants namely *Curcuma longa* (local name- Haldi) and *Berberis aristata* (local name - Daru haldi, kashmal) were evaluated for their antioxidant activity by *in vitro* assays. Rhizomes of *Curcuma longa* were procured from Dept. of Vegetable Science and Floriculture, CSKHPKV, Palampur and stems of *Berberis arista* were procured from Distt. Mandi, H.P. and got identified at the Dept. of Biodiversity, IHBT, Palampur. The collected parts of plants were shade dried and subjected to methanolic extraction. Both extracts were dried and percent recovery was calculated. Antioxidant activity was studied by measuring Total antioxidant activity by ABTS method and DPPH free radical scavenging assay at different concentrations i.e. ranging from 62.5 µg/ml to 500 µg/ml and IC_{50} values of both the extracts were calculated. BHT and Trolox were used as standards for DPPH and ABTS assays respectively. The results revealed that the recovery of methanolic extract of *C. longa* rhizomes and *B. aristata* stems was 16.2% and 9.79% respectively. Both the extracts exhibited antioxidant action and methanolic extract of *B arista* stems was proved to have better antioxidant activity with IC_{50} value 43.05 µg/ml through DPPH free radical scavenging assay. The revealed antioxidant property of the extracts may provide potential therapeutic intervention against oxidative threats, both in healthy and diseased conditions.

ISVIB 2013/ TAIV/3

Synthetic cell penetrating peptide comprising polyarginine sequence and hydrophobic viral domain efficiently deliver nucleic acid into cells

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The delivery of nucleic acid into cells is useful not only as research tool but also as potential therapeutic agent. However, selective permeability of the cellular membrane always restricts entry of foreign molecules. Cell penetrating peptides (CPPs) constitute very promising tools for non-invasive cellular import of different exogenous biomolecules and have been successfully applied for *in vitro* and *in vivo* delivery of therapeutic molecules like plasmid DNA, siRNA, oligonucleotide, peptide-nucleic acid (PNA), peptides, proteins and liposomes. In the present study a delivery peptide was synthesized chemically using solid phase peptide synthesis methodology by incorporating a hydrophobic transmembrane domain derived from the N-terminal portion of membrane associated NS5 protein of *Dendrolimus punctatus* cytoplasmic polyhedrosis virus and a hydrophilic positively charged sequence (seven arginine residues) separated with tri glycine spacer. The in vitro cell penetrating ability of the FITC labeled peptide was assessed in MDBK cells by fluorescence microscopy and flow cytometry. The secondary structure of the delivery peptide was observed by performing CD spectroscopy in three different solvents showing predominantly α-helix and β-structures. The synthesized peptide showed strong and stable interaction with plasmid DNA as observed by EMSA/gel retardation and heparin competition assay. The peptide was also able to protect cargo DNA from nuclease as determined by nuclease protection assay. The CPP was used to deliver fluorophore labeled oligonucleotides efficiently inside MDBK cells with 85% transfection efficiency. This delivery peptide was further used for expression of plasmid construct containing GFP reporter gene which showed about 28% transfection efficiency. In all experiments, appropriate negative controls were kept using a random linear peptide. The Cytotoxicity of the delivery peptide was evaluated by MTT assay and more than 80% viability was observed indicating that this peptide based delivery vehicle can be used as an efficient delivery system for intracellular trnsfection of biomolecules.

ISVIB 2013/
TAIV/4

Immunomodulatory responses of nano-emulsified and tlr ligand adjuvanted fmdv antigen in guinea pigs

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Foot and Mouth Disease is a highly contagious, acute vesicular viral

disease of cloven hoofed animals. Vaccination is the major strategy for control and prevention of Foot and Mouth disease in the developing countries like India. Recent advances in non-inflammatory Nanoemulsion as well as TLR mediated molecular adjuvant helps to stabilize and better presentation of antigen to T lymphocytes. In an effort to induce good systemic immune response and protection, the present study was undertaken. In this approach, Inactivated whole virus (146 'S') FMDV type 'O' antigen was incorporated with *rec*HSP60 protein in Nanoemulsion, and this formulation were immunized through intra muscular route in Guinea pigs with appropriate control groups. The efficacy of vaccine formulation was assessed by evaluating the serum neutralizing antibodies, total IgG, IgG1, IgG2 for humoral immune response in Guinea pigs. The group which immunized with FMDV type 'O' antigen incorporated with recHSP60 protein in Nanoemulsion induced high SN titre (1:128) and IgG response which were comparable with SN titre and IgG response of conventional vaccine group, and also it conferred 66.66% of protection, But the groups which immunized with FMDV type 'O' antigen+Nanoemulsion or FMDV type 'O' antigen+*rec*HSP60 proteins were induced less humoral and cell mediated immune response. The IgG2 antibody responses of these groups were high when compare to IgG1 response, it indicates Th1 mediated immune response were played major role in protection against homologous virus challenge. Therefore the present study has shown that the stable, non-inflammatory Nanoemulsion could helped to stabilize and consistent release of antigen to stimulate the APCs (especially Dendritic cells), at the same time, the *rec*HSP60 protein could helped for better processing and presentation of antigen to T-lymphocytes through MHC-class-I mediated pathway.

ISVIB 2013/
TAIV/5

Myostatin gene silencing by shrna constructs and its effect on expression of myogenic genes in caprine myoblast cells

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Myostatin belongs to the transfroming growth factor (TGF-β) superfamily and is a potent negative regulator of skeletal muscle development and growth. Genetic manipulation (knockout and knockdown) has been used to increase muscle mass in mammalian species. In this study, we investigated the silencing of myostatin (MSTN) gene by shRNA constructs and studied it's effect on the expression

profile of myogenic genes. 29-mer HusH shRNA constructs were used.The shRNA cassette was cloned in the vector in between the *BamHI* and *HindIII*siteand purified plasmids were used for transformation in the competent cells. The host cells harbouring the positive clones were used for isolation of Hush plasmid DNA. The culture myoblast cells were transfected by Lipofectamine transfection reagent. Transfection of all constructs was performed in 1:3 ratio of plasmid and lipofectamine. RNA was isolated from transfected cells and a fixed RNA of 400 ng was converted into cDNA for expression profile study of MSTN and other myogenic genes by real time PCR. The samples were the amplified in real time RT-PCR and the cycle threshold (C_T) values obtained were normalized to GAPDH. It was found, the expression of MSTN gene significantly downregulated (pd"0.01) by shC and showed highest level of downregulation in shD (54.0 %) followed by shB (41.0 %), shC (39.0%) and shA (31.0%) in reference to mock control. Except decorin (DCN) all the myogenic genes taken in this experiment namely, follistatin, MyoD, myogenin and Myf5 were upregulated. But the DCN showed downregulation.

ISVIB 2013/
TAIV/6

Effect of co-transfection of anti-myostatinshRNA constructs on caprine fetal fibroblast cells.

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RNAi induction with shRNA is a powerful reverse genetic tool for suppressing the expression of gene in mammalian RNAi. The direct introduction of shRNA introduced into the plasmid targeting a particular mRNA sequence has been successfully used in gene knockdown studies. In the present study, we investigated MSTN gene knockdown by co-transfection of two 29-mer HuSH vectors in three different ratios i.e. 1:1, 1:2 and 2:1 in caprine fetal fibroblasts and also evaluated interferon responsive genes (IFN-α and OAS1), apoptotic gene (Caspase 3) and anti-apoptotic (MCL-1) to find the least interferon responsive and apoptotic ratio. We reported 75-89% silencing of myostatin mRNA by co-transfecting two shRNA constructs in transiently co-transfected fetal fibroblasts. The transfection efficiency was significantly higher ($p<0.01$) as compared to single transfection and mock control. At the same time we also demonstrated high level expression of shRNA lead to induction of 1.05- to 2.61- fold induction of OAS1 ($p<0.01$) and 0.66- to 2.13- fold induction of IFN-α ($p<0.01$). In addition we also reported due to induction of interferon response there was 0.49- to 3.21-fold induction of caspase 3

($p<0.05$) and 0.33- to 1.42- fold MCL-1 gene ($p<0.01$) as compared to single transfected cells and mock control. On the basis of efficiency of downregulatingmyostatin transcripts and least IFN response and apoptosis induction, 2:1 ratio was found to be the best ratio as it displayed 89% downregulation of myostatin and revealed an inclination of reduction in potency to induce IFN response and apoptosis at lower concentration. It was concluded that co-transfecting shRNA using two vectors is a better approach to silence myostatin gene for developing stable cell line.

ISVIB 2013/
TAIV/7

Construction and characterization of MsrA gene deletion mutant in *Salmonellatyphimurium*

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*Salmonella enterica*serovar Typhimurium (ST) is the major cause of gastroenteritis in human. ST not only survives but also replicates inside the phagocytic cells. Amino acids, methionine (Met) in particular are the primary targets of phagocyte generated oxidants. Oxidation of Met leads to the methionine sulfoxide (Met-SO) formation. Methionine sulfoxidereductaseA (MsrA) can repair protein bound Met-SO to Met thus maintain protein function. To determine the role of MsrA in ST virulence, we generated an MsrA gene deletion mutant strain. The deletion was confirmed by PCR using primers located flanking to the MsrA gene sequence. Western blot analysis using anti-MsrA antibodies confirmed the absence of MsrA protein band in the mutant strain which was present in ST wild type strain. In Luria Bertani media mutant grew comparable to wild type suggesting that MsrA gene deletion doesn't affect the growth of ST. Bacterial counts (cfu/ml) were similar in both wild type as well as in mutant strain cultures. Stability of deletion was confirmed by growing mutants for many generations or by co-culturing with ST wild type bacteria. Studies regarding attenuation and vaccine potential of mutant in mouse and poultry model are underway.

ISVIB 2013/ TAIV/8

A comparative study for development of goat parthenogenetic embryos under two culture media

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Parthenogenesis is the process by which a single oocyte can develop without the presence of the male counterpart. Artifical stimuli, like exposure to ethanol, ionophore ,ionomycin, or direct electric pulses, can elevate the cytoplasmic Ca^{2+} levels and cause mammalian oocyte activation. These activation treatments are combined with protein synthesis inhibitors, such as cycloheximide, and phosphorylation inhibitors, such as 6-dimethylaminopurine. Although these embryos cannot develop upto it's full term in mammals however, under optimum culture conditions these embryos can be used for embryonic stem cell production which can be used as a potential source for autologous cell therapy in the female and bypass the need for creating a competent embryo. The present study is an attempt to compare the developmental potency of parthenogenetic goat embryos under two different culture media. The *in vitro* matured oocytes were activated by Ca-CHX-6DMAP. The activated goat oocytes (n=512) were cultured in two media viz. 1) RVCL and 2) RVCL-Blast. In the first protocol, activated oocytes were cultured in RVCL medium till blastocyst formation. Whereas, in the second protocol activated oocytes were cultured in RVCL (Cooks Australia) for three days and further in the Blastocyst medium (Cooks, Australia) till blastocyst formation. Embryonic development in both culture medium, RVCL and RVCL- Blast media, were recorded to assess the degree of support provided by the media. The comparative developmental potential of embryos in RVCL and RVCL Blast media for 2-4 cell (81.60±0.70 vs 80.47±0.94%), 8-16 cell (70.56±0.94 vs. 70.92±0.71%), 16-32 cell (55.59±0.95 vs.63.00±0.66%) were found to be statistically non significant. However, the rate of morula (39.71±0.73 % vs.48.54±0.94%) and blastocyst (12.21±0.55 % vs. 22.26±0.73 %) embryo production was significantly higher (Pd" 0.01) in case of RVCL-Blast medium group when compared against RVCL medium alone. Our study concludes that RVCL in combination with Blastocyst medium resulted into better embryonic developmental rate in case of goat parthenogenetic embryos.

Keywords- Parthenogenetic goat embryos, RVCL-BSA-Blast, morula, blastocyst

ISVIB 2013/ TAIV/9

Expression of pluripotent marker genes Sox2 and Klf4 in putative parthenogenetic goat embryonic stem (PES) cells

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Embryonic stem (ES) cells are derived from the inner cell mass of blastocyst stage embryos and can be expanded in vitro, while retaining the pluripotency to give rise to all cell types of the embryo. These features have made ES cells a powerful tool for potential applications in regenerative medicine by targeted differentiation into needed cells and tissues. Recent success in reprogramming mouse somatic cells into pluripotent ES cell-like cells by the simple retroviral overexpression of four transcription factors Oct4, Sox2, Klf4, and c-Myc has established these genes as four key pluripotent marker genes. Keeping these facts in view, the present study was designed to find out the expression of two key pluripotent marker genes Sox2 and Klf4 in putative embryonic stem cells derived from parthenogenetic goat embryos (PES). The *in vitro* matured goat oocytes were chemically activated by using the calcium ionophore (5µM) for 5 min and 6- Dimethyl amino purine (6DMAP) for 4h. The activated oocytes were cultured in RVCL (Cooks Australia) medium for 3 d and further cultured in Blastocyst medium (Cooks, Australia) till blastocyst formation. Embryonic stem cells were cultured from the inner cell mass of blastocyst using DMEM high glucose, β-mercpatoethanol, essential and non-essential amino acids and LIF. After 15-20 d of primary culture, total RNA was isolated from the cell colonies using Trizol method, in which glycogen was added at a concentration of 20ug/ul. DNase treatment was given to total RNA to eliminate the DNA contamination. cDNA was prepared from total RNA using fermentas single strand cDNA synthesis Kits. These cDNA were used for expression study of both Sox2 and Klf4 genes using RT-PCR. Distinct bands of desired amplicon size were observed for both the genes, whereas β-actin was used as an endogenous control expressing in a usual manner in the test sample and in feeder cell line, which was taken as a negative control.

Key words- Goat, Blastocyst, Parthenogenetic embryonic stem cells (PES), Sox2, Klf4

ISVIB 2013/
TAIV/10

Isolation and identification of tannase producing *Klebsiellaoxytoca*strain SHD-1 from the rumen of migratory goat and sequencing of its tannase gene

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Tannase is regarded as one of the most versatile biocatalyst with enormous applications in food, beverages, chemical and pharmaceutical industries and analytical science. A number of microorganisms (bacteria and fungi) with ability to produce tannase have been reported from various niches such as soil, compost and gastrointestinal tract of animals. However, commercial production of tannase by traditional method is expensive and recovery is low. It is desirable to explore novel microbial ecosystems as potential sources of tannase. Present investigation was undertaken to explore rumen ecosystem of migratory goats and sheep with ability to browse on unconventional forages. Efforts were aimed to isolates tannase producing microorganisms and molecular characterization of the tannase genes therein. A total of 146 isolates were purified from the rumen of goats and sheep and were screened for their ability to degrade tannin extracted from fruits and leaves of various native forages. The elite microorganisms with higher tolerance to tannins (2.0% w/v or more) were selected for further studies. Five isolates were finally selected for their identification and molecular characterization of the tannase genes. Isolate G-25 possessing tannase genes and higher ability to tolerate tannins was studied in details and was identified as *Klebsiellaoxytoca*strain SHD-1. The tannase gene of *Klebsiellaoxytoca*SHD-1 was found to be of 621 bp. The RFLP (restriction fragment length polymorphism) analysis of the amplicon suggested that it contains restriction sequence for *Eco*RI only. Restriction digestion of the tannaseamplicon generated two fragments of 429 and 192 bp. Similar pattern was observed for all other isolates studied suggesting that no polymorphism could be detected among them.

ISVIB 2013/ TAIV/11

Effect of siRNA on cytokine gene expression in IBD virus infected cell culture

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RNA interference (RNAi) is a promising method for targeting gene expression on mRNA level.VP2 gene of Infectious Bursal Disease virus (IBDV) is an obvious target for applying the molecular techniques for detection and manipulation of the virus as VP2 gene encodes a protein involved in capsid formation, cell entry and induction of protective immune responses against the virus. In the present study, effect of siRNA on cytokine gene expression in IBD virus infected cell culture was seen. IBDV isolated from field cases was adapted on chicken embryo fibroblast cells (CEF) and inhibited by shRNA construct against VP2 gene, shRNA which was expressed from modified CMV promoter wasused to silence the gene. Silencing was carried out at gradual dosage of shRNA construct; sh3 IBD against VP2 gene. We observed down regulation of mRNA of VP2 gene by 73%, also RT PCR analysis of the gene indicated that, the gene was less expressed in sh3 IBD transfected cells as compared to positive control infected cells at dose 1 ug. The expression of IL-2, IL-4 and IL-6 genes for evaluation of cytokines response during VP2 silencing was also observed. These cytokines showed down regulation as compared to the positive control indicating that the level of viral load or virus replication was closely associated with level of cytokine expression and since silencing of the VP2 by RNAi was done, so the replication of virus was lesser as compared to positive control in CEF and thus down regulation of genes was seen, also cell viability as indicated by MTT reduction assay and NBT assay was more in test group as compared to positive control due to silencing of the VP2 by RNAi mechanism. Thus it was concluded that RNAi is an effective mechanism for modulating host immune response.

ISVIB 2013/ TAIV/12

Production of world's first cloned pashmina goat through hand made cloning technique using a continuous culture system

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Application of advanced assisted reproductive technology like Cloning through somatic cell nuclear transfer in goats has been elucidated to a limited extent. This study was aimed to produce cloned embryos of pashmina goats through economical and efficient hand-guided cloning technique. Reconstructed embryos containing nucleus from cultured donor goat fibroblast cells were cultured in vitro in four different continuous media supplemented with B-27. Significantly higher cleavage and blastocyst percentages were observed in G1-G2 and RVCL media respectively. Well in drop (WID) culture system was found to be better for in vitro culture of cloned embryos giving higher cleavage and blastocyst percentages. Embryos cultured in vitro for 7 days were laparoscopically transferred into synchronised recipient goats. Embryos transferred into 19 recipients led to the establishment of 3 pregnancies, out of which one successfully culminated into the live birth of a cloned kid. The identity of the kid to its genetic donor was established by DNA profiling using 10 microsatellite markers.

ISVIB 2013/
TAIV/13

Establishment of three-dimensional (3-D) culture of mammary epithelial cells towards developing a suitable *in vitro* mammary gland model in riverine buffaloes

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The primary mammary epithelial cells (MEC) grown on plastic surface usually losses its tissue specific function, and are low responsive to lactogenic hormones. On the other hand, the use of extracellular matrix for growing MEC in three-dimensional (3-D) condition could provide a unique opportunity to model the architecture of epithelium *in vitro*. Therefore, matrix-based three-dimensional (3-D) *in vitro* model of mammary epithelial cells that recapitulate the structural and functional context of normal tissues could provide a relevant alternative to understand the biology of buffalo mammary gland. The aim of this study was to establish and characterize buffalo mammary epithelial cells (MEC) in 3-D culture using Geltrexextracellular matrix. Mammary tissue collected from the slaughter house was processed enzymatically to obtain a heterogenous population of cells containing both epithelial and fibroblasts cells. Epithelial cells were purified by selective trypsinization and were grown in a plastic substratum in DMEM: F12 supplemented with 10 % FBS, antibiotic-Antimycotic solution (1X), 5µl/mL insulin, 50µM hydrocortisone, 1µg/ml β-estradiol, 5µg/mL holotransferrin, 1µg/mL progesterone. The purified

mammary epithelial cells generated after several passages were used to establish 3-D culture using Geltrex matrix. This is a soluble form of basement membrane purified from Engelbreth-holm-Swarm tumor that gels at 37°C forming a reconstituted basement membrane. The major components of Geltrex included laminin, collagen IV, entactin and various growth factors. MEC were characterized by immunofluorescence using cytokeratin-18 antibodies which is specific marker of this cell type. Our initial results demonstrated that MEC grown on Geltrex matrix formed polarized acinus like structure within 15 days of culturing. The MEC were organized into characteristics epithelial structures within first 5 days of culturing, indicating a more controllable growth pattern on extracellular matrix. By day 7, some acinar and tubule-like structures were generated. The size of the acinus and duct-like structures in the culture increased over time, especially during the second week. In addition, the immunoflourescence data generated using several mammary specific antibodies exhibited typical mammary epithelium differentiation. These results suggested that Geltrex or any other extra cellular matrix can be utilized for appropriate buffalo mammary epithelial cell morphogenesis and functional differentiation in culture condition. Efforts are underway to further characterize the mammary epithelial cells morphology and functioning in 3-D culture environment. In future, this type of cell culture system would prove to be a quite relevant cellular model to understand the buffalo mammary gland biology in a better way.

Thematic Area V

Veterinary Vaccines & Adjuvants

ISVIB 2013/ TAV/1

Genetic and antigenic analysis of H5N1 viruses for selection of vaccine seed strains

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Genetic and antigenic analysis of H5N1 viruses of clade 2.2 isolated in India revealed antigenic diversity that needs to be taken into account for selection of vaccine seed strains for poultry vaccine as well as pandemic vaccine for human beings. Based on the phylogenetic analysis of haemagglutinin (HA) gene of H5N1 viruses using MEGA 5.1 version of the software, 14 genetically diverse viruses were selected for antigenic characterization by cross haemagglutination inhibition (HI) analysis. Sera were raised in specific pathogen free (SPF) embryonated eggs against the genetically diverse 14 H5N1 viruses. The sera were tested in cross-HI test against the inactivated viral antigens from the 14 viruses. The mean values of three independent titrations were calculated and were used for further analysis. Using antigenic cartography software, an antigenic map was constructed based on the data of cross-HI titration of 14 sera vs. 14 viruses to visualize the relatedness among the antigens and protective coverage of the sera. Sera against five H5N1 viruses (A/crow/Assam/142119/2008, A/chicken/West Bengal/100879/2008, A/chicken/ West Bengal/155505/2009, A/chicken/West Bengal/80995/2008 and A/chicken/ West Bengal/81760/2008) exhibited maximum (100%) antigenic coverage, hence, were selected as the potential H5 vaccine strain. The present study demonstrates a stepwise methodology for logical selection of vaccine strains for H5N1 using genetic and antigenic data.

ISVIB 2013/ TAV/2

"Broad-Spectrum" Prophylactic Vaccine for Bluetongue - Possible Approaches

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Bluetongue (BT) disease caused by BT virus (BTV) is an acute hemorrhagic fever of domesticated and wild ruminants. 26 BTV serotypes have been isolated so far throughout the world with 21 circulating in India conferring no cross protective immunity. Hence, the objective of

the current study is to investigate the possibility of developing a recombinant subunit protein based vaccine for BT that is cross protective to all the known serotypes of BTV. Given that BTV protein VP2 is viral serotype determinant, that vaccination with VP2 recombinant protein induced neutralizing antibodies, and that virus circulates as 26 immunologically distinct serotypes, conserved VP2 protein domain among all the serotypes was determined by compiling and aligning complete VP2 sequences using the MUSCLE program. The amino acid sequence alignment showed that, although VP2 was very variable among serotypes, several regions were relatively more conserved between serotypes. Previous studies reported that a neutralizing epitope had a location in VP2 spanning amino acids 321–346. According to our analysis most conserved region of VP2 (cVP2) is evident from 338-383aa. Moreover, selection pressure analysis revealed that all the codons in cVP2 region were under strong negative selection (*dN/dS*=0.16) suggesting strong structural and functional constraints on cVP2 region. In addition, two MHCI, MHCII, and B cell epitopes each were predicted based on the physicochemical properties of the amino acids in cVP2 region. This suggests strong immunogenicity of cVP2 making it a suitable vaccine candidate. Taken together, cVP2's proximity to the neutralizing epitope, rather conserved nature, evolutionary pressure, and strong immunogenicity makes it a promising target for a subunit vaccine moiety. Therefore, findings from our current study can be extended into developing a "broad-spectrum" subunit vaccine for BT that can confer protection against all the available serotypes of BTV. Since all the structural proteins of BTV will not be used in such subunit vaccines, observations from current study can also be extended to develop a DIVA vaccine strategy for BT.

ISVIB 2013/
TAV/3

WSA-MP immunostimulator in the augmentation of immune response to *Brucella abortus* killed antigen in adult lactating cows

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Effective control and eradication of brucellosis is in the national agenda now to tackle this wide spread and zoonotically very important disease in India. In spite of conventional attenuated (Strain 19 & RB51) and killed (Strain 45/20) vaccines, proper immunization of adult lactating cows is lacking though they serve as the main source of spreading the pathogen. Use of killed vaccine primed with immunostimultion is expected to be the best possible option for more effective immunization of adult cattle. In this context, newer and better immunostimulators such as WSA-MP has immense untapped potential with lucrative opportunities of improvement in the immune response to poor antigens. WSA-MP is a user's friendly preparation derived from *Mycobacterium phlei*, a well known

saprophytic mycobacteria with immunostimulation activities in animals and birds against various infectious diseases and neoplacia. The present investigation was undertaken to elucidate *in-vitro* the augmented specific and non specific immunity to killed *B. abortus*antigen in WSA-MP primed adult lactating cows. WSA-MP was prepared by immunochemical fractionation and lysozyme digestion of *M. phlei.*Immunostimulation experiment was conducted in 10 cross-bred unvaccinated lactating Jersey cows of 4 to 5 years age, in two equal groups. The test group was treated with WSA-MP immunostimulator once weekly for two weeks, at dose rate of 10 mg/kg b. wt., S/C. Subsequent immunization was done in both the groups with phenol killed *B. abortus (Strain 99)* antigen (10^8 cells/ml, 5 ml S/C). The WSA-MP treated group has shown augmented antibody response (7.8 $\pm$ 1.03 Log2 titer) and cell mediated immune response (33.56 $\pm$ 3.24 % LMI) to the *B. abortus*antigen as compared to the control group with antibody response (6.6 $\pm$ 0.82 Log2 titer) and cell mediated immune response (21.95 $\pm$ 2.76 % LMI). Non specific immunity in test group with increased phagocytic index of macrophages (216.44 $\pm$ 8.21%) was observed as compared to the control group (168 $\pm$ 6.53 %). Thus, the user's friendly WSA-MP immunostimulator could successfully augment specific as well as non specific immune response to killed *B. abortus* organigmin the adult lactating cows, opening up a better strategy for more effective immunization.

ISVIB 2013/
TAV/4

Flow cytometric analysis of peripheral blood lymphocytes of calves vaccinated with HS saponified vaccine

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HaemorrhagicSepticaemia saponified vaccine (SV) against *Pasteurella multocida* infection in cattle was tested for the duration and mechanism of immunity. Immune responses were analysed by measuring the levels of peripheral blood lymphocytes expressing CD4, CD8, CD21 and WC1. Twelve jersey cross bred cow calves were immunized with a single 2 ml dose of HS saponified vaccine intramuscularly and blood was collected at one and six month post-vaccination. Fluorescein isothiocyanate labelled monoclonal antibodies CD4, CD8, WC1 and CD21, were used to examine the peripheral blood lymphocytes in whole blood of these calves by single-parameter flow cytometry. The percentage of peripheral blood lymphocytes expressing CD8 and WC1 were higher at one month (CD8 25.46% and WC1 13.38%) and six month (CD8 23.04% and WC1 18.19%) post-vaccination in calves than in control (CD8 13.82% and WC1 9.84%). The

percentage of CD4+ T cells was higher at one month (43.42%) post-vaccination compared to control (32.01%) and at six month post-vaccination (21.42%). CD21+ B cells showed a little rise only on six month post-vaccination (17.49%) compared to control (15.31%) and one month post-vaccination (11.39%). The increased percentage of monoclonal antibodies at one and six month post-vaccination compared to control calves indicated that the HS saponified vaccine was able to confer immunity for six months. The higher percentage of these monoclonal antibodies at six month compared to control further suggest to analyse these cell populations at nine and twelve month post-vaccination.

ISVIB 2013/
TAV/5

CpG-ODN-induced effect against *Trypanosoma evansi* in Peripheral Blood Mononuclear Cells (PBMCs) of Marwari horse *in vitro*

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Trypanosma evansi is the causative agent of surra, one of the most common and widespread of the trypansomal diseases. This trypanosome can infect most mammals, although horses and camels are the principal hosts and represent the most significant sources of economic loss. Trypanosomes are unusual among protozoan parasites with regard to their unique property of possessing the thick immunogenic surface coat which is known as variant surface glycoprotein (VSG). These parasites modify their VSG constantly leading to antigenic variation and thus evade the immune system of the host. Because of antigenic variation and the limited success in vector control, there is currently no effective control against trypanosomiosis. Synthetic oligodeoxynucleotides containing CpG-motifs (CpG-ODN) are capable of driving immunity toward a Th1 bias. No study till date was carried out on the use of CpG-ODN for enhancing immune response against *Trypanosoma evansi* in equines. Considering the importance of Th1 mechanisms in resistance against parasite, *in vitro* immunostimulatory effect of CpG-ODN Class-A and C in Peripheral Blood Mononuclear Cells (PBMCs) of horses was studied. Increased Th1 cytokine production (IFN-α, TNF-α, IL12) in response to both classes of CpG-ODN was observed. The significant proliferative responses in terms of stimulation indices were observed with ODNs as well as whole cell lysate antigen of *T. evansi.* The highest stimulation index was observed when

horse PBMCs were co-cultured with CpGs and whole cell lysate antigen of *T. evansi* showing synergistic effect. A number of factors produced by mononuclear cells including nitric oxide, reactive oxygen species, and tumor necrosis factor alpha, have all been shown to produce CpG-ODN-induced enhancement of host resistance to trypanosomes.

ISVIB 2013/ TAV/6

Comparison and efficacy of cell culture adapted field infectious bursal disease virus (IBDV) vaccine strain in chicken

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A local field isolate of IBDV from an outbreak with low mortality with absence of kidney damage was adapted in chicken embryo fibroblast cell culture. An experimental vaccine trial was conducted and found as effective as a commercial vaccine in protecting chickens. The vaccinated birds showed higher Geometric mean titre (GMT) of 9.8 compared to the commercial vaccine group of 8.0.The bursa body weight (B/BW) ratio of vaccinated birds were significantly different to that of control birds. The bursal scores of both the vaccinated groups were indicating that they were equally effective in protecting chicks whereas the control birds showed a bursal score of 4.

ISVIB 2013/ TAV/7

In vivo studies on the therapeutic efficacy of brucellaphage and its endolysin against *brucella abortus* infection

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Brucellosis is a zoonotic disease affecting a broad range of animals and humans. Due to the intracellular nature treatment of brucellosis with antibiotics is ineffective. The advantages of phages and endolysin therapy over antibiotics are their specificity for the pathogen without disturbing the normal flora, the low chance of bacterial resistance and their ability

to kill colonizing pathogens in various tissues, capabilities that were previously unavailable. Thus, phages and their lysins have now been successfully used in animal models as effective antibacterial agents to control pathogenic antibiotic resistant bacteria. In the present study a bacteriophage against *Brucella abortus*S 19 was isolated from the sewage samples (n=15). The phage was lytic against *Brucella abortus*S 19, *Brucellaabortus*field isolates (n=9) and *Brucellamelitensis*but was non-lytic for any of the heterologous species tested. Phage associated lysin (PAL) was extracted from the brucellaphage and this lysin was specific to *B. abortus*and non-lytic to heterologous species of bacteria tested. Therapeutic efficacy of brucellaphage, phage adsorbed *B. abortus*S19, PAL and PAL lysed *B. abortus*were tested *in vivo* using mice model. After experimentally infecting the mice with field isolate of *Brucella abortus*for five days, the therapeutic efficacy of brucellaphage, phage adsorbed *B. abortus*S19, PAL and PAL lysed *B. abortus*were tested *in vivo* in mice by subjecting the organs (liver, spleen, testicles and ovaries) to enumeration of bacterial load, histopathology and immunohistochemistry. Maximum reduction in bacterial load was observed in *Brucella* infected mice that were treated with PAL lysed *B. abortus*followed by phage adsorbed *B. abortus*. These results were positively correlated with the results of histopathology and Immunohistochemistry.

ISVIB 2013/ TAV/8

Evaluation of Immune response to vaccination against Johne's disease: In therapeutic and prophylactic models

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Mycobacterium avium subspecies *paratuberculosis* (MAP), the cause of Johne's disease (JD) is responsible for huge economic losses in domestic livestock productivity worldwide. JD, though primarily a disease of domestic ruminants but has also been associated as principal etiology of inflammatory bowel disease or Crohn's disease in human beings. More than 70% of dairy cattle herds in United States are infected with MAP, causing an annual economic loss of about $200 million to $1.5 billion to dairy industry. The annual economic losses per sheep/farmer is approx. Rs. 1840 (US$ 38.33) due to paratuberculosis in India. Though mortality rates are low but >50% animals in a herd/flock may be asymptomatic shedders, resulting in reduced productivity. Control of the MAP infection through test-cull strategy combined with hygiene management practices has not produced the expected results in the control of disease in herds/flocks world-wide. However, different countries have used vaccine to control JD in their animals. An ideal vaccine would provide prophylactic immunity

so that animals do not develop clinical disease on exposure to MAP. This helps the animals to contain the infection so that there is no spread of infection (horizontal). In chronic infections like JD, which is usually endemic in herds/flocks, the vaccines (in-activated or live) help to cure the infection (therapeutic effect). The infected animals get rid of infection and improve their health and productivity. Currently, most of the MAPvaccines use mineral oil adjuvants to evoke more active immune responses. Most of the studies used strain 316F, strain 18 and virulent field strains with oily adjuvant as killed vaccines for the control of Johne's disease (JD). 'Indigenous vaccine' developed from native 'Indian Bison Type' strain ('S5') of *Mycobacteriumavium* subspecies *paratuberculosis* (MAP) of 'goat origin' was evaluated in goat, sheep, cattle and buffaloes farm endemic for the JD. Therapeutic and prophylactic efficacy of the vaccine was evaluated on the basis of improvements in productivity (body weights, reproductive efficiency, growth rate, body weight and kidding rate), physical condition (diarrhea, weakness, body coat color and shining), clinical symptoms (shedding, mortality and morbidity), immune responses (cell-mediated and humoral) and pathological symptoms (gross and microscopic lesions, presence or absence of visceral fat). Since, the morbidity and mortality due to Johne's disease was calculated in terms of EADR (Equivalent Average Death rate). Lymphocyte transformation test (LTT) and Nitric oxide (NO) estimations showed protective cell mediated immune responses. Information on bio-load of MAP infection at animal, species, region, state and National levels is limited in India. In the absence of National estimates on prevalence and initiation of control programs, the disease has not received due attention and priority for the control. Thus, vaccination against MAP may serve as therapeutic and prophylactic model for other developing and poor countries of the world where status of MAP infection is not known and infected animals are not sacrificed for religious reasons. However, need for a DIVA test to differentiate vaccinated and infected animals is closely linked with use of vaccination in the livestock population of the country.

Miscellaneous

ISVIB 2013/
M/1

Bacterial contamination of various veterinary anaesthetic drugs

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This study was done to evaluate different anaesthetics for their microbial contamination. The study was performed in three parts. In part 1, aliquots from unopened vial/ampoules were cultured. In part 2, aliquots from opened vial/ampoules which were left after routine clinical use were cultured. In part 3, aliquots from unopened vial/ampoules were drawn into sterile syringes stored at room temperature (24-26°C) and at 37°C. Then room temperature stored samples were aerobically cultured at 72, 96 hrs and 24, 48 hrs for those stored at 37°C. Results: In Part 1 culture of unopened containers of anaesthetics were negative while in part 2 anaesthetics i.e propofol and xylazine were contaminated with bacteria. In part 3 propofol and butorphanol supported bacterial growth.Strict aseptic techniques are essential during the handling of these agents to prevent extrinsic contamination. They should be used immediately after drawing up. Microbial contamination can occur at any stage, thus attention to asepsis remains important throughout the administration period. This is clinically significant as contamination can have dangerous postoperative infectious complications.

ISVIB 2013/
M/2

Multiplex PCR Assay for Identification of beef, pork and chicken in Meat and Meat Products

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The multiplex PCR assay was developed and evaluated for simultaneous identification of beef (513 bp), pork (276 bp) and chicken (442 bp) meat processed under different processing conditions using self-designed three sets of primer pairs from mitochondrial D-loop gene. The developed multiplex PCR assay was also found to be suitable for species identification in cooked and autoclaved (121°C/15 psi/15-20 min) meat and meat products. No adverse effects of heat treatments, processing conditions and ingredients used for emulsion preparation were found on PCR assay. Even the developed PCR assay was also successfully used for

detection of beef, pork and chicken in admixed meat products processed under different processing conditions. Although weak amplification was observed with pork DNA, but amplicon was clear enough to differentiate pork in admixed meat. Finally, multiplex PCR assay was successfully used in this work for detection of meat species in admixed meat products containing varying proportion of beef, pork and chicken. The developed multiplex PCR assay was able to detect less than 1 percent of adulteration of the above species in complex heat treated meat metrics and products.

ISVIB 2013/ M/3

Biochemical characterization of efficient rhizobial isolates

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A survey was conducted in Kalpa (block-I), Nichar (block-II) and Pooh (block-III) of Kinnour district of Himachal Pradesh taking five villages from each block to find out the nodulation status of off-season pea crop. In Kalpa block (block-I), the highest average number of nodules per plant was recorded in Chitkul village (34.80), in Nichar block (block-II), the higher nodulation was (29.44) seen in Kaphanu village, and in pooh block (block-III), the highest nodulation was observed in Janghi village (45.24). The plants showing highest nodulation from each village were used for the isolation of rhizobial strains. The effectiveness of rhizobial strains isolated from the root nodules of pea were tested under pot culture conditions. From each block most efficient rhizobial strains were selected and designated as S1 (block-III), S2 (block-II) and S3 (block-I), respectively. These rhizobial isolates were biochemically characterized and found as *R. leguminosarum.* These efficient rhizobial isolates were further employed to study their effect on various growth parameters of off- season as well as on main- season pea crop. The strain S1 inoculation resulted in higher average plant height (89.13 cm), nodule number (38.40 nodules/plant), nodule dry weight (87.66 mg/plant), plant biomass (49.67 g/plant), pod number (30.86 pod number/plant) and seed yield (1400.73 kg/ha) as compared to S2 and S3 isolates in off-season pea crop. Whereas, when the main season pea crop inoculated with rhizobial strains S1, S2, and S3 of off-season pea crop, there was no significant difference in nodule number, dry weight and crop yield as compared to uninoculated control.

ISVIB 2013/
M/4

Clinical management of an outbreak of Sheep Pox in mid-hill pastures of District Kangra (H.P.)

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An outbreak of a disease affecting sheep only (with 50 reported deaths) of all age groups (kids / adults) in a mixed flock of migratory sheep and goat and clinically manifested as inappetance, anorexia for 2-3 days, rectal temperature of 105-106° F, recumbency, nasal discharge, dyspnoea and ultimately ending in death was attended in winter season of 2012 at mid hill pastures near village Daka Bandal of tehsil Kangra. Clinical and Pathological examination (post-mortem) revealed presence of pock lesions (macules, pepules, vesicles and scab formation) in areas around axilla, sternum, perianal region, oral cavity, jugular furrow and internal organs like abomasum. Round to circular whitish grey umblicated lesions were observed throughout the lung and kidney. Ulcers were present on gums, cheeks and tongue along with scab formation by the discharges at area around nostrils and eyes. Respiration rate ranged from 14-25/ minute and heart rate was between 72-100 beats/minute. The moribund sheep showed marked dysponea with pulmonary rales audible from a distance. Hanging nasal discharge was observed in 6 ailing animals (including one kid) while all ailing animals showed discharge from the eyes. The disease was later confirmed as Sheep Pox at IVRI Mukteshwar. Treatment included Inj. Ceftriaxone with Tazobactam (562.5 mg) for six days along with Inj. Meloxicam with paracetamol (Melonex-P), Inj. Avil Vet and Inj. Vetade for 5 days in standard doses and route. Oral rehydration therapy with electrolytes was also advised. Complete and strict isolation of ailing animals, segregation of in-contact animals, disinfection of pasture area, restriction of movement of animals & attendants and proper disposal of carcass by deep burial method was advised.

ISVIB 2013/
M/5

Malori (*Rumex hastatus*) Leaf Powder as an Alternative to Antibiotic Production Promoter in Laying Hens from 14 to 17 Months of Age

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Comparative egg production response by replacing deoiled rice bran with that of dried powdered Malori (*Rumax hastatus*) laves was studied in one hundred and twenty, commercial laying chicken. The treatments consisted of control (C), and malori leaves @ 3% (M_3), 4% (M_4), 5% (M_5) and 6% (M_6). Standard managemental practices were followed for rearing the chicks. A total of 120 layer chicks were divided randomly into 5 groups with 30 birds per group. The birds were offered feeds formulated with BIS specifications and were kept in Californian cages. The daily feed intake and production data was recorded from the age of 14 months to 17 months. There was a significant ($P< 0.05$) increase in percent hen-day egg production in treatment M_4, M_5 and M_6 compared to that of the control and M_3 treatments. Similarly, egg size was also significantly ($P<0.05$) higher in these treatments, whereas the feed consumed per kg egg mass production and per dozen egg production was not significantly affected upto 6% addition of malori leaves. Therefore, it was concluded that the addition of Malori leaf powder upto 6% in the diet of layer chicken improved the profits through decrease in cost of production of egg and more work is needed to be done for establishing the maximum level of inclusion of malori leaves.

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