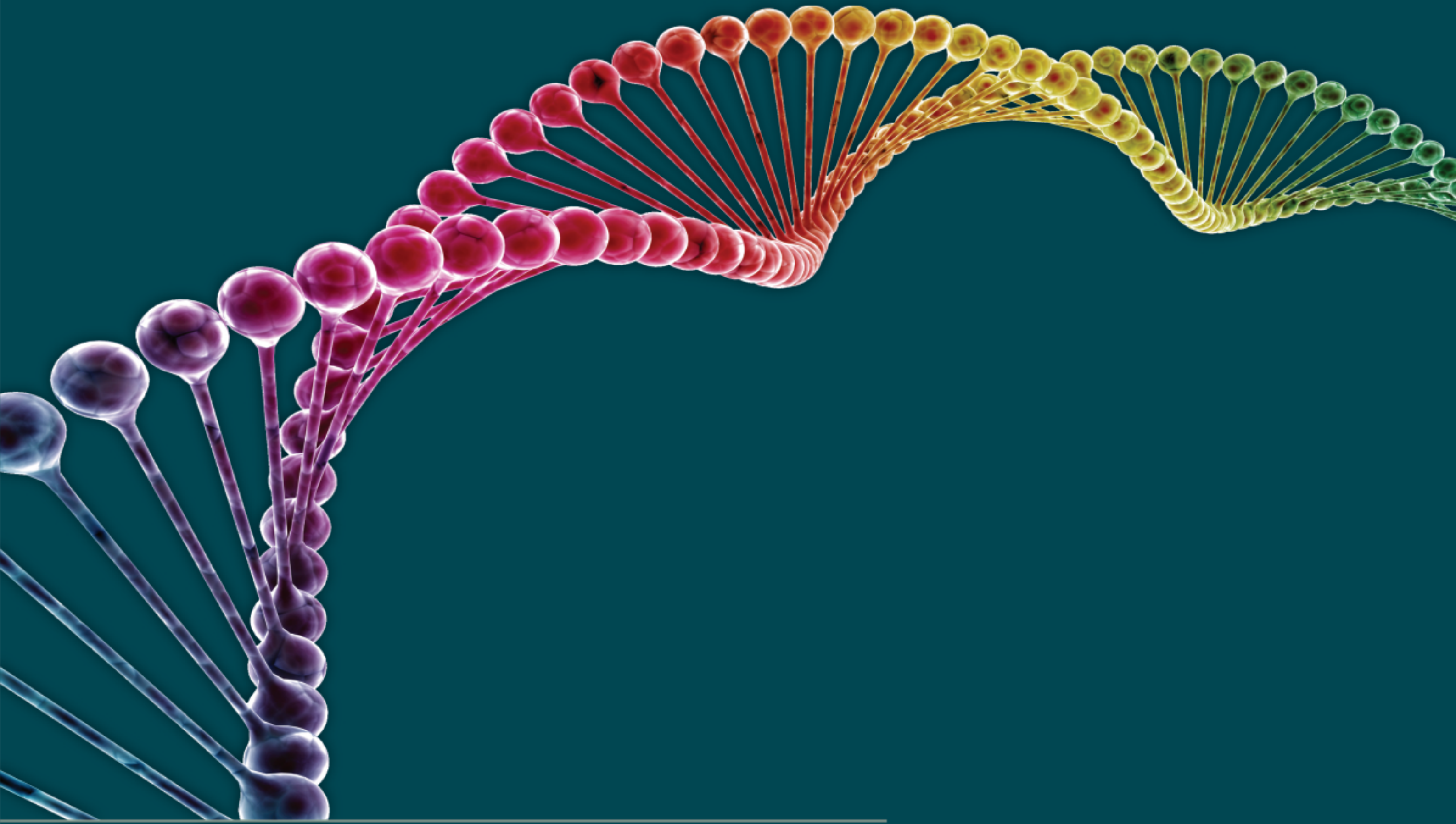
XXV ANNUAL CONVENTION OF ISVIB AND NATIONAL CONFERENCE

VIBCON-2018

on

Innovative Biotechnological Approaches for Improving Animal Health and Productivity

13th – 15th December 2018



Organized by:
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www.nrcmithun.res.in

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XXV Annual Convention of Indian Society of Veterinary Immunology & Biotechnology (ISVIB) and National Conference

VIBCON-2018

on

"Innovative Biotechnological Approaches for Improving Animal Health and Productivity"

13th-15th December 2018

Supported by

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ICAR

NABARD

DEPARTMENT OF
BIOTECHNOLOGY
Ministry of Science & Technology

Organized by

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P.B. ACHARYA
Governor of Nagaland

सत्यमेव जयते

RAJ BHAVAN
Kohima - 797001

November 19, 2018

MESSAGE

I am pleased to learn that ICAR – National Research Centre on Mithun, Medziphema is organizing the XXV Annual Convention of Indian Society of Veterinary Immunology and Biotechnology (ISVIB) & National Symposium, VIBCON-2018 in Medziphema, Dimapur on December 13-15, 2018.

Animal health is a crucial factor in ensuring food safety and an essential component of animal welfare. There is need to draw attention to the economic, social and environmental consequences of inadequate support for animal health and production.

I hope that the symposium will witness conglomeration of scientists, research scholars and students from across the nation resulting in productive deliberations and innovative biotechnological approaches for improving animal health and productivity.

I am sure the compendium containing lead papers and abstracts of scientific presentations will be a source of invaluable information and bring added insights to the veterinary sector.

I convey my best wishes for the success of the Convention.

(P.B. ACHARYA) 19/11/18

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CHIEF MINISTER
NAGALAND
KOHIMA

MESSAGE

23/11/2018

It gives me immense pleasure to learn that ICAR-National Research Centre on Mithun is organizing the XXV Annual Convention of Indian Society of Veterinary Immunology and Biotechnology (ISVIB) & National Symposium (VIBCON-2018) on 'Innovative Biotechnological Approaches for Improving Animal Health and Productivity: in Medziphema, Dimapur, Nagaland on December 13-15, 2018 and is bringing out a Souvenir cum-Compendium of VIBCON 2018.

Mithun is the State Symbol of Government of Nagaland and is also of great importance to the people of North Eastern Region which is its home terrain. It has considerable potential for meat and milk under the special climatic and topographic conditions of the region and is intricately associated with the socio-economic, religious and cultural lives of the people of the North Eastern Region.

I am happy to learn that scientists from ICAR, CSIR, IITs, Professors from Central and State Universities, Research scholars and students from all over India will be attending the Annual convention and National Symposium. I am confident that the deliberations for Improving Animal Health and Productivity related to Mithun Health and productivity will go a long way in enhancing its population in the region.

I convey my best wishes to the organizers all success for the consortium and the Silver Jubilee Celebrations.

23/11

(NEIPHIU RIO)

राधा मोहन सिंह
RADHA MOHAN SINGH

D.O. No. 2578 /AM

सत्यमेव जयते

कृषि एवं किसान कल्याण मंत्री
भारत सरकार
MINISTER OF AGRICULTURE
& FARMERS WELFARE
GOVERNMENT OF INDIA

07 DEC 2018

MESSAGE

I am very pleased to learn that ICAR-National Research Centre on Mithun, Nagaland and Indian Society of Veterinary Immunology and Biotechnology (ISVIB) are jointly organizing the Silver Jubilee Annual Convention and National conference "VIBCON 2018" on **"Innovative Biotechnological Approaches for Improving Animal Health and productivity"** from 13th to 15th December 2018 at Medziphema, Dimapur, Nagaland.

Animal husbandry sector can play a pivotal role in doubling farmers income by 2022. Development of innovative technologies and their effective applications is essential for improved and sustained production to provide livelihood, nutritional and economic security to the animal keepers.

VIBCON-2018 can be an important forum to discuss and draw a road map for addressing the required strategies including recent biotechnological approaches for improving of animal productivity in our country.

I also congratulate the ISVIB for successfully completing 25 years and I extend my best wishes for their endeavor in hosting this event in the North-East Region of the country.

I wish **Silver Jubilee ISVIB Annual Convention** & **VIBCON 2018** a grand success.

Radha Mohan Singh
(RADHA MOHAN SINGH)

Office : Room No. 120, Krishi Bhawan, New Delhi-110 001 Tel.: 23383370, 23782691 Fax : 23384129

त्रिलोचन महापात्र, पीएच.डी.
एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
सचिव एवं महानिदेशक
TRILOCHAN MOHAPATRA, Ph.D.
FNA, FNASc, FNAAS
SECRETARY & DIRECTOR GENERAL

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली 110 001
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AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
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MESSAGE

I am extremely happy to learn that ICAR-NRC on Mithun, Nagaland is organizing the 25th Annual Convention of Indian Society of Veterinary Immunology and Biotechnology (ISVIB) and National conference (**VIBCON 2018**) on **"Innovative Biotechnological Approaches for Improving Animal Health and productivity"** on 13-15th December 2018 at Medziphema, Nagaland.

Biotechnological tools are now globally recognized as a rapidly emerging and far-reaching innovations aptly described as the "Technology of Hope" for promising sectors like food, health, and environmental sustainability. Biotechnology is the third wave in biological science and represents such an interface of basic and applied sciences, where the gradual and subtle transformation of science into technology can be witnessed. The recent and continuing advances in life sciences clearly unfold a scenario energized and driven by the new tools of biotechnology.

I hope that the deliberation that will be made during the three-day event will come out with the recommendations for formulating policies and might serve as a roadmap for future research initiatives.

I congratulate the team ICAR-NRC on Mithun for taking this initiative and organising this on 30th foundation day and the ISVIB for choosing the State of Nagaland as the venue for this important seminar which will give the required impetus to the animal husbandry of the North-Eastern hilly region and I wish the VIBCON 2018 a grand sucess.

(T. MOHAPATRA)

Dated the 19th November, 2018
New Delhi

डॉ. जे. के. जेना
उप महानिदेशक (मत्स्य विज्ञान)
Dr. J. K. Jena
Deputy Director General (Fisheries Science)

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MESSAGE

It is a matter of great pleasure and satisfaction to learn that ICAR-National Research Centre on Mithun, Nagaland and Indian Society of Veterinary Immunology and Biotechnology (ISVIB) are jointly organizing the Silver Jubilee Annual Convention and National conference "VIBCON 2018" on **"Innovative Biotechnological Approaches for Improving Animal Health and productivity"** from 13th to 15th December 2018 at Medziphema, Dimapur, Nagaland.

Application of biotechnological tools to support livestock production has now become an integral part of sustainable livestock farming in multi-enterprise systems. Biotechnology is largely dictated by commercial considerations. Nevertheless, it should be used for the overall improvement of the productivity as well as to address the issues of animal health by developing cheap and dependable diagnostic kits and vaccines for the vast animal resources owned by resource-poor people. Looking into the increased need of protein in coming future, biotechnological tools like molecular markers, genome-wide selection, assisted reproductive technologies including embryo transfer and animal cloning need to put in for commercial use for the genetic improvement of livestock.

I am hopeful that these three days will be dedicated to cover a wide spectrum of themes related to innovations in the biotechnological tools through which animal health and productivity can be enhanced. Further, the conference is being held in the North-Eastern region, which is a bowl of biodiversity, the discussions held in the area of the conference will greatly benefit the participants as well may will lay the path for the future research.

I am very happy to note that ISVIB has decided to organize it's Silver Jubilee Convention in the North-East Region of the country. I wish the ICAR-NRC on Mithun as well as ISVIB a great success in conducting **Silver Jubilee ISVIB Convention** & **VIBCON 2018** a grand success.

(Dr. J. K. Jena)
Deputy Director General (AS),
Indian Council of Agricultural Research (ICAR),
Krishi Bhavan, New Delhi-110001.

DR. K. M. BUJARBARUAH,
ARS, PhD. FNAAS, Dsc(Hc)
VICE-CHANCELLOR

ASSAM AGRICULTURAL UNIVERSITY
JORHAT - 785 013
ASSAM (INDIA)

(Recipient of Sardar Patel Outstanding Institution Award)

Dr. K.M. Bujarbaruah
Vice Chancellor
Assam Agricultural University

Message

I am extremely happy to learn that ICAR-NRC (Mithun), Nagaland is the host institution this time to hold the XXV Annual Conference of the Indian Society of Veterinary Immunology and Biotechnology with an associated National Symposium (VIBCON-2018) at Medziphema Nagaland from13-15 December, 2018. I will miss my participation because of an International Seminar on the same dates in our university.

ISVIB has been spear heading research in advanced Veterinary Immunology and Biotechnology area and we have already noted some significant deliverables in terms of products and processes. As the sector is advancing almost on daily basis, the unfolded research opportunities are to be tapped for better technological assistance to the livestock health and such conferences provide a very good platform to the researchers to benefit from each other's work. I hope the conference will achieve its intended purpose and shall also open up newer collaboration particularly for biotech related research on animals like Mithuns /Yak etc.

I complement Dr. Abhijit Mitra and his team shouldering this responsibility and wish the conference all success.

(K.M. Bujarbaruah)

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"Healthy Soils for a healthy life"

CENTRAL AGRICULTURAL UNIVERSITY
LAMPHELPAT, IMPHAL-795004, MANIPUR (INDIA)

Prof. M. Premjit Singh,
Vice-Chancellor

Tel : (0385) 2415933(O)
Gram : AGRIVARSITY
Fax : 2410414

No. SVC/CAU/178/2018/
Imphal, the 27th November, 2018

MESSAGE

It gives me immense pleasure to learn that ICAR-NRC on Mithun, Nagaland. is organizing the 25th Annual Convention of Indian Society of Veterinary Immunology and Biotechnology (ISVIB) and National conference on **"Innovative Biotechnological Approaches for Improving Animal Health and productivity"** from 13th to 15th December 2018 in its Medziphema campus of Nagaland.

I hope this conference will bring together many eminent speakers covering a wide array of topics in the field of Veterinary Immunology and Biotechnology and will provide a platform for enriching academic feast during this three-day meeting. A comprehensive scientific programme has been designed covering all issues of current interest with emphasis on recent biotechnological approaches for improving livestock productivity.

I take this opportunity to congratulate the Director and staff of ICAR-NRC on Mithun for rallying together, dedicating their valuable time and energy towards making this conference both successful and memorable. I must congratulate ISVIB for completing 25 years and choosing North-East as a venue of their Annual Convention.

I invite all the eminent speakers, academicians, scholars and students across the country to participate and deliberate on the scientific agenda as well as enjoy the natural beauty of the North-East.

I wish VIBCON 2018 a grand success.

27/11/2018

(M. Premjit Singh)

WEST BENGAL UNIVERSITY OF
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Prof. Purnendu Biswas, Ph.D.
Vice-Chancellor

No. : VCS/WBUAFS/ M-5/656
Date : 19.11.2018

MESSAGE

I am extremely happy to learn that ICAR- National Research Centre on Mithun, Medziphema, Nagaland is organizing the XXV Annual Convention of Indian Society of Veterinary Immunology and Biotechnology(ISVIB) and National Symposium (VIBCON-2018) on " Innovative Biotechnological Approaches for Improving Animal Health and Productivity" during 13-15th December, 2018 at your esteemed Organization.

The Topic chosen for the National Symposium is very relevant particularly in the context of the animal health and productivity prevalent in India, which needs extensive research for improvement of the animal health and increasing the productivity of farm animals.

I am confident that the learned delegates attending the Annual Convention and the National Symposium (VIBCON-2018) will share their experience and advancement in their relevant fields, which will be of immense benefit to the Veterinary Immunologists and Biotechnologists and also to the field level workers.

I wish that the above programme will be a grand success. I also wish the Organizing Committee members all the best.

(Purnendu Biswas)

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY
Madhavaram Milk Colony, Chennai - 600 051, India

Dr. C.BALACHANDRAN, M.V.Sc., Ph.D., PGDAJ., PGDEVP., DICVP,
FAO Fellow, FIAVP, FNAVS, FASAW.
VICE-CHANCELLOR

MESSAGE

Greetings!

It is indeed a pleasure to note that the ICAR - National Research Centre on Mithun is organizing the XXV Annual Convention of the Indian Society of Veterinary Immunology and Biotechnology (ISVIB) and National Conference on "Innovative Biotechnological Approaches for Improving Animal Health and Productivity" during 13-15 December 2018.

Innovative technologies are needed to improve the productivity in livestock and poultry to reach the goal of doubling the farmer's income by 2020. The research undertaken with such mandate should be 'translational' and 'transformational'. Innovation is primarily an outcome of cross fertilization of ideas which requires functioning among researchers crossing 'discipline' barriers. This culture needs to be nurtured among present day research workers and they should not suffice in thinking that "What I can do" but start thinking in terms of "What needs to be done".

Research conducted using the modern biotechnological tools can lead to genetic improvement of animals and low cost feeds. Emergence and reemergence of diseases remain a constant threat. Biotechnology offers a great 'tool box' that can help in the development of more-efficient, thermostable and cheap vaccines and early diagnostic tools. Early specific and rapid diagnosis always results in control of disease spread thereby largely limiting economic losses. A classical example in recent times was the Nipah outbreak in Kerala wherein the confirmatory diagnosis was done within 2 days which was the major reason for such limited fatalities due to this outbreak.

In North Eastern India livestock rearing is mostly by small holders. There is a need for enhancing animal husbandry activities in North Eastern area to produce more animal products for human consumption. New technologies can help small farms to achieve more productivity and encourage more farmers to take up animal and poultry rearing. In this context, conduct of this national conference at Dimapur is a timely event which is likely to foster animal agriculture in this geographical location.

I appreciate the efforts taken by the organizers to conduct this conference in the North Eastern region of our country inviting scientists from all over the country. I offer my warm felicitations to the organizers and participants of the National Conference.

I WISH THE EVENT A GRAND SUCCESS.

10/1/2018
(C. BALACHANDRAN)
VICE-CHANCELLOR

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Email : vc@tanuvas.org.in; Website : www.tanuvas.ac.in

अध्यक्ष
Chairman

नाबार्ड

Message

I am happy to know that the National Research Centre on Mithun of ICAR is organizing seminar on innovative biotechnological approaches for improving animal health and productivity.

The livestock sector, is an important source of income for small and marginal farmers. The growing demand for livestock and livestock related products provides an opportunity for increasing the farmers' income by improving production and productivity in the sector. Productivity improvements address the production constraints of small and marginal farmers without depleting the natural resource base of the country. Recent breakthroughs in biotechnology have enhanced possibilities for achieving such productivity improvements.

I hope that the discussions and ideas shared in this seminar will be useful for the participants and that the papers contained in this souvenir will reach a larger audience .I take this opportunity to wish the event a grand success.

Dr. Harsh Kumar Bhanwala

राष्ट्रीय कृषि और ग्रामीण विकास बैंक
National Bank for Agriculture and Rural Development

प्लॉट क्र. सी-'24', जी ब्लॉक, बान्द्रा-कुर्ला कॉम्प्लेक्स, बान्द्रा (पूर्व), मुंबई - 400 051. • टेलि : +91 22 2653 0000 • फैक्स : +91 22 2653 0113 • ई-मेल : chairman@nabard.org
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डॉ आर.के. सिंह
Dr R.K. Singh
निदेशक एवं कुलपति
Director & Vice-Chancellor

MESSAGE

Recent times have witnessed a paradigm shift in farming and husbandry practices, especially in the densely populated developing countries in tropical regions, primarily on account of sweeping global climate changes and emerging threats of food insecurity, nutritional security and biosecurity, which have adversely affected the agricultural ecosystems in many ways, and caused huge losses. These factors have also led to the emergence or re-emergence of many infectious diseases including zoonoses, increase in vector population and vector borne diseases, change in disease patterns and, alarming increase in heat stress-related deaths amongst livestock, poultry and wildlife as well as extinction of many flora and fauna in different ecosystems across the globe. The problem is further likely to worsen as the global population is projected to surpass 9.6 billion by 2050, requiring nearly a doubling of agricultural output to provide an adequate food supply. Summarily, the agricultural system as a whole will be increasingly challenged by water scarcity and climate change, thereby, raising the risk of production shortfalls in a world, where over 1 billion people are already undernourished. In this context, the rapidly developing biotechnology basket has unprecedented opportunities to offer for increasing the animal productivity and health, especially in the areas of animal disease diagnostics, veterinary medicine and animal breeding.

The Golden dream of our Hon'ble Minister to double the income of farmers by the year 2022 encompasses a holistic view to strengthen the Indian farming as an integrated enterprise. Livestock farming, one of the key components of Indian farming with a significant contribution of 25.6% of the value of agriculture output, holds the key to achieve this feat in realistic terms. In fact, no other sector is more important to the lives and livelihoods of the poor than the livestock. Therefore, increased livestock, poultry and aquatic food yields are needed in order to feed a growing population. The biotechnology holds huge potential to address these core issues through the innovative approaches aimed at developing improved crops by genetic engineering, genomic-assisted breeding and the use of recycled wastewater; bio-fortification of foods either through transgenic animals and poultry, or by altering the physiology of plants; besides developing mitigation strategies for augmenting animal productivity and health. These efforts need to go hand-in-hand with adoption of biotechnology for reducing greenhouse gas emissions from agriculture, livestock and poultry sectors by boosting carbon sequestration besides manipulating rumen microflora in livestock and developing suitably modified diets for animals.

I am quite optimistic that the eminent biotechnology researchers, policymakers and other stakeholders participating in **VIBCON-2018** will generously share their findings, views, experiences, knowledge and wisdom for effectively addressing the aforesaid core issues for realistic, eco-friendly and beneficial solutions and recommendations. I congratulate the organizers for having taken this initiative to address this contemporary and pertinent issue in the larger interest of the world community, ecosystem and environment. I also convey my best wishes for the successful organization of this important endeavour and its meaningful outcome.

[R. K. Singh]

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On the service of nation since 1889

From the Desk of Organizing Secretary

On behalf of the organizing committee, I am delighted to welcome you all to the ***Silver Jubilee*** Convention of Indian Society for Veterinary Immunology and Biotechnology (ISVIB) & National Conference VIBCON-2018 on "Innovative Biotechnological Approaches for Improving Animal Health and Productivity" being hosted in ICAR-NRC on Mithun, Nagaland, from 13– 15 December 2018.

Our association, ISVIB, has Special Consultative Status on the United Nations' Economic and Social Council, which brings people and issues together to promote collective action for a sustainable world, and this conference will strengthen links between ISVIB and the Scientists and other stakeholders working in this field with common efforts to achieve greater sustainability and developing innovative biotechnological techniques for improving animal health and productivity.

It is indeed a pleasure & honor to organize the Silver Jubilee convention and a National conference of such a great magnitude in Nagaland. This is our first endeavor to host any National conference in the Institute but we affirm you that this special event will be hosted with great acclaim, with active participation from all the stakeholders, supporting a culture of innovation and entrepreneurship that addresses the great challenges facing our community today.

I am indeed grateful to the members of our association for reposing faith in us. The organization of this conference has been made possible with the guidance of our senior's, office bearers of ISVIB, help from our esteemed friends and efforts from juniors.

We express our heartfelt gratitude to Indian Council of Agricultural Research (ICAR), National Bank of Agriculture and Rural Development (NABARD), Department of Biotechnology (DBT) for the financial help kindly rendered.

I must acknowledge my colleagues who are actually doing all the ground work and I am just coordinating the affair. My sincere thanks to all of them. Any lacunae in the part of the organizers are deeply regretted and I take all the responsibility.

Once again, I extend my warm welcome to all the delegates who have participated from various part of the country and I am sure this event will be a great knowledge-sharing opportunity. Hope this conference will linger with golden memories of three days stay in Nagaland- "***The Land of festivals***".

Abhijit Mitra
Director, ICAR-NRC on Mithun

National Bank for Agriculture and Rural Development

NABARD

Our Mission: Promote sustainable and equitable agriculture and rural prosperity through participative financial and non financial interventions, innovations, technology and institutional development for securing prosperity.

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About ICAR-NRC on Mithun, Nagaland

The Institute

ICAR-National Research Centre on Mithun, established in the year 1988, being the only research organization in the world, is exclusively working for the continual improvement and conservation of Mithun (*Bos frontalis*). During the last more than 29 years, the Institute has not only generated invaluable scientific data towards the understanding of this unique species but also developed several packages of practices and technologies. Conservation efforts including taming of mithun and demonstrating an alternative system of semi-intensive rearing of mithun resulted in the complete domestication of the species. Popularization efforts led to the adoption of scientific rearing of mithun by the tribal communities of North Eastern Region (NER) with better returns. Some of the salient achievements of the Institute are:

- Genetic and molecular characterization of different mithun populations and delineating evolutionary relationship of mithun with gaur (*Bos gaurus*).
- Protocol for collection and freezing of semen, estrus synchronization and AI in mithun, and successful implementation in the institute farm and farmers' fields.
- Protocol for collection and cryopreservation of embryo in mithun leading to the birth of the World's first embryo transfer mithun calf from a cryopreserved embryo "Mohan"
- Area-specific mineral mixture and feed block using locally available tree leaves/shrubs and industrial by-products
- Chemical and nutritional evaluation of 260 feed resources (e.g., tree leaves/ shrubs/grasses) for incorporation in the total mixed ration (TMR).
- Supplementation of spent grain and wheat bran/ rice bran in the diet of mithun increased dry matter and gross energy intake
- Methods for drying high moisture content agro-industrial by-products (wet cake) and successful incorporation in paddy straw based feed blocks
- Determination of age by dentition pattern of mithun under field conditions

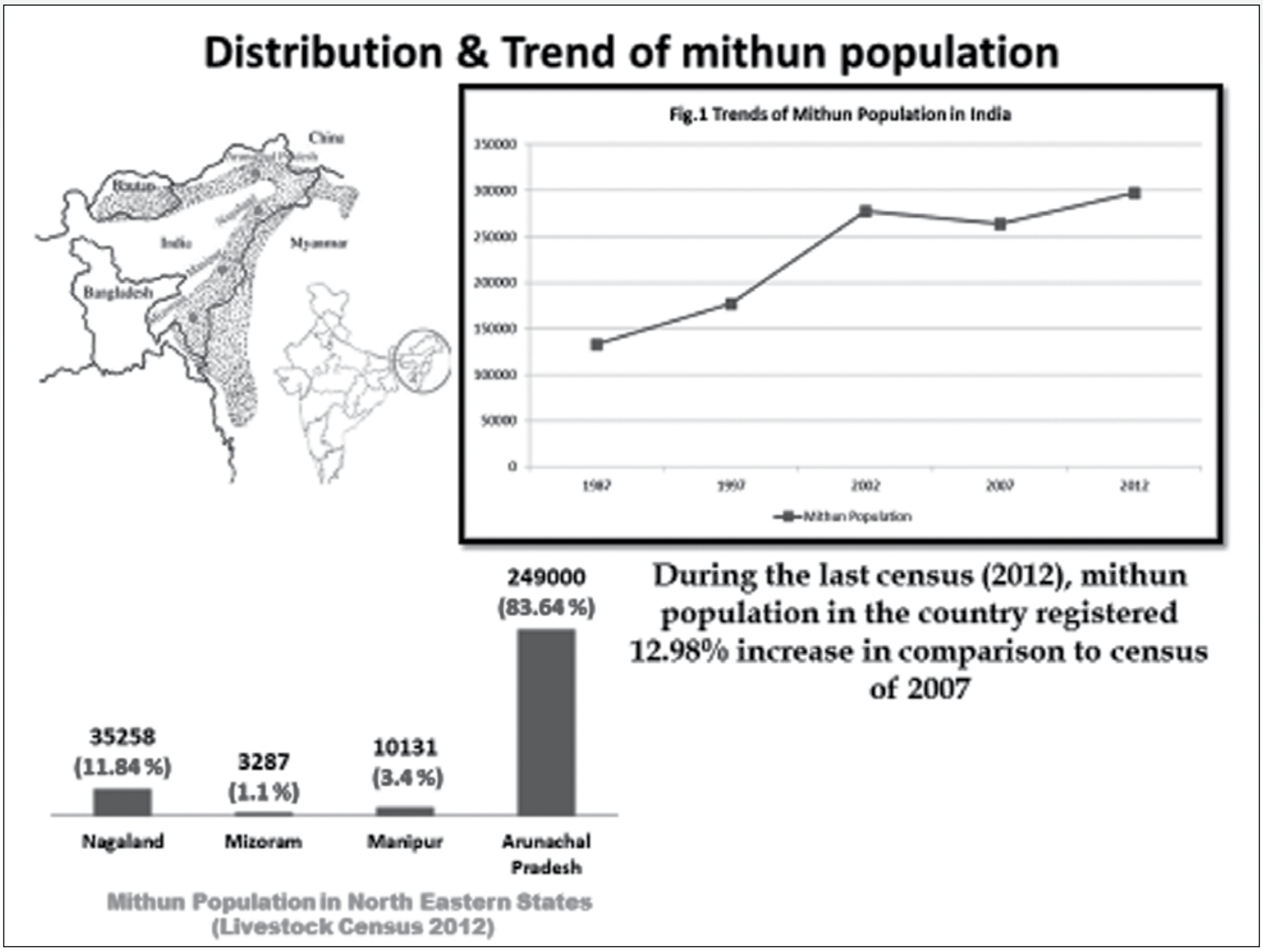
- Surveillance of important parasitic, bacterial, and viral diseases in mithun and development of control measures
- Diversified use of mithun:
  - value-added milk products (paneer, lassi, dahi and rasgolla)
  - meat (meat block, patties, nugget and meat powder)
  - skins and hides (leather jacket, ladies bag, shoe, wallet, portfolio bag)
  - Draftability
- Semi-intensive system of rearing of mithun as a farming system model

Geographical Distribution and Population Status of Mithun

Mithun is distributed within a limited geographical boundary. It is mainly found in the North-Eastern Region of India viz., Arunachal Pradesh, Mizoram, Nagaland, and Manipur. It is also found in Myanmar, China, Bangladesh and Bhutan. It is difficult to ascertain the world total population of mithun as no systematic population record is available in mithun inhabited areas except in India. According to the 19th Livestock Census (2012), out of 298264 total population of mithun in India, 249000 mithuns are available in Arunachal Pradesh, followed by 34871 in Nagaland, 10131 in Manipur and 3287 in Mizoram.

Mithun is traditionally reared under a forest ecosystem and can easily thrive at an elevation of 300–3000 MSL. Several factors including inbreeding, indiscriminate slaughter, cross-breeding with cattle and habitat destruction are responsible for poor or slow population growth of mithun. Considering the social as well as economic importance, this animal species deserves a special attention for the conservation, improvement, and propagation.

In the following sections, a brief account of the research and extension activities undertaken by the Institute, and man-power, funding status, ongoing projects, and other information during the period of 2017-18 is presented..



During the last census (2012), mithun population in the country registered 12.98% increase in comparison to census of 2007

Mithun Population in North Eastern States (Livestock Census 2012)

About Indian Society for Veterinary Immunology and Biotechnology (ISVIB)

Indian Society for Veterinary Immunology and Biotechnology (ISVIB) was started in the year 1990.The founder members included Drs. B.B.Mallick, S.Krishnaswamy, M.V.Subba Rao, V.D.Padmanabhan and A.S.Grewal. Scientists from Veterinary Microbiology, Biotechnology and other disciplines are life members in this society. The organization has grown over the years with a current membership of over 1000 scientists from India and abroad. Indian Society for Veterinary Immunology and Biotechnology motivates the members to discuss, analyze and formulate suitable strategies for safeguarding animal health and thus to ensure improved animal production. The society strives to highlight the role of veterinary scientists in achieving availability of balanced nutrition for all people and freedom from zoonotic diseases by applying biotechnological tools in animal and fisheries sphere of agriculture. To achieve this annual conventions and national/international symposia are organized to critically analyse and update our knowledge and understand issues concerning Immunology and Biotechnology based technologies in animal production and health. So far 24 conventions and one colloquium were organized in association with different institutions. Last convention (24th) was conducted in theCollege of Veterinary and Animal Sciences, Parbhani, Maharastra during 5-7th December 2017. Many scientists from different parts of our country participated in the convention.

Apart from organising conventions with National and International symposia/ seminars ISVIB also honours and encourages senior and young scientists by instituting many awards. The highest award of the society is the Dr.P.Richard Masillamony oration award. Scientists are also recognised by the Fellow of ISVIB awards. The other awards are ISVIB Young and Mid career scientist awards, ISVIB -GADVASU Woman scientist award, Sir. F.M. Burnett memorial award (Team research), ISVIB – IVRI Mukteswar Albert Linghard Memorial award (Product development), ISVIB Scientist award (Research article in high impact factor journal) and poster awards. To encourage UG and PG students to participate and present research abstracts in the conventions ISVIB has instituted two student travel grants.

The society has got a website,www.isvib.org, containing member details, office bearers, upcoming events etc.The society has started a peer reviewed, open access research journal entitled "Veterinary Immunology and Biotechnology" to publish original research studies on basic and applied aspects of veterinary immunology and biotechnology.

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VIBCON-2018

ICAR-NRC on Mithun, Medziphema, Nagaland

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SESSION – I

Dr. Richard P. Masillamony Oration Award Lecture

‘Triangle’ of Translational Veterinary Medicine: Nano-, Immuno-, and Bio-technologies

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We all know the disease triad - disease occurs due to the interaction of host, pathogen and the environment. Similarly, the technology response to the prevention, therapy or control of disease has to encompass nanotechnology, immunotechnology or biotechnology for rapid and effective results. The application of these technologies, either alone or in combination, for protective and therapeutic interventions in translational veterinary medicine is required for the animal sciences sector, to achieve the goal of doubling the farmers income and to accelerate growth of this sector.

NANOTECHNOLOGY

When Dr. Richard Feynman suggested that “There’s Plenty of Room at the Bottom”, this room also included veterinary and animal sciences. Many nanotechnology-based applications are beginning to be harnessed in this less-affordable sector. To name a few that our laboratory has been engaged in developing / producing nano-vaccines, nano-medicine and nano-formulations. A new live Newcastle disease (ND) vaccine formulation was developed using calcium phosphate (CaP) coupling to improve the vaccine efficacy. The CaP coupled ND vaccine elicited significantly higher and earlier protective immune titers even with 100 times lesser virus dose in chickens. CaP coupled vaccine had increased infectivity and spread, in target organs.

Progesterone was impregnated in nanofibers prepared using zein which is applied for estrus synchronization in bovines. This is applied as a dermal patch thereby ensuring a farmer-friendly application process eliminating the need for a veterinarian for implant delivery for a similar purpose.

Nano salt was prepared using membrane filtration or coating with magnesium glutamate. The sodium reduction strategies were found to be highly product dependent showing no reduction in sodium when applied to popcorn but showed significant reduction in processed dry fish.

The main concerns with nano vaccinology technologies are:

- Biocompatibility
- Upscaling and reproducibility of nano-vaccine formulation
- Toxicity particularly cumulative toxicity in organs

The toxicity of nanoparticles is hard to assess since the potential adverse effects are likely to result from long-term, low-level exposure, which requires very long trials to determine. Our understanding of the long-term effect of nanoparticle exposure is still limited.

Nanotechnology applications extend to all disciplines and needs to be harnessed properly and adequately for the benefit of animal health and productivity!

IMMUNOTECHNOLOGY

Pathogens are successful in causing infections, because they have developed an amazing variety of virulence factors that allow them to evade, inhibit, or otherwise subvert host immune responses. Improved immunological understanding of such pathogens has resulted in vaccines and immune-therapeutics which have led to eradication of some of the dangerous pathogens from the face of the earth. The notable achievements are the eradication of small pox in man and rinderpest in cattle.

Modern immunology has contributed significantly not only in increasing the life span of man but also in improving the wellbeing of the livestock and in turn quality of animal based food.

Recent advancements in immunology focuses not only on the cell mediated immune mechanisms, but also on innate immune systems. Innate immunity, perhaps a lesser explored area, is now the focus of researchers trying to address the problems of emerging variant pathogens and antimicrobial resistance.

This has all been possible only due to the advent of modern technologies in the field of immunology. Few such breakthrough technologies include conventional ELISA, Luminex based multianalyte profiling, flowcytometry and cell sorting technologies etc.

For decades, ELISA has been the mainstay for scientists to detect the various protein analytes and specific antibodies. A variety of formats such as competitive ELISA, indirect ELISA, sandwich ELISA and liquid phase blocking ELISA were invented. However, the assay system had the limitation of detecting one analyte at a time. This was inadequate in situations where there are limited samples and one needs to get as much information from the sample as possible. Immunoassays based on Luminex multi-analyte profiling technology allows for the simultaneous detection and quantitation of multiple protein targets such as cytokines. This high-throughput technology produces results comparable to ELISA but with greater efficiency in terms of number of proteins detected at a given time and the speed of the assay.

Flowcytometry was another breakthrough in immunology. Cell populations can be characterized by flowcytometry using a combination of surface and/or intracellular antigens. There are a number of practical applications regularly used by immunologists including immunophenotyping, measuring intracellular cytokine production, cellular proliferation, assessing cell viability and analysis of cell cycle, rare events, stem cells and fluorescent proteins. Flow cytometry has advanced in recent decades and is used not only to identify the cell types but also to sort them for various purposes. Fluorescence activated cell sorting (FACS) technologies resulted in isolation and study of immune cell types to even sorting sperm cells to get desired gender of the progeny. The advent of magnetic nanobeads also has contributed to the cell sorting technologies by providing an alternative to fluorescent tags with inert magnetic nanoparticles, the magnetic-activated cell sorting (MACS). However, there are more antibodies commercially available for FACS than for MACS.

Immunotherapy is the most notable advancement in the field of immunology. Immune therapies are fast becoming paradigm-changing treatment options for a variety of disorders especially the malignancies. Cancer immunotherapies, which rely on harnessing a patient's immune system to kill malignant cells, are booming as a treatment option for patients with hematologic malignancies. The field has grown exponentially since Science magazine designated cancer immunotherapy, including checkpoint inhibitors and chimeric antigen receptor T cells (CARTs), as "breakthrough therapy" of the year for 2013.

Cellular immunotherapy is evolving rapidly for high-risk hematologic disorders. Recent advances include CART, mesenchymal stromal/stem cells, dendritic cell tumor vaccines, cytokine-induced killer cells, and virus-specific T cells. The cellular immunotherapy is also highly beneficial for patients for whom organ or tissue transplantation has failed and for relapsed/refractory patients. Immunotherapies have now shown efficacy in hundreds of patients with hematological malignancies refractory to conventional therapies, including some targeted therapies.

BIO-TECHNOLOGY

Biotechnology has dramatically advanced during the last three decades. The understanding of DNA and advancement of technologies that allowed this knowledge to be exploited for manipulation of genetic material has contributed significantly for such giant leap. The term biotechnology has overlapped and spread across diverse disciplines such as animal health, human health, agriculture, defence and even space exploration.

Since its discovery in 1983 by Kary Mullis, the polymerase chain reaction (PCR) has become an indispensable technique and a widely applied tool in medical and biological research. PCR was as a qualitative test that could only be used to detect the presence of a specific gene segment which in turn will confirm the presence of pathogen.

However, over the years, PCR has diversified into various sub-techniques with important applications and is widely available in the market as diagnostic kits. The principal advantage of PCR is that nucleic acid amplification allows the detection of lower levels of pathogen genetic material providing high sensitivity.

Of late, PCR is often followed by a number of post-amplification analysis methods such as sequencing, deep sequencing, hybridization and Luminex. Widely applicable, real-time PCR is another important methodology not only for the molecular diagnosis of viral, bacterial and parasitic infections but it also allows for the quantification of the target sequence. In fact, the real time PCR by virtue of its specificity ensured by the use of internal probes and prevention of carry-over contamination using UNG-glycosylase have led to large scale use of this technology in disease diagnosis. The recent Nipah virus outbreak in Kerala was diagnosed within 2 days of the first outbreak that largely resulted in limiting the virus spread and thereby human mortality.

Following the application of genome amplification and analytical (PCR-related) techniques in the field of disease diagnosis, now the capability of sequencing of the genomes of pathogens is permitting the identification of individual genetic variants. Sequencing of large genomes could now be completed in hours or days. With the power to sequence even individual nucleic acid strands, the **concept of focused detection** of an organism has now been **widened to identify unknown organisms** as well. Such technological advancements have led to deeper investigations and understanding of the infectious/ genetic aetiology of diseases, biology of the organisms and helps in designing of effective control strategies.

Considered as one of greatest achievements in the human history, the Human Genome Project was coordinated by the National Institutes of Health and the U.S. Department of Energy. Since its official start in 1990, the project's goal of determining the sequence of nucleotides that make up human DNA completed in April 2003 with the researchers announcing the preliminary sequence of the entire human genome. This work has inspired researchers to begin to understand the genetic blueprints of other animal species, which resulted in complete genome data for cattle, pig etc. Once the blueprint is ready researchers turned their attention on the functions of genes and proteins, which aided in identifying genes that cause diseases, and in turn looking for effective treatment.

Gene therapy is another advancement of biotechnology where a normal (functioning) copy of the gene could be inserted into a cell to replace a non-functioning gene. As genes will not enter cells on their own, researchers exploited the capability of viruses (vectors) to infect and insert their genetic information into cells. Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) is a recent innovation in gene-editing system, which is considered as a ground-breaking tool in biotechnology research. With this tool in their arsenal, microbiologists and geneticists can actively test newly found mutations and edit them to provide customisedand targeted therapies.

Synthetic genome is another example of biotechnology making another giant leap. In 2010, scientists synthesized the 1.08 million base pair genomes from *Mycoplasma mycoides* that infects goats. This process involved, synthesizing DNA to created genome fragments and assembling them into an entire genome and inserting it into a host Mycoplasma cell whose own DNA was destroyed. When the host cells continued to grow and multiply, they did so under the direction of the synthetic genome.

Sum up….

There would be a dramatic increase in the demand for livestock products, driven largely by human population growth, income growth and urbanization. However, the changing scenarios and the need to adapt to climate change and to mitigate greenhouse emissions will add to the costs of production. Livestock production would become industrialized.

The "vicious circle" involving the challenges of teaching, researching, publishing and competing for competitive grants, coupled with pursuing career aims and ambitions, can seem daunting. However, it can also be deeply satisfying when the fruits of the experimental laboratory research are translated into improved healthcare delivery to our animal patients. To do this, the basic motive to do research has to change in terms of addressing a pressing problem and not to start with another publication.

We need to be up to the challenge and above technologies is our tool box!!

SESSION – II

MOLECULAR DIAGNOSTICS

LEAD PAPERS

MD–L–01

Technology Innovations in Diagnosing Enteric Viral Infections

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Enteric infections are among major problems and responsible for colossal losses worldwide, majorly in resource poor low- and middle-income developing countries. Extraordinarily, 95% of deaths from respiratory infections and 98 % deaths from diarrheal diseases occur in these countries. Its impact is much higher among young and neonatal stage animals. Among various causes of infectious diarrhea the viral aetiology is of much more significance and to better comprehend and confront these problems there is an urgent need to acquire more scientific knowledge on these pathogens including their reservoir hosts, immunopathobiology, genetic diversity and the development of state-of-art diagnostic tools.

For the effective prevention and management of enteric conditions an early and accurate detection of pathogen is needed. Only, the accurate and confirmatory lab results assurances effective management of disease. Albeit, inaccurate diagnosis on several occasions lead to great economic loss. During lab testing we have to assess precisely the existence of the pathogen or its antibodies, confirming status of the disease (current/past). Since the first report on rotavirus being the cause of acute gastroenteritis during 1972 by Ruth Bishop, list is being expanded with the discovery of several other viruses (picobirnavirus, astrovirus, calicivirus, kobuvirus, bocavirus, sapelovirus, teschovirus, coronavirus, etc.) with use of biotechnological tools. The biotechnological tools have revolutionized the field of enteric virus diagnostics.

The advances in enteric virus research has led to recognise the aetiology of acute viral gastroenteritis, co-infectious status, pathogen transmission modes, facilitating timely adoption of appropriate disease prevention and control strategies. The enteric virus research progressed greatly with use of electron microscopy for the first time in late 19th century. Since the first description of enteric virus based on the use of electron microscope (EM), the diagnostic and research laboratories are now relying on a diverse range of techniques to diagnose enteric viral infections. The culture-based methods has shown great contribution in the diagnosis of viral infections, along with serology testing (Complement fixation test, Haemagglutination based tests, precipitation assays) for detection of antibodies targeted against the virus. The diagnostic industry using state-of-art technologies world over are engaged in bringing latest instruments within low budget having more compliance in low and middle income countries. Now, a number of molecular techniques are available and their improved modifications are being evolving. The field of pathogen detection revolutionized with introduction of the nucleic acid amplification by polymerase chain reaction (PCR). Nucleic acid amplification tests (NAAT) term was used for these new variants. NAAT are very popular in the diagnosis and management of viral infections because they allow determination of the viral load. Developments have been seen in the availability of several nucleic acid-based amplification assays viz., reverse transcription-polymerase chain reaction (RT-PCR), real-time qPCR, loop-mediated isothermal amplification (LAMP), polymerase spiral reaction (PSR), with the aim of improving the assay capabilities. The real-time PCR (quantitative PCR) is nowadays becoming benchmarks in detecting and assessing the viral load. Other amplification-based tests such as nucleic acid sequence-based amplification (NASBA) and transcription-mediated amplification (TMA) are suited for detection of RNA viruses by amplification of the mRNA instead of conversion to cDNA. To detect multiple viruses or subtypes in a single run multiplexed NAAT have been designed which detects several pathogens simultaneously. Furthermore, the utility of Next-generation sequencing (NGS) is unparalleled to other assays and has become an assay of choice these days. It has allowed discovery of novel viruses responsible for unknown diseases.

The modern diagnostic assays have several advantages including high sensitivity and specificity, multiplexing possibility, automation of the assays, use of small sample size, work efficiency, use of freezing–thawed samples/formalin fixed samples, rapidity, etc. Epidemiological monitoring of enteric viral diseases is indubitably a global public health desire. Nevertheless, several diagnostic methods have been developed over the last two decades, given the constant evolution of viruses, newer, sensitive, efficient and rapid diagnostics are still need of the hour. Efforts are in-line towards developing novel field and point-of-care quality diagnostics which are economic and affordable in low- and middle-income countries. The availability of such assays would substantively reinforce disease control strategies. This lecture will provide information on the conventional methods and currently in-use, as well as advanced tools available worldwide for the effective detection of enteric viruses.

MD–L–02

Japanese Encephalitis: An appraisal of current research

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Japanese encephalitis (JE) is a vector borne viral encephalitis widely prevalent in eastern and southern Asia. The causative virus, JEV belongs to family Flaviviridae, genus flavivirus. The 11 kb positive sense, single strand RNA genome of the virus encodes for two structural proteins and seven non-structural proteins. JE generally affects children of 0-15 year's old age group and rarely adults. Following infection, after 5 to 15 days incubation period, neuronal damage occurs either due to multiplication of virus inside the neurons or due to microglia activation and release of reactive oxygen species and pro-inflammatory cytokines such as IL-6, TNF-α, MCP-1 and RANTES. This results in infiltration of MNCs in the brain and neuronal apoptosis by TRADD pathways. Also production of IP-10 by activated astrocytes causes NK and MNCs infiltration in the brain. JEV affects neuronal progenitor cells in the sub ventricular areas of brain and causes neurological defects in children. Various micro RNAs have also been implicated in JEV pathogenesis. Up-regulation of micro RNA 29b, miRNA155, miRNA146a, miRNA15b and miRNA19b-3p and down-regulation of miRNA 432 and miRNA370 by JEV promotes JEV pathogenesis either by playing role in evasion of antiviral host response or by up-regulating pro- inflammatory cytokines causing severe neuroinflammation. Over expression of miRNA 124 causes deprivation of cellular membrane vesicular system inhibiting viral propagation. Flaviviruses interfere with formation of stress granules and processing bodies (PB) in oxidatively stressed cells, thereby enhancing flavivirus messenger RNA synthesis. The sub-genomic flavivirus RNA produced due to incomplete degradation of flavivirus RNA in PB cause cytopathic effect.

Less than 1% people infected with JEV develop clinical illness. Initial symptoms are fever, headache and vomiting, few days' later neurological signs, weakness, movement disorders, changes in mental status and seizures in children are observed with death rate being 20-30% among encephalitis patients and 30-50% survivors face neurological and psychological consequences. Miscarriage in pregnant women is also observed. Pigs principally develop reproductive problems, decreased sperm motility and count in boars; abortion, stillbirth and mummification in sows at term and hydrocephalus and cerebellar hypoplasia in foetuses. The viable piglets develop tremor, convulsions and die shortly. In horse, subclinical signs and non-specific signs develop in most of the cases. Cattle rarely show nervous signs with very low level of viraemia.

JEV circulates in an enzootic cycle involving pig, birds and mosquitoes mainly *Culex* species. Pigs and birds are reservoir and amplifying hosts, mosquitoes are bridging vector and human, cattle and horse are dead end hosts. Viraemia is reported in about 90 domestic and wild bird species. Among mammals, pig is the amplifying host and maintenance host because of high natural infection rate, high viraemia for 4 days, high birth rate and tendency of vector mosquitoes to feed on pig etc. Around 17 mosquito species belonging to *Culex*, *Anophele*s, and *Armigeres* spp. have been implicated as main vectors in India. *Culex tritaeniorhynchus,* a major paddy breeding mosquitoe is the main vector. JEV infective rate in mosquitoes varies according to time and space which depends on changing environmental condition and availability of amplifying host. Around 30-40% pig feeding rate has been reported in Northern India while humans account for only 5% in Asia. Multiplication of virus in vector is temperature dependant and at low temperature multiplication is reduced. *Cx. quinquefasciatus* is the main vector in Uttar Pradesh and West Bengal. In Kerala, Mansonia spp. has been implicated in JEV transmission. *Cx. gelidus* is emerging as a major emerging vector for JEV in India, SE Asia and Australia due to its insecticide resistance, high anthropophily, invasive nature, and ability to breed in fresh and dirty water etc. Overwintering mechanism of JEV has been reported experimentally in bats, snakes, lizard, frogs and Culex mosquitoes where low level of virus is maintained in adverse winter condition. In temperate region, migratory birds, bats and wind borne mosquitoes play an important role in virus reintroduction. Recent report suggests vector free transmission of virus between pigs is also possible as infected pigs shed JEV in oronasal secretion and can be infected by oronasal route. Also persistence of JEV in the tonsil of pigs for 25 days inspite of high neutralizing antibody has been suggested.

In India, several factors are responsible for emergence of JEV. Climate change like heavy rainfall after extended hot period increases mosquitoes breeding rate and subsequent spill over to human and change in the pattern of bird migration might be responsible for increased JE incidence in India. Uncontrolled population growth and poor socioeconomic status are also important contributing factors. Pig rearing which increases contact between human and pig might be responsible for emergence of JEV in rural and urban areas. India is world's largest (20%) producer of rice. Irrigated systems of rice farming have increased both cropping area and cropping intensity providing breeding ground for mosquitoes. Around 108 million people live in close proximity to paddy cultivated area at higher risk for JEV infection. So in areas where rice and pork is consumed, the risk for JEV transmission is presumably more.

Emergence of new JEV genotypes and change in vector dynamics are responsible for JE emergence in India. JEV is categorized into five genotypes such as GI, GII, GIII, GIV and GV. JEV genotypes (GI, GII, GIII) circulating in endemic and epidemic are recent types and those circulating in Indonesia-Malaysia region including GIV and GV are ancestral types. The Muar strain of JEV which was isolated from a patient in Singapore in 1952 belongs to genotype V. In 2009, in China, genotype V was isolated from *Cx. tritaeniorhynchus* which suggest that JEV is re-emerging in Asian countries after 57 years which may be due to various factors related to changing environment. Genotype III is the most widely distributed genotype in Asiatic countries including India. All the JEV isolates in India until 2007 belonged to genotype-I. Both GI and GIII of JEV were detected in West Bengal in 2010 outbreak. GI isolates in India showed genetic closeness to GI circulating in Japan and Korea. In India, neutralizing antibodies have been detected in herons and cattle egrets. Also perennial lakes, swamp and rice fields favour wintering and staging of waterfowls and mosquitoes breeding and JEV maintenance. Genotype-I virus may have been introduced into India through migratory birds.

Confirmatory diagnosis of JE can be done by virus isolation from CSF and fresh brain tissues in BHK, MDBK, and Vero and C3/36 cell lines and in suckling mice. Serological tests likes HI, ELISA, VNT and CFT also help in diagnosis. IgM capture ELISA (Dipstick and JEV-Chex) is most widely used for JE detection to measure the level of silent infection and immunity level in the local population. Now-a-days, RT-PCR and real-time RT-PCR are commonly used in the laboratories. Histopathology and antigen detection by IHC and IF are also helpful.

Control of JEV transmission can only be done by minimizing contact between humans and mosquitoe vectors. Use of insect repellent, insecticide bed nets, thermal fogging, use of personal protectives, larvicidal measures such as use of larvivorous fish and biolarvicidal compound and novel water management measures like intermittent irrigation in rice field and use of neem leaves in rice field, lowering water levels and constant drainage system are crucial for control of mosquito breeding. Vaccines are available for use in humans to combat JE. Presently used vaccines confer protection against all genotypes, though some reports suggested weaker immune response against heterologous genotypes. In India, live attenuated SA-14-14-2 vaccine imported from China, JEEV

(Indian variant of IXIARO by Biological E. Ltd.) and indigenous vaccine of India (JENVAC) are used for vaccinating children and adults. Government of India launched JEV vaccination campaign in order to provide single dose of live attenuated JE to 1-15 years age group children in 2006. Since 2013, after expert recommendation, two dose of JE vaccine have been used, one at 9 months age and other at 16 to 24 months age. Effective disease surveillance, proper case management, pig vaccination and rearing pigs away from human settlement, improving agricultural practices, improvement of living standard and health education/ community awareness are crucial steps for eradication of JE. Incidence of JE is showing an increasing trend in India and in future, there are chances of larger epidemics. Effective disease surveillance, control and preventive measures should be strictly implemented.

MD–L–03

Leptospira putative lipoprotein LIC11966 is a fibrinogen binding protein of diagnostic importance

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Leptospirosis is a worldwide zoonotic infection of human and veterinary concern, with approximately one million cases reported every year (Costa et al., 2015). The disease is caused by spirochetes of the genus *Leptospira*, with higher occurrence in the tropical and subtropical regions. Humans are infected with these bacteria through their contact with contaminated environments and exhibit a diverse range of clinical manifestations, ranging from a mild influenza-like febrile illness to a severe disease that affects multiple organs. In large animals, reproductive complications may result in abortion, stillbirth, birth of weak newborns, and decrease in the growth rate as well as in milk production resulting in huge economic losses (Ellis, 2015). The clinical diagnosis of leptospirosis is difficult due to its similarity with other hemorrhagic diseases. The problem of the disease has not been completely addressed even though it has been recognized for decades, the primary reason being the presence of the major burden of the disease in poor, developing countries (Victoriano et al., 2009).

Transmission of leptospirosis occurs by both direct and indirect contact with the causative agent. Infection propagates directly from the contaminated urine of reservoir hosts including chronically infected wild or domesticated carrier animals and indirectly by entry from environments which support leptospiral growth or survival (Ko et al., 2009). However, successful transmission of these spirochetes by direct or indirect entry requires the bacteria to adapt and survive in various types of host tissues, diverse host immune response and with the habitat of low nutrient environments of swamps, streams, rivers and alkaline soil (Izurieta et al., 2008; Patarakul et al., 2010). Under these conditions, pathogenic *Leptospira* have developed mechanisms to sense these diverse environments and respond by synthesizing appropriate proteins and other cellular constituents necessary for its survival in each habitat (Artiushin et al., 2004; Matsunaga et al., 2007; Nally et al., 2001; Qin et al., 2006). Recently, using high-throughput RNA sequencing of *Leptospira* transcripts, 166 genes were identified during mammalian host-adapted state to be differentially expressed (Caimano et al., 2014). Such phenomenon was reported in another infectious spirochete *Borrelia burgdorferi* where differential expression of various genes was observed during its enzootic cycle between vertebrate and invertebrate hosts.

The outer membrane proteins of pathogen are targeted to understand the host-pathogen interaction and are centrally implemented in the diagnostics development. The molecular mechanisms of *Leptospira* pathogenesis are not well elucidated. However, it is believed that the adhesion of *Leptospira* to host tissue components is a necessary step for infection and pathogenesis. The extracellular matrix (ECM) is a major versatile structure produced by resident cells of the host. These host matrices are known to facilitate bacterial cell attachment and in some cases to pathogen dissemination. Several leptospiral proteins were shown to interact with blood plasma proteins to reduce fibrin clot formation that could facilitate the bacterial dissemination inside the host (Vieira et al., 2011). In the last two decades, many known leptospiral adhesins as the virulence factor of *Leptospira*, have been reported that may be critical in the pathogenesis of leptospirosis (Vieira et al., 2014). In addition, virulence factors of pathogens expressed during host infection are expected to elicit specific humoral immune responses and, thus, may serve as candidate markers for a recombinant protein-based serodiagnostic test.

We report that *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 genome harbours a gene *LIC11966*, which is predicted to encode an outer membrane surface lipoprotein. On performing bioinformatics analysis, orthologs of LIC11966 was found to be present only in pathogenic *Leptospira* species with protein sequence conservation from 57-100%. The presence of conserved *LIC11966* gene was demonstrated by PCR and RT-PCR in serovars of pathogenic *Leptospira* species viz. *L. interrogans* serovar Copenhageni and *L. interrogans* serovar Lai. The gene *LIC11966* was cloned into expression vector pET28a and the recombinant protein (r-LIC11966) was purified using nickel affinity chromatography from *Escherichia coli* BL21 (DE3). Polyclonal anti-LIC11966 antibodies raised against r-LIC11966 in BALB/c mice, detected LIC11966 expression in the *Leptospira* lysates suggesting that the antigen LIC11966 is immunogenic. To know the location of this protein in *Leptospira*, cellular localization studies using Triton-X-114 phase-partition and proteinase-K accessibility assay were performed in which LIC11966 was found to be an outer membrane surface exposed protein. The recombinant protein showed its ability to attach to diverse host extracellular matrix (ECM) components with relatively higher affinity towards fibrinogen suggesting that LIC11966 may interfere with the host fibrin based blood clotting mechanism. *Leptospira* spp. may use these interactions as a possible mechanism for the establishment of infection. The enzyme-linked immunosorbent assay performed using human (n=50)/bovine (n=50) and canine (n=18) serum, tested positive for leptospirosis by microscopic agglutination test (MAT +ve), recognized r-LIC11966 with 100% sensitivity and specificity, suggesting that LIC11966 is expressed in diverse hosts during *Leptospira* infection. Thus, the present findings suggest that the *Leptospira* LIC11966 antigen is an outer membrane adhesin of diagnostic importance.

In conclusion, we have identified a conserved immunogenic LIC11966 lipoprotein in pathogenic *L. interrogans* serovars. Recombinant LIC11966 can be serologically detected by antibodies present in the serum of human, bovine and canine infected with *Leptospira*. The characterization of novel diagnostic antigen markers for diagnosing leptospirosis may help for the accurate diagnosis of a disease which is mostly misdiagnosed in the present scenario.

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MD–L–04
Caprine Mycoplasmosis : An overview

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Introduction

Goat farming is one of the enterprise which has been practiced by a large section of human population especially in developing world and it also plays an immensely important role in employment generation, capital storage and improving house hold nutrition.Inspite of the enormous technological and developmental efforts in Livestock improvement sector in the country, the status of goats has largely remained as resource for poor farmers. A number of factors are responsible for economic losses to the goat industry,among them the health of goats is utmost important.The goat population in our country is frequently exposed to ravages of infectious diseases.Amongst,Pneumonia has been noticed as one of the most frequently encountered condition and is responsible for around 28.7% mortality (Kumar and Paliwal,1995).Amongst various pneumonic conditions, Contagious caprine pleuropneumonia(CCPP)is one of the most dreaded disease of goats and belongs to OIE list-B which has a significant constraints owing to its high mortality rate from 15.09 to 100 percent (Iyer*et al.*,1968: Kumar *etal.*, 2011a). CCPP is a disease of major economic importance in Africa,Middle east,Mediterranean Littoral and Asia.The direct losses of the disease result from its high mortality, reduced milk production and meat yield, cost of treatment,control,disease diagnosis and surveillance. In addition to these,there are indirect losses due to imposition of trade restrictions.In 1873, CCPP was first time reported in Algeria by Thomas and known under the local name of *boufrida* because lung was affected in majority of the diseased goats. The first detailed description of classical contagious caprine pleuropneumonia was given by Hutcheon (1881). It all started with the shipment of rams and ewes from Angora to Port Elizebeth in South Africa on 1st Oct,1880.Within a short period of their arrival, the disease was occurred in the imported animals and several hundreds of animals died in the outbreaks.

Etiology

A number of *Mycoplasma* sp. have been reported as cause of Mycoplasmosis in goats in India causing various diseases and are listed (Thiaucourt&Bolske, 1996; Chakraborty *et al.*, 2014; Jain *et al.*, 2015) inTable 1.

Predisposing factors

Respiratorydisease of high severity caused by *M.capricolum*subsp. *capripneumoniae*has been observed in goats and mainly predisposed by– stressful conditions viz., transportation, overcrowding; sudden changes in environment .

Species Affected

Goats are the primary hosts for *M. capripneumoniae,* and the only domesticated animals proven to be affected by this organism. However, cross species transmission to sheep have also been reported by many others. There is also a possibility that this organism might have been involved in an outbreak of acute respiratory disease among goats and sheep in Ethiopia in 2002. Recently, *M. capripneumoniae*was isolated from an outbreak of severe respiratory disease in captive wild goats (*Capra aegagrus*), Nubian ibex (*Capra ibex nubiana*), Laristan mouflon (*Ovisorientalislaristanica*), and gerenuk (*Litocraniuswalleri*). Whether it occurs in other wild ruminants is uncertain.

Species and subspecies of *Mycoplasma*	Host	Predilection site	Disease
M. capricolum subsp. capripneumoniae	Goat (sheep)	Lungs	Contagious caprine pleuropneumonia (CCPP)
M. mycoides subsp. capri	Goat (sheep)	Respiratory tract (joints)	Caprine Pleuropneumonia, Arthritis, Conjunctivitis
M. mycoides subsp. mycoides type LC	Goat and Sheep	Respiratory tract	Pleuropneumonia
M. capricolum subsp. capricolum	Goat, sheep	Joints/resp. tract (udder)	Pleuropneumonia , Mastitis , Arthritis
M. ovipneumoniae	Sheep, goat	Respiratory tract	Pneumonia
M. conjunctivae	Sheep, goat	Eyes	Keratoconjunctivitis
M. agalactiae	Sheep, goat	Udder (joints, eyes)	Mastitis , Arthritis, Keratoconjunctivitis, Pneumonia
M. putrefaciens	Goat (sheep)	Udder (joints)	Mastitis , Arthritis
M. arginini	Ubiquitous	Respiratory tract	None

Epidemiology

Occurrence of Mycoplasmosis in India has been noted from time to time with the report of Walker (1914) being the earliest however some believe that in India outbreaks of CCPP occurred as early as 1889 from Bombay (Verma, 2000).

In India, first record of the existence of CCPP disease was made by Steel (1889) in Khandesh, Bombay followed by several authors in different states of India. The disease still exists in most states of the country. The causative agent of CCPP, *Mycoplasma mycoides*subsp.*capri* was first isolated by Shirlaw in 1949 in India and later on by Uppal and Kumar (1981) and then Singh *et al.*,(1997) from kids and goats. The disease often occurs during the rainy reason and also after cold spells, which is attributed to shedding of *Mycoplasma* by recovered animals after the stress or sudden climatic change. The goats of all ages and either sex are susceptible to infection, however, mortality was found higher among the younger animals than adults.The outbreaks of CCPP have largely been reported in young kids. The investigation of different *Mycoplasma* disease outbreaks are being routinely carried out at this University and screening of clinical samples are being processed with the available facility and the following are the details of samples processed and diagnosed from field disease outbreaks reported in Tamil Nadu state.

Prevalence of Mycoplasmosis in Tamil Nadu:

Period	Sheep		Goat	
	Received	Positive	Received	positive
2011	27	02	40	01
2012	40	02	53	04
2013	112	05	112	14
2014	116	06	118	15
2015	118	08	120	16
2016	120	09	123	18
2017	60	04	50	10
2018	20	01	25	05
Total	613	37	641	83

Economic impacts

Mycoplasma sp. cause severe contagious diseases in small ruminants, with significant economic impact (OIE 2017a).Morbidity causes constraints in livestock management, overburdens with costs of treatment, and imposes restriction on trade or transport. CCPP is severe and highly contagious in naive animals. During outbreaks, goat flocks have morbidity rates up to 100% and mortality rates as high as 80%.

Mode of transmission

Contagious caprine pleuropneumonia is highly contagious. This disease is transmitted during close contact by the inhalation of respiratory droplets. Chronic carriers may exist, but this remains unproven. Some outbreaks have occurred in endemic areas when apparently healthy goats were introduced into flocks.

Incubation Period

The incubation period is commonly six to 10 days. Some experimentally infected goats develop fever as soon as three days after inoculation and respiratory signs as early as five days, but others become ill up to 41 days after exposure.

Clinical signs of Mycoplasmosis

Clinical signs of Mycoplasmosis are highly variable and usually not specific hence confusing. They range from respiratory, genito-reproductive, mastitis, arthritis, conjunctivitis, skeletal, and nervous signs due to involvement of various systems.

Clinical signs can be variable as per subspecies also as contagious caprine pleuropneumonia (CCPP) is caused by *M. capricolum* subspecies *capripneumoniae* (Mccp), atypical pneumonia by *M. ovipneumoniae*, and infectious keratoconjunctivitis by *M. conjunctivae*. Clinically, *Mycoplasma* infections are usually difficult to ascertain as clinical signs resemble other infections.In *Mycoplasma agalactiae* infection, the inflammation of mammary gland is initially caused due to connective tissue involvement in the udder. Ultimately there is development of mastitis (catarrhal/ parenchymatous) that subsequently leads to atrophy and then agalactia. Contagious caprine pleuropneumonia is strictly a respiratory disease. Peracute, acute and chronic forms may be seen in endemic areas. Peracutely affected goats can die within 1 to 3 days with minimal clinical signs. In acute disease, the initial signs are a very high fever (41-43°C [106-109°F]) lethargy and anorexia, followed within 2 to 3 days by coughing and labored respiration. The cough is frequent, violent and productive. In the final stages of disease, the goat may not be able to move and stands with its front legs wide apart, and its neck stiff and extended. Saliva can drip continuously from the mouth, and the animal may grunt or bleat in pain. Frothy nasal discharge and stringy saliva may be seen terminally. Pregnant goats can abort. Acutely affected goats generally die within seven to 10 days. Chronic CCPP is characterized by a chronic cough, nasal discharge and debilitation.

Morbidity and Mortality

CCPP is severe and highly contagious in naive animals. During outbreaks, goat flocks have morbidity rates up to 100% and mortality rates as high as 80%.

Post Mortem Lesions

The lesions of contagious caprine pleuropneumonia are limited to the respiratory system. Acute disease is characterized by unilateral or bilateral pneumonia and serofibrinous pleuritis with straw-coloured fluid in the thorax. On cut surface, the lung is granular with copious straw-coloured exudate. Pea-sized, yellow nodules may be found in the lungs; these nodules are surrounded by areas of congestion. Varying degrees of lung consolidation or necrosis can be seen, and the regional (bronchial) lymph nodes are enlarged. Some long-term survivors have chronic pleuropneumonia or chronic pleuritis, with encapsulation of acute lesions and numerous adhesions to the chest wall. The interlobular septa is not thickened in domesticated goats.

Samples to be collected

From Live animals- nasal swab, conjunctival swab and tracheal swab may be collected in PPLO medium or in NSS.

At necropsy, samples from active lung lesions should be collected for culture and histopathology. These samples should be taken from the interface between consolidated and unconsolidated areas. Samples of pleural fluid, exudates from lung lesions, and regional lymph nodes should also be collected.

Diagnosis

1. Clinical

Contagious caprine pleuropneumonia should be suspected when severe respiratory disease, with a high morbidity and mortality rate, is seen in goats. The typical necropsy lesions aid in diagnosis.

2. Isolation

A definitive diagnosis can be made by isolating *M. capripneumoniae*from lung tissue and/or pleural fluid at necropsy using PPLO broth/ agar

3. Identification:

a) **Microscopic appearance:** This organism has a branching, filamentous morphology in exudates, impression smears or tissue sections examined under the microscope.

b) **Colony morphology:** Colony morphology varies with the medium, the passage level and the age of the culture; although small, irregular colonies may occur in early passage, the classic 'fried egg' colony morphology can be seen in older cultures.

c) **Biochemical testing:** is usually done by digitonin sensitivity, glucose fermentation, sorbitol digestion, arginine decarboxylation, casein and serum digestion for identification of Mycoplasma species.

d) **Serological tests:** Serological tests include complement fixation, Rapid plate agglutination test, latex agglutination, indirecthemagglutination and enzyme linked immunosorbent assays (ELISA).

e) **Polymerase chain reaction (PCR):** assays are used to identify cultures of *M. capripneumoniae*, as well as to identify this organism directly in tissue samples.

Differential diagnosis

The differential diagnosis includes Pasteurellosis and other forms of bacterial pneumonia, *Peste des Petits Ruminants* and caseous lymphadenitis.

Prevention and Control

Outbreaks can be eradicated with quarantine, movement controls, slaughter of infected and exposed animals, and cleaning and disinfection of the premises. Some countries have included vaccination in their eradication procedures. In endemic areas, care should be taken when introducing new animals into the flock. Flock testing, slaughter, and on-site quarantine may be helpful in controlling the spread of disease. Vaccines are of prime importance for the prevention and control of mycoplasmosis by using either locally or widely prevalent strains of the mollicute. Vaccine development for CCPP was attempted in India as early as 1950 (Verma, 2000). Few experimental attempts have been made for the development of caprine pleuropneumonia vaccine for goats. These experimental vaccines against caprine pleuropneumonia have been evaluated from time to time (Singh 1990; Manimaran ,2003; Manimaran*et al.*,2006a;2006b;2015) but its study on larger goat population is still required. At present there is no commercial vaccine available to combat CCPP in India and to prevent morbidity and mortality losses in goats.

Treatment

A wide range of antibiotics have been tried in therapeutic management of mycoplasmosis in sheep and goat under field conditions in India. They include oxytetracycline, enrofloxacin, tylosin, florfenicol, tiamulin and danofloxacin.

Public Health

Humans are not susceptible to infection with *M. capripneumoniae*.

ORAL PRESENTATION

MD–O–01

Detection of *Brucella* species in an organized farm by Polymerase chain reaction

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Brcellosis is a major bacterial zoonosis of global, economic and public health significance which poses a serious threat to the livestock economy. The current study was aimed to detect Brucellosis in an organized farm located in Chintaladevi, Nellore district of Andhra Pradesh. Out of 266 sera samples tested by RBPT and STAT, seven (2.6%) were found positive with STAT titres of 1:80 to 1:320. PCR was applied for 7 blood samples from seropositive animals and 22 serologically negative blood samples for comparative analysis. Out of 29 whole blood samples, seven were found positive. The primer set B4/B5 used in the present study was able to amplify a target sequence of 223bp in a gene that encodes a 31KDa immunogenic OMP of *Brucella* species.

MD–O–02

Peste des petits ruminants (PPR) seroprevalence in large ruminanats of Tamil Nadu

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PPR is an economically important contagious disease of goats and sheep. It is generally considered that cattle alone are naturally infected sub-clinically, although disease and death were recorded in calves experimentally infected with PPRV-infected tissue. During 1995, PPRV was isolated from an outbreak of rinderpest-like disease in buffaloes. With the successful eradication of Rinderpest, global strategy for the control and eradication of PPR is being contemplated. The aim of the present study is to assess PPRV antibodies in cattle and buffaloes sera in Tamil Nadu.

To study the prevalence of PPRV antibodies in the state of Tamil Nadu, six districts were chosen and 240 serum samples were collected from domesticated large ruminants (120 each from cattle and buffaloes). Competitive enzyme linked immune sorbent assay (cELISA) and micro serum neutralization test (mSNT) were employed to screen the presence of antibodies against PPRV. Both the tests showed 8.3% and 15% positivity for PPRV antibodies in selected herds of cattle and buffaloes in the areas without previous history of PPRV disease incidence and PPRV vaccination in goats and sheep reared nearby. Whereas both the tests showed 18.3% and 28.3% positivity for PPRV antibodies in selected herds of cattle and buffaloes in the areas with previous history of PPRV disease incidence and PPRV vaccination in goats and sheep reared nearby under field/farmers holdings, indicating infection of PPRV without clinical signs.

The presence of PPRV antibodies demonstrated that bovines are exposed to PPRV infection and it implied the importance of cattle and buffaloes as subclinical hosts for the virus, besides widespread presence of the disease in goats and sheep. Though the cattle and buffaloes are considered to be the dead end host, vaccination of large ruminants has to be considered, when they are reared in the premises along with sheep and goats for a successful National PPRV control programme.

MD–O–03

Identification of *Staphylococcal species* from Bovine mastitis cases by PCR and MALDI-TOF MS

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A study was undertaken for the identification of *Staphylococcal* isolates obtained from bovine mastitis cases by MALDI-TOF MS. Isolates of *Staphylococcus aureus* (n=28), *S. epidermidis* (n=3), *S. intermedius* (n=4) identified by phenotype and species specific PCR and 25 isolates of coagulase negative *Staphylococcal* species (identified by Coagulase gene PCR and tube coagulase test in a previous study) were analyzed by MALDI-TOF MS to assess the suitability of MALDI-TOF MS for their identification. MALDI-TOF MS is found to be a rapid and reliable tool for the accurate identification of *Staphylococcal* species. The results of MALDI-TOF-MS were in perfect agreement with PCR based identification of *S. aureus*, *S.epidermidis* and *S. intermedius*. Among the 25 coagulase negative staphylococcal isolates 14 were identified as *S. sciuri*, 6 as *S. warneri*, 3 as *S. simulans* and 2 as *S.chromogens*. MALDI-TOF-MS is valuable tool for identification of bacteria in infections like bovine mastitis where multiple etiological agents are responsible, when compared to conventional phenotypic techniques or PCR.

MD–O–04

Isolation and molecular characterization of Mannheimia haemolytica from sheep pneumonia.

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Mannheimia haemolytica is one of the major causative agents of sheep respiratory diseases leading to huge economic losses in sheep industry. In this aspect, a total of 196 pneumonic lung samples were collected from sheep slaughter house and attempted for isolation of M. haemolytica. Preliminary confirmation of the sample was done by amplifying PHSSA (325 bp) and Rpt2 (1022 bp) genes by multiplex PCR which resulted in 15 positives. The positive percentage was 7.6%. Later, PCR positive samples were processed for isolation using spot culture method on Brain heart infusion agar, blood agar, Mac conkey agar and biochemical tests like indole production and lactose fermentation. The pure cultures once again confirmed by conducting multiplex PCR with PHSSA and Rpt2 primers. Out of 15 preliminary positive samples 10 pure cultures were isolated (5.2%). The isolates were tested for antibiotic sensitivity for 14 different antibiotics phenotypically and 7 resistance genes genotypically. 100% sensitivity was noticed in gentamicin, cotrimoxazole, ceftriaxone and resistance to amoxicillin, colistin, and enrofloxacin was noticed. Genotypically, amplification of only tetB gene (40%) was noticed. The virulence genes of leukotoxin (lkt A) which is the major virulence factor identified in 30% of the isolates, with band length of 206bp. Histopathology of infected lung tissue showed bronchopneumonia with infiltration of inflammatory cells.

MD–O–05

Evolutionary conservation of OmpW protein sequence and structure among members of *Pasteurellaceae* including pathogenic *Pasteurella multocida* strains

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The family; *Pasteurellaceae is comprised of more than* 100 species and species-like taxa including 18 genera have been documented till date. Family members act either as specialized commensals or potential pathogens of animals/avians, with lesser survivability in external environment. Several bacterial outer membrane proteins (OMPs) are known to play diverse role in physiology including adoptive response to adverse environmental conditions and contribute for enhanced virulence. *Pasteurella multocida,* a causative agent of multiple infections in animals and avians, is also known to possess several uncharacterized OMPs as virulent factors. OmpW protein, a member of ubiquitous small family of OMPs, characterized well in different Gram-negative bacterial species and attributed its rolein multi-functions such as anti-phagocytosis, osmoregulation, transporter, anchor molecule as well as its utility in developing diagnostic assay and subunit vaccine.

In the present study, hitherto uncharacterized *OmpW* gene encoding for OmpW protein of *P. multocida* strains (n=7) have been cloned, sequenced and comparatively analyzed with existing OmpW sequences/homologues (n=98) by multiple bioinformatics tools. A comparative phylogenetic, multiple sequence alignment and structural features of OmpW protein of *P. multocida* with that of homologues in other bacterial species revealed the presence of evolutionarily conserved residues forming a pore like structure in outer membrane. Structurally, OmpW protein of *P. multocida* had eight strands (β1-β8) with four external loops (L1-L4). Analysis of transmembrane pore (hydrophobic) features as well as predicted functionality by docking studies using fumerate molecule indicated similarity with that of OmpW protein of *E. coli*. Further, the study indicated the potential possibilities to target OmpW gene/protein of *P. multocida* either for development of alternative specific diagnostics or vaccines against pasteurellosis in livestock.

MD–O–06

Molecular diagnosis of *Klebsiella pneumoniae* from cases of neonatal calf pneumonia in dromedary camels.

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In an organized dromedary camel farm mortalities due to pneumonia were reported in total 6 neonatal camel calves aged between 5 to 10 days during the winter season. The affected camels showed symptoms of weakness, fever, respiratory distress and sudden mortality. Grossly the lesions were focal to diffuse congestion and consolidation of lungs and histopathology revealed thickened alveolar and bronchial wall, mononuclear cellular infiltration and congestion of blood vessels. The bacterial culture in blood agar from lung tissue revealed isolation of *Klebsiella pneumoniae* in 4 cases and isolation of *E. coli* from 2 cases. The genomic DNA was extracted from lung tissue as well as bacterial culture and PCR reaction was performed for amplification of 16S–23S rDNA internal transcribed spacer (ITS) sequence specific for *K. pneumoniae*. A 260 bp DNA fragment was amplified from ITS of *K. pneumoniae* from lung tissue and bacterial culture of all the 4 cases. These findings confirmed the role of *K. pneumoniae* as an important etiological agent from cases of neonatal calf pneumonia in dromedary camels.

MD–O–07

PUBLIC SERVICE DELIVERY SURVEY IN REFERENCE TO FMD-CONTROL PROGRAMME IN MATHURA, UTTAR PRADESH

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Public sector organizations or Government policies face common challenges in implementation of programme in developing countries. Appropriate model for public service delivery should be chosen focusing on the benefits for citizens and increasing awareness taking into account the constraints in terms of structure, capacity of people, public sector, culture and finances. To control FMD, Government of India initiated FMD-Control Programme in 2003-04. Uttar Pradesh is having 23rd round covering all districts. To evaluate effectiveness of service delivery mechanism of FMD-CP in UP, present study was undertaken in Mathura. It involved development of a structured questionnaire for beneficiary as well as service provider. Livestock owner (n=222) from 27 villages and Veterinary Officer (n=26) participated in the survey. Data received in form of questionnaires was processed and analyzed. The success of the FMD-CP in Uttar Pradesh was compared to the neighbouring states of Rajasthan, Haryana and Punjab. It was found that majority livestock owner is aware about the disease, its control programme and free vaccination available. Veterinary Officers are playing major role in dissemination of information. Vaccine has been found to be safe, protective and with minimum side effects. FMD-CP was found to be successful in states like Haryana and Punjab with a good seroconversion in vaccinated animals and near zero occurrence of disease, but in UP seroconversion is poor and disease has been reported to occur regularly. Therefore, to improve FMD-CP in a large state UP, it was concluded that design of the policy is optimum but there is a need for transformation in implementation practices of the policy. Extensive information dissemination is required using TV, radio, newspaper, goshthi, seminar etc. Release of adequate budget and timely intervention with sufficient staff and necessary items will help in vaccination coverage. With private players in the vaccine manufacturing, it is important to have quality control of vaccine periodically. Issue of vaccination certificate to the livestock owner will help in effective monitoring of the programme. An independent audit system for the implementation agency (state Animal Husbandry Department) and screening agency (ICAR-DFMD) should be undertaken to remove any discrepancy.

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MD–O–08

Evaluation of virus like particle vaccine against a fatal viral disease of dogs in target species

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Canine Parvoviral enteritis is one of the fatal diseases of dogs and many other carnivores. The disease is prevalent worldwide and the etiological agent of the disease is canine parvovirus (CPV). CPV causes a highly contagious disease in dogs and it is characterized by hemorrhagic gastroenteritis, vomiting and high temperature. The mortality rate is very high in young pups. Live modified vaccines are being used widely to control the disease. Interference from maternal antibodies and less than the protective level of antibody titer were the common factors responsible for vaccine failures. Virus Like Particle (VLP) based vaccines are more immunogenic and much safer for administration. We have developed a virus like particle (VLP) based vaccine for CPV using baculovirus expression vector system (BEVS). The CPV-VLP production was optimized in small scale fermenter and the VLP was purified and characterized by performing 1. ELISA reactivity with specific monoclonal, 2. Immunoblot, 3. MALDI-TOF, 4. Circular Dichroism analysis, 5. Fluorescence spectra and 6. TEM analysis. The CPV-VLP based vaccine was further administered at different doses in beagle dogs having various levels of maternal antibodies. Our results indicated that there is an improved serum antibody response in vaccinated animals and we have found that higher doses of CPV-VLP vaccinated groups could able to overcome the interference of maternal antibodies.

MD–O–09

Multilocus variable number tandem repeat analysis (MLVA) of *Dichelobacter nodosus* isolates the causative of Ovine footrot

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Ovine Footrot is a contagious, debilitating hoof disease caused by *Dichelobacter nodosus* (Beveridge, 1941) an obligate anaerobe. The disease is primarily confined to the epidermal components of the interdigital skin and the horn of the hoof subsequently leads to progressive necrosis of intermediate layers of the epidermis and separation of horn from the soft tissues of the hoof with varying degrees of lameness. MLVA analysis of five *D.nodosus* isolates targeting DNTR-02, DNTR-10 and DNTR-19 genes resolved into five different MLVA types viz., MLVA type 4(MBNR-B), 9 (KDP-I), 28 (Mon-'C'), 36(V.pet'I') , and 42 (Che 'I'). The simpson's diversity index for allelic variation was found to be 0.960, 0.917 and 0.800 for DNTR-02, DNTR-10 and DNTR-19. Minimum spanning tree showing clustering of *D.nodosus* isolates at level -2 double locus variants revealed the presence of MLVA types of isolates (4, 9, 28, 36 and 42) at the terminal nodes and J&K and Australian isolates in the internal nodes suggest that footrot was present in Australia and J&K prior to the AP, Telangana and Tamilnadu. The results also suggest that Indian isolates were closely related with Australian isolates and this might be due to importation of sheep from Australia.

MD–O–10

Appropriateness of molecular tools for detection of cancers in dogs

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Cancer is the leading cause of death in dogs. Traditional diagnostic methods like X-ray, ultrasonography, CT-scan, blood test etc. detect only presence and location of neoplastic conditions, but their confirmation relied upon histopathological or immunocytochemical examination. Histopathological examination is correct in most cases but may be doubtful in many cases, especially if neoplastic condition is of high grade. This limitation could be overcome by molecular diagnostic tools. In the current study, prevalence of Mast Cell Tumor (MCT), Lymphoma and Chronic Myeloid Leukaemia (CML) were recorded making use of different molecular assays. Polymerase Chain Reaction for Antigen Receptor Rearrangement (PARR) assay for detection of lymphoma, PCR for detection of mutation at juxtamembrane domain of c-kit gene in mast cell tumors and a two-step nested PCR for detection of bcr-abl fusion in CML were used. Out of a total 123 blood samples, two blood samples were found positive for T cell lymphoma using PARR assay. Out of 21 tissue samples, one was positive for MCT. In all three out of 10 blood samples processed from suspected dogs were found positive for CML. These molecular tools were able to detect insiduos cancerous conditions in dogs which were otherwise could not be detected clinically or using imaging or cytology techniques.

MD–O–11

Antibiogram of *Pasteurella multocida* isolates of Buffalo.

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Pasteurella multocida is a significant bacterial pathogen responsible for Haemorrhagic Septicemia in Cattle and Buffalo. Phenotypic antibiotic resistance pattern of 16 *P. multocida* isolates were tested for their antibiotic sensitivity pattern by Disc diffusion method against commonly used antibiotics and the antibiogram of these isolates revealed 100% sensitivity to enrofloxacin, gentamicin, tetracycline, ampicillin, amoxicillin, ceftriaxone, Cephalothin, Piperacillin+tazobactam and penicillin-G, 93.75% to chloramphenicol, ciprofloxacin, streptomycin, cotrimoxazole and amoxicillin + clavulanic acid, followed by meropenem, sulphafurazole, aztreonam, nalidixic acid. The isolates found highly resistant to lincomycin (100%), clindamycin (93.75%), followed by sulfadiazine (25%). The genotypic antibiotic resistance pattern of *P. multocida* isolates was done by PCR amplification of antibiotic resistance genes *i.e. sul1, sul2, catA1, strA and strB* resulted *sul2* gene in 11 isolates, whereas 5 isolates had both *sul1* and *sul2* genes and none of the isolates harbored *catA1, strA* and *strB* genes and compared the resistance patterns both phenotypically and genotypically. The results showed Cap B isolates had 100% phenotypic resistance to sulfadiazine and sulphafurazole and also showed *sul 1 and sul 2 genes* but Cap A isolates showed ambiguity.

MD–O–12

Selection of peptide ligands against Pasteurella multocida using iterative subtractive selection

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Haemorrhagic Septicaemia is an acute fatal septicaemic disease of cattle and buffaloes and is mostly caused by serotype B: 2 in India and of great economic importance mainly due to the high mortality in susceptible populations. Despite its economic importance, there is no specific field level diagnosis of this disease. The pathogenicity of the organism is associated with various virulence factors such as the. They facilitate the colonisation and invasion of the host tissue. Antigens-comprising surface structures such as capsule, lipopolysaccharides, adhesins, outer membrane proteins etc. could be a target for both therapeutics as well as diagnostics. Keeping this in mind, the current study was carried out to select ligands using phage display peptide library against major structural components of the *Pasteurella multocida* and characterized them by using phage ELISA. Phage display library was tittered and concentration was found to be 1.8×10^{11} pfu/ml. Amplification of stock library was done to the concentration of $2.1\text{x}10^{12}$ pfu/100 µl. These amplified phages were then subjected to the alternate selection methodology of panning by suspension method in which alternate rounds of positive and negative selection were done. In starting of selection $2.1\text{x}10^{12}$ pfu/100 µl were employed and after 3 round of alternate positive and negative selection 4.8×10^{11} pfu/100 µl were recovered. Out of these 48 phages, 16 phages were selected for Indirect phage ELISA and 7 were found to highly specific to P. multocida and did not cross-react with *Haemophillus* spp. and *Actinobacillus* spp.

SESSION – III

FOOD BIOTECHNOLOGY AND BIOINFORMATICS

LEAD PAPERS

FBB–L–01

Host-microbe interactions and pathogen exclusion mediated by the surface proteins of probiotic lactic acid bacteria

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Background

Gastrointestinal infections are rampant in human and animals and cause a debilitating impact on the health of the infected subject. Gut infection in the children or animal calf has a lifelong impact on their growth and health. Gut infections have a high economic burden on the economy of a country. Given the poor living and hygiene conditions in the third world countries, the impact is much more stronger on the subjects' health and economy of the country. The first line of the treatment involves antibiotics. However, because of the over usage of antibiotics, development of resistance against them in the pathogens is becoming even a more serious concern in the medical and veterinary sciences. Therefore, strategies other than antibiotics are the need of the hour [1]. To manifest the disease, the first step in the infection is the adhesion and colonization of the hosts' gastointestinal tract (GIT) and is pivotal for sustenance of microbes, including pathogens and commensal community. Probiotics therapy has been used successfully against some gut pathogens. The efficient adhesion of probiotics in the GIT can be exploited to prevent or compete with the pathogen binding. In recent times, microbial interference therapy involving whole cell probiotics has shown promise[2], but it suffers with certain limitations or risks. The adhesion property of probiotics is mediated through their surface receptors[3,4]. We studied the potential of surface receptors/proteins of the probiotics through which they interact with the host cells in preventing pathogen adhesion in the GIT. Mucus binding proteins (Mub) are a group of surface adhesins found in gut-inhabiting microbes like Lactobacillus species. These proteins have multiple Mub domains occurring in tandem. Similarly, fibronectin-binding protein (Fbp) is also involved in binding with GIT cell lining. These proteins are implicated in adhesion but the underlying mechanism is not well understood. We expressed truncated recombinant Mub domains of *Lactobacillus plantarum*[5] and Fbp of *L. acidophilus* NCFM[6] to check their efficacy in preventing the Enterotoxigenic *E. coli* (ETEC) adhesion to host mucosa.

Objective(s)

To understand the host-microbial interaction and develop a strategy to break the host-pathogen cross-talk by using the probiotics surface proteins.

Methods

Two domains (domain No. 5 and 6, here in called rec-Mub) out of the six tandemly arranged domains present in a large Mub protein (2200 residues) of *Lactobacillus plantarum* Lp9 were cloned and expressed in *E. coli.* The purified fusion protein rec-MBP-Mub (*mub* gene tagged with maltose binding protein gene - *mbp*), which helped in the affinity purification of rec-MBP-Mub protein and where MBP also served as a detection system, showed good adhesion to human intestinal tissue sections[5]. We studied adhesion of rec-Mub to the host mucosa components by using different substrata and solution conditions. The potential of rec-Mub to preclude ETEC binding with cultured human intestinal cells was evaluated by pre-incubating enterocytes with rec-Mub under *in vitro* condition. The adhesion between rec-Mub and porcine gastric mucin (PGM) was determined by using the indirect ELISA method. Few other binding partners of Mubs5s6 were identified by combining the pull-down assay and LC-MS/MS.

The recombinant Slp was also expressed in the form of inclusion bodies and refolded to biologically active form[6]. The rec-Fbp binding with human intestinal tissues and cell lines was studied by using

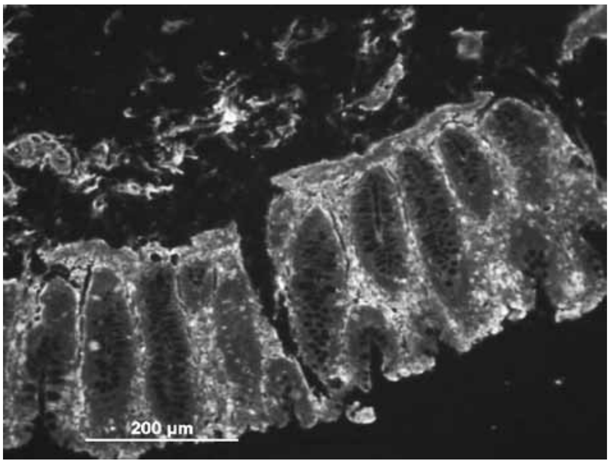

Figure 1: Immunofluorescence (phycoerythrin conjugated with anti-MBP protein) showing binding of rec-MBP-Mub fusion protein to human intestinal tissue sections.

immunofluorescence. The inhibition of pathogen binding was examined by allowing binding of the recombinant Mub or Fbp to enteric cell lines Caco-2 or HT-29 cells followed by challenging with pathogens.

Results

The rec-Mub protein showed binding to different substrata including PGM, human intestinal tissue sections (**Figure 1**), rat intestinal mucus and human intestinal cell lines: Caco-2 and HT-29. Upon, pre-incubation of the Mub protein (25-400 μg) there was a dose-dependent proportional decrease (52-81%) in the pathogen adhesion to HT-29 and Caco-2 enterocytes. The source probiotic *L. plantarum* Lp9 was only able to inhibit ETEC binding by 36-49% to these intestinal cell lines. The binding of rec-Mub protein with intestinal tissues was decreased at low pH, while it was unaffected at neutral and alkaline pH. The binding of Mub to PGM was reduced by 36% (45 min exposure) and 11% (120 min exposure) over treatment with simulated gastric fluid and simulated intestinal fluid, respectively. We also identified binding partners like laminin, Hsp90, Calcium and Glucose to rec-Mub protein. These partners might be involved in mediating host-microbial binding. The rec-Mubs protein was successfully encapsulated in 1.75% Chitosan - 2% TPP microspheres with encapsulation efficiency of 65% and controlled release over a period of 20 hours[7].

The rec-Fbp protein also showed strong binding with the human intestinal tissues and inhibited binding of pathogen (ETEC and *Salmonella* Typhimurium) to Caco-2 and HT-29 cell lines by around 60% (unpublished results).

Conclusion

The rec-Mub and rec-Fbp protein could inhibited binding of pathogens to human enetrocytes cell lines more efficiently than their source probiotic whole cell. The recombinant form of these proteins could be used as prophylaxis agents to prevent enteric infection after more stability and safety studies.

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FBB–L–02

RECENT ADVANCES ON ANIMAL FEED BIOTECHNOLOGY

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INTRODUCTION

Animal feed biotechnology will provide new and unprecedented opportunities to accelerate the productivity and nutritional base of livestock through the development of new and improved feedstuffs as well as in microbiology related to food and bioremediation. The technology utilizes scientific knowledge on genetics of organism to improve a certain trait of organism for particular purpose. The applications covered not only modification of microorganisms but also for efficient feed utilization. Several areas of application were presented which includes feed additive production (Enzymes, probiotics , prebiotics, immune supplements etc), feed supplement (bypass protein, bypass fat), modification of rumen micro organisms, newer trait of various animals include chicken, pig, fish, and new fodder crops. Addition of vaccine and antibodies in feeds can also be used to protect the animals from disease. The use of biotechnological products is relatively well established in the feed industry and shows considerable potential for further growth. The present article is an attempt to focus the role of biotechnology in animal feed to improve the nutritional base of livestock which ultimately will reflect in the production.

PROTECTION OF PROTEINS, AMINO ACID AND FAT

Rumen degrades the protein to form ammonia, then the ruminal microbes use this non proteinious nitrogen to synthesize the microbial protein. To increase the efficient utilization of degradable protein, it should be protected from ruminal degradation through chemical treatments such as formaldehyde and physical treatments like heat treatments and extrusion cooking (Kumar et al 1994). Some researcher have reported 33% reduction in protein degradability by formaldehyde treatment of groundnut cake. The use of crystalline amino acids produced through industrial fermentation is extensive (Bercovici and Fuller, 1995) and has resulted in improved diet formulation and lower feed cost. Fat can be protected by using the saponification of fats with calcium salt. Feeding calcium soaps of fatty acids, which are inert in rumen can enhance dietary energy density without compromising the activity of rumen miocroflora in negative energy balance animals (Thakur and Shelke, 2010).

DIETARY AMINO ACIDS

Essential amino acids are added as supplement to the feed to get a balanced amino acid profile. The new trend is to formulate diets on digestible amino acid levels for reducing the requirement of protein. Lysine is produced by microbial fermentation and methionine chemically synthesized to add as supplement. Genetically enhanced micro-organisms are being used to produce threonine and tryptophan on a commercial basis. Non-traditional applications of amino acids may involve the use of arginine (Hurson et al., 1995) and aspartic acids (Kuhara et all., 1991) as potent stimulants of pituitary somatotropin release and enhance growth and carcass quality. Using all these amino acids it is possible to lower dietary crude protein level by 2 – 3 %, which is substantial saving for the farmer.

USE OF ENZYMES

There is an increasing trend of use of enzymes to enhance the feed utilization. Enzymes are used to remove compounds naturally present in feed grains and forages which are potentially harmful to animals or which interfere with their nutrition. It can also be helpful by reducing the methane production thus help in reducing the carbon footprint most of enzymes. They are cocktail of various enzymes (pentosanase, pectinase and agalactosidase activity and phytase) of bacterial or fungal origin. Enzymes can be used for removal of antinutritional factors (e.g 13glucans and arabino-xylans in barley grain cell wall), increasing the digestibility of nutrients (e.g, phytate phosphorus in grains) and non starch polysaccharides (e.g xylose and arabinose in plants). The enzyme phytase is added to feed to improve digestion of phytate and reduce the need for phosphorus supplements. When grain like corn is fed to pigs and chickens, phytate inhibits the animals' absorption of phosphorus. Producers must then use phosphorus supplements, which increase the amount of phosphorus in the manure. Using phytase prevents the build-up of phosphorus in the environment. An enzyme called beta-glucanase is added to the diets of broiler chickens and piglets. Grains such as barley and oats contain sticky carbohydrates that make the cereals hard to digest. Beta-glucanase reduces this stickiness, allowing pigs and poultry to properly digest their feed. Bhatt et al (1991), reported the improvement in weight gain and feed efficiency with supplementation of enzymes such as cellulose and hemicelluloses in diet.

The benefits of enzyme supplementation of ruminants diets is variable, probably because of complex interactions due to the presence of the rumen fermentation system and the much greater variability in the quality of the feedstuffs, particularly of forages and silage.

Feed enzyme supplementation has good potential for broader application, which largely depends on development of new enzymes, better identification of the optimal conditions for feed processing including physicochemical interaction and identification of the optimal condition for use in animals. Feed enzymes also have considerable potentials to improve the availability of nutrients from by-products, such as rice bran, which as an important source of livestock feed in developing nations.

PREBIOTICS AND PROBIOTICS

Prebiotics are some oligosaccharide like fructo-, gluco- and galacto-oligo saccharides resist attack by the digestive enzymes of animals and thus are not metabolized directly by the host and act as bed for growth of beneficial microbes. Probiotics are live microbial feed supplements which beneficially affect the host animal by improving the intestinal microbial balance(Madan, 2005). Manipulation of microflora in the intestine through the use of prebiotics and probiotics represents an additional opportunity for the improvement of nutrient digestion, disease resistance and health (Kelly et al. ,1994, Salminen et al.,1998). Prebiotics may be defined as compounds, other than a dietary nutrient, that modify and balance the microbial flora, promote the growth of beneficial bacteria and thus provide a healthier intestinal environment for a better absorption of nutrients. The composition of the intestinal microbial population and competitive exclusion of pathogens has progressively been recognized as an significant factor impacting on health and growth performance. The shift in microbial populations as a result of pre- and pro- biotic treatments then leads to a reduction in the proliferation and attachment of pathogenic organisms and reduces the incidence of disease. Generally, pre- and pro- biotic products have provided inconsistent results, and research to better define optimal feed processing and application in animals is ongoing. The effects appear greatest in young fast growing animals during specific periods when microbial flora is subject to large change, such as after weaning, and diminish with age. These age effect is consistent with the capacity of the normal gut flora to resist change as the animal grows.

ADDITION OF VACCINES OR ANTIBODIES IN FEEDS

Nowadays many feeds and fodder are from crop plants that have been modified for characteristics such as disease or pest resistance and their nutritive value remain unaffected. Secondly plants are used as bioreactors for the production of recombinant biopharmaceuticals like cytokines, hormones, monoclonal antibodies, bulk enzymes and vaccines (Miele, 1997).

METABOLIC MODIFIERS

Metabolic modifiers like recombinant bovine somatotropin (rBST) have been used to increase efficiency of production such as weight gain or milk yield (per feed unit), improved carcass composition (meat fat ratio). In developed countries its use increases about 10-15% of milk yield. Similarly researchers have also developed porcine somatotropin that increases muscle growth and reduces body fat deposition resulting in pigs that are leaner and of greater market value.

GENETIC MANIPULATION OF MICROBES

The rumen microbes can be altered genetically to increase their cellulolytic ability and reduction in methanogenesis to improve the overall utilization of feed and fodder. This can be done to eliminate the antinutritional factors in feeds and also increase the essential amino acids specially limiting amino acids synthesis by rumen microbes. Attempts are being made to introduce the lignin breakdown property into ruminal microbes. Depolarization of lignin by ligninase enzymes which is produced by the soft-rot fungus (*Phanerochaetye chrysosporium*) (Tien and kirk, 1983) can be useful for the animals. Efficiency and stability of lignase gene has been modified by recombinant DNA technology (Tien and Tu, 1987)

Toxin Binders

Present day methods are generally the use of organic acid sand their salts like propionic acid or adsorbents like bentonites, zeolites, hydroxyl aluminosilicates. In the future, biotechnology based products like microbes, herbal extracts or esterified glucomannan could be used. Aqua extracts of garlic, onion, turmeric, neem have been shown to exert antifungal activity or inhibit aflatoxin production.

Transgenic plant and animal for improved nutrition and metabolism:

Researchers are interested in taking the traits which can improve the sustainability and efficiency of the entire production system from crop agronomy to animal production to meat processing. Typical examples are incorporation of phytase in crops and development of hulless grain with improved processing characteristics and feeding value both of reduce the nutrient density of animal waste. Edible vaccines, antibodies, enzymes and hormones can also be incorporated in plants that could influence gut function. Silage is which has been fermented by naturally occurring bacteria to enhance its nutritional value. Microbial inoculants are now used to improve the formation of silage, but there is a possibility that genetically modified species could be used to further improve the process.

Transgenic introduction of metabolic pathways may remove inherent nutritional and metabolic limitation, leading to substantial improvement in feed utilization efficiency.In ruminant metabolic efficiency can be improved through transgenic introduction of metabolic pathway for converting acetate to glucose- particularly when forage based diet are fed. Ward and Nancarrow(1992) are targetting sheep rumen epithelium fot the transgenic expression of enzymes serine acetyl transferase and o-acetyl serine sulphhydrylage. this pathyway enables de novo synthesis of cysteine from inorganic sulpher and removes a nutritional limitation to wool growth .

CONCLUSIONS

In developing countries, use of biotechnology in animal production is limited to some like conservation, animal improvement, healthcare (diagnosis and control of diseases) and increase supply of feed resources. By the adoption of biotechnology the animal owners, livestock entrepreneurs will be benefitted. However we have address some issues like political will, infrastructures funds and trained human resource.

So, this is the time, when investment in biotechnology and animal nutrition is important for sustainability of human and animals, food security, rural health and wealth creation and for upliftment of poor people living in the villages.

Finally, the use of biotechnology in animal production should be mostly beneficial for humanity. In many cases, biotechnology is not accepted because people do not understand how it works and what is really at stake. The public needs to be educated on the reality of biotechnology and be informed about the positive and negative of any given application of biotechnology. On that basis, people can make an educated choice on whether or not they can accept it.

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FBB–L–03

Application of Proteomic Technologies for Understanding Meat Quality and Ensuring Safety

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We are now in an exciting period where many new opportunities are presented to researchers through the application of genomics, proteomics and other 'omic' approaches. The great progress in biotechnology in recent years has resulted in the development of new scientific research areas such as genomics and proteomics, which are used to study the complex patterns of gene and protein expression in cells and tissues. Ability to simultaneously analyze hundreds or thousands of genetic polymorphisms (genomics), transcripts (transcriptomics), proteins (proteomics) and metabolites (metabolomics) on dedicated arrays or with specific tools have increased our knowledge of the molecular organization of living organisms. These tools have been implemented in recent years to reveal genes, proteins or metabolites whose expression level or abundance is associated with a phenotype of interest such as the quality of the meat.

After genomics, proteomics is considered the next step in the study of biological systems. Proteomics tools consist of 2-Dimensional gel electrophoresis (2-DE), mass spectrometry (MS) and bioinformatics. Proteomics permits visualisation of the protein content of the cell under varying conditions, combining powerful separation techniques with highly sensitive analytical mass spectrometry. Proteomic analyses describe the identity, relative quantity, and state of proteins in a cell, under a specific set of conditions (Vaidyanathan and Goodacre, 2003). All the proteomic studies require protein separation and purification of crude sample before further analysis and characterization.

Protein separation

A. Gel-based separation

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has long been the method of choice for resolving proteins based on molecular weight for a variety of biochemical analyses (Laemli, 1970). Evidence from the available database (http://www.expasy.ch) suggests that 2-DE is the most widely used tool for large scale proteomics. Established in the mid 1970s, modern 2DE technology make use of first dimension, isoelctric focussing (IEF) using highly reproducible immobilized pH gradient (IPG) strips. In 1st dimension (IEF), proteins are separated based on their charge followed by separation based their molecular weight using SDS-PAGE (2nd dimension). The DIGE (Differential gel electrophoresis) technology has more recently been used for direct quantification of abundance changes on a global scale without interference from gel-to-gel variation. This is done using spectrally resolvable MW and charge-matched fluorescent dyes (Cy2, Cy3 and Cy5) to pre-label protein samples which are then multiplexed onto 2D gels. These dyes offer sub-nanogram detection limits. Lametsch and Bendixen (2001) demonstrated that when optimal extraction, focusing, and staining conditions were used, 1000 well-separated individual protein spots can be separated by 2-DE in each gel, as estimated by computer-assisted image analysis.

B. Liquid chromatography mass spectrometry (LC/MS) based separation

Liquid chromatography coupled with mass spectrometry (LC/MS) based approach offer greater sensitivity than is typically offered by the protein-staining detection limits from gel-based strategies. Here, protein identification is performed at the level of peptide fragmentation pattern acquired during tandem MS (LC-MS/MS), and which are indicative of amino acid sequence (Wolters et al., 2001).

Protein identification by mass spectrometry

With the rapid progression of many ongoing and completed genome sequencing projects, there is a growing demand for rapid and reliable identification of proteins. Mass spectrometry is a powerful technique for the identification of proteins at nanogram quantities. Proteomic samples can be exceedingly complex - a proteolytic digest of a simple cell lysate can contain several hundred thousand peptides. Many proteomic strategies contain protein identification as a major component, and this is routinely performed using MS followed by a statistical comparison of the mass spectral data with theoretical data generated from protein sequence databases which are generated from genomic sequences (Friedman, 2011).

The most commonly used method for identification of proteins from 2-DE is peptide-mass fingerprinting by matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS). This technology is sensitive, allowing identification of femtomole quantities of proteins and peptides, in a high-throughput mode. The MS produces a data characteristic of individual proteins, usually at the level of aminoacid sequence or peptides that are generated after digestion with a site-specific protease (Henzel et al., 1993). Other commonly used mass spectrometer include electrospray-ionisation triple quadrupole (ESI-QTOF). Powerful bioinformatics algorithms can then be applied to search databases that match these experimentally derived mass spectral signatures (Eng et al., 1994).

Bioinformatics

The use of computer science, mathematics, and information theory to model and analyze biological systems, especially systems involving genetic material is called as bioinformatics. Comprehensive information regarding complete and ongoing genome projects around the world can be obtained from ‘genome online database (GOLD)’ (http://wit.integratedgenomics.com/GOLD/). The ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) is dedicated to the analysis of protein sequences and structure as well as 2-D PAGE analysis data (http://us.expasy.org/ch2d/2d-index.html). Information on biologically significant protein domains, patterns and profiles that help to identify the protein family a sequence belongs to can be obtained from the PROSITE database (http://ca.expasy.org/prosite/).

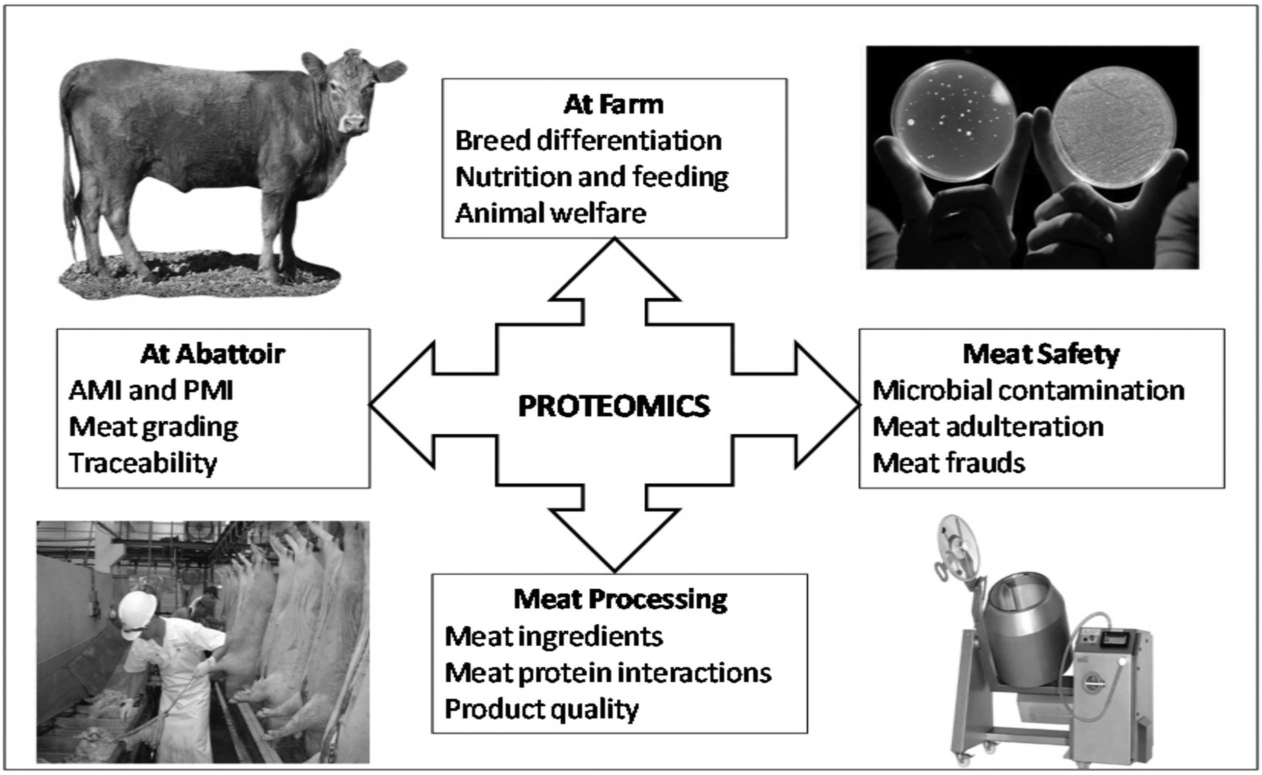


Fig. 1 Schematic representation of the use of proteomics in meat science

The Munich Information centre for protein sequence (MIPS), hosted by the Institute for Bioinformatics (IBI) supports and maintains generic protein databases (http://mips.gsf.de/).

Application of proteomics in meat science research

Modern proteomic technologies for understanding muscle biology have been successfully used for a series of investigations including mapping of muscle proteins, muscle disorders, muscle physiology, conversion of muscle to meat, understanding meat colour and texture, meat speciation, sensorial and technological meat quality traits etc. (Figure 1). Proteomics and Meat Quality Laboratory at ICAR-NRC on Meat has been working towards answering complex questions relating to meat colour and texture, detection of meat adulteration, identification of peptide biomarkers, understanding muscle food quality etc. using state-of the art proteomic approaches. Knowledge gained from these approaches are beneficial in defining and optimising management systems for quality, providing assurance of meat quality and safety and in tailoring quality to suit market needs.

Proteomic characterization of meat colour

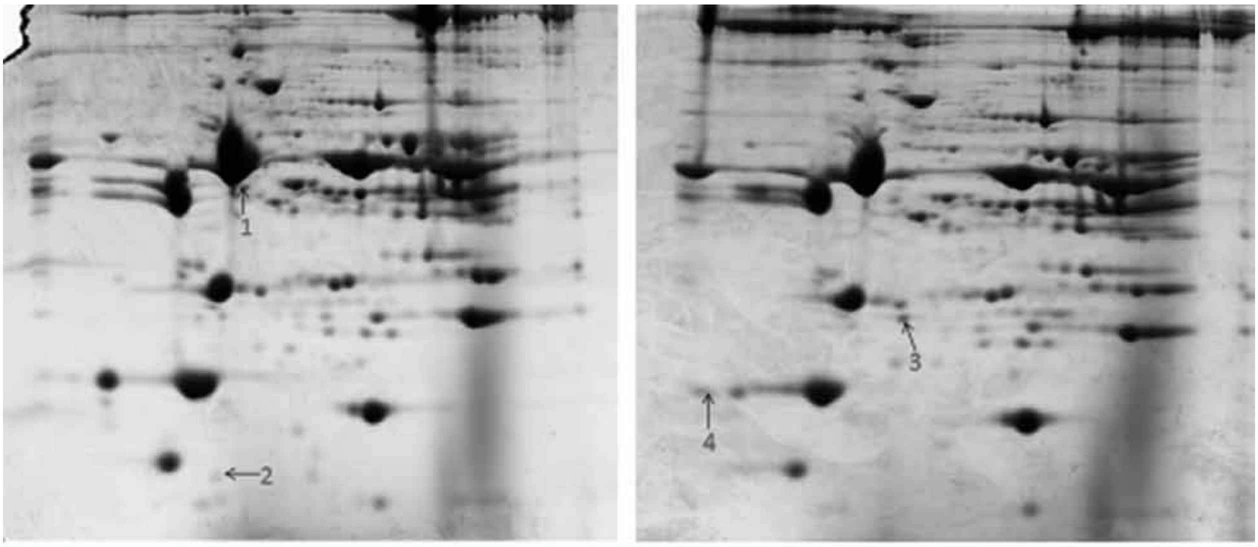
Myoglobin (Mb) is a sarcoplasmic heme protein primarily responsible for meat color and its chemistry is species specific. 4-hydroxy-2-nonenal (HNE) is a cytotoxic lipid derived aldehyde detected in meat and was reported to covalently adduct with nucleophilic histidine residues of Mb and predispose it to greater oxidation (Faustman et al., 2000). We characterized the Mb extracted from water buffalo and goat cardiac muscles using two-dimensional gel electrophoresis (2DE), OFFGEL electrophoresis and mass spectrometry (MS). Purified buffalo and goat Mb samples revealed a molecular mass of 17,043.6 and 16,899.9 Daltons, respectively. The 2DE analysis exhibited 65 (sarcoplasmic protein extract) and 6 (pure Mb) differentially expressed protein spots between buffalo and goat samples. In-vitro incubation of HNE with bright red buffalo and goat oxymyoglobin's at pH 7.4 and 37 °C resulted in pronounced oxidation and formation of brown metmyoglobin. MALDI-TOF MS analysis of Mb-HNE reaction mix revealed covalent binding (via Michael addition) of 3 and 5 molecules of HNE with buffalo and goat Oxy-Mb's, respectively. ESI-QTOF MS/MS identified seven and nine histidine (HIS) residues of Mb that were readily adducted by HNE in buffalo and goat, respectively (Naveena et al., 2016).

Understanding tenderness variability and ageing changes

Proteomic tools were extensively used to unravel the meat texture and variation in meat quality from different meat animal species. Our laboratory at NRC on Meat, Hyderabad has characterized the biochemical basis for understanding the variation between different muscles, age group and ageing periods. Our study on characterizing proteome profile of *Longissimus dorsi* muscle between buffaloes (*Bubalus bubalis*) of different age groups (young vs. old) has revealed higher myofibrillar and total protein extractability, muscle fibre diameter, and Warner-Bratzler shear force (WBSF) values in old buffalo meat relative to meat from young buffaloes. Proteomic characterization using two-dimensional gel electrophoresis (2DE) found 93 differentially expressed proteins between old and young buffalo meat. Proteome analysis using 2DE revealed 191 and 95 differentially expressed protein spots after 6 days of ageing in young and old buffalo meat, respectively. The MALDI-TOF/TOF analysis of selected gel spots helped in identifying molecular markers of tenderness mainly consisting of structural proteins (Kiran et al., 2016). In another study we have unravelled the variation in meat quality between tender (*Psoas major*, PM) and less tender (*Longissimus lumborum*, LL) muscles of Indian water buffaloes (*Bubalus bubalis*). Higher muscle fibre diameter and Warner-Bratzler shear force was observed in LL, whereas higher water holding capacity and myofibrillar protein extractability was observed in PM. Proteome analysis using 2-DE revealed 123 differentially abundant proteins in PM and LL (Figure 2). The MALDI TOF-TOF MS analysis of selected protein spots from LL and PM with significant differences identified the proteins mainly consisting of Calcium transporting ATPase (Kiran et al., 2015).

Authentication of meat species using proteomic technologies

Proteomic-based method by utilizing the



***Longissimus lumborum* (LL)**   ***Psoas major* (PM)**

Fig. 2 Total proteins from LL and PM muscles of water buffaloes separated through 2-dimensional gel electrophoresis

species-specific peptide biomarkers capable of providing information about the meat species has been reported by Sentandreu et al., (2010) and few other researchers. We developed the proteomic-based technology using in-gel (two-dimensional gel electrophoresis, 2DE) and OFFGEL-electrophoresis for authentication of meat species from three closely related ruminant species viz, water buffalo, sheep and goat in both raw and cooked conditions. The MALDI-TOF/TOF MS analysis of proteins separated using 2DE or OFFGEL electrophoresis delineated species-specific peptide biomarkers derived from myosin light chain 1 and 2 (MLC1 and MLC2) of buffalo, sheep and goat meat mix in different proportions that were found stable to resist thermal processing. The 2DE and tandem mass spectrometry based in-gel method can detect up to 1.0 per cent substitution of sheep and goat meat in buffalo meat, whereas OFFGEL electrophoresis and tandem MS approach can detect even up to 0.1 per cent substitution of sheep and goat meat in buffalo meat (Figure 3). The effectiveness of OFFGEL electrophoresis over in-gel based method is its efficiency to concentrate and enrich the low abundant proteins mainly originating from myosin light chain 1 and 2. We demonstrated the accuracy

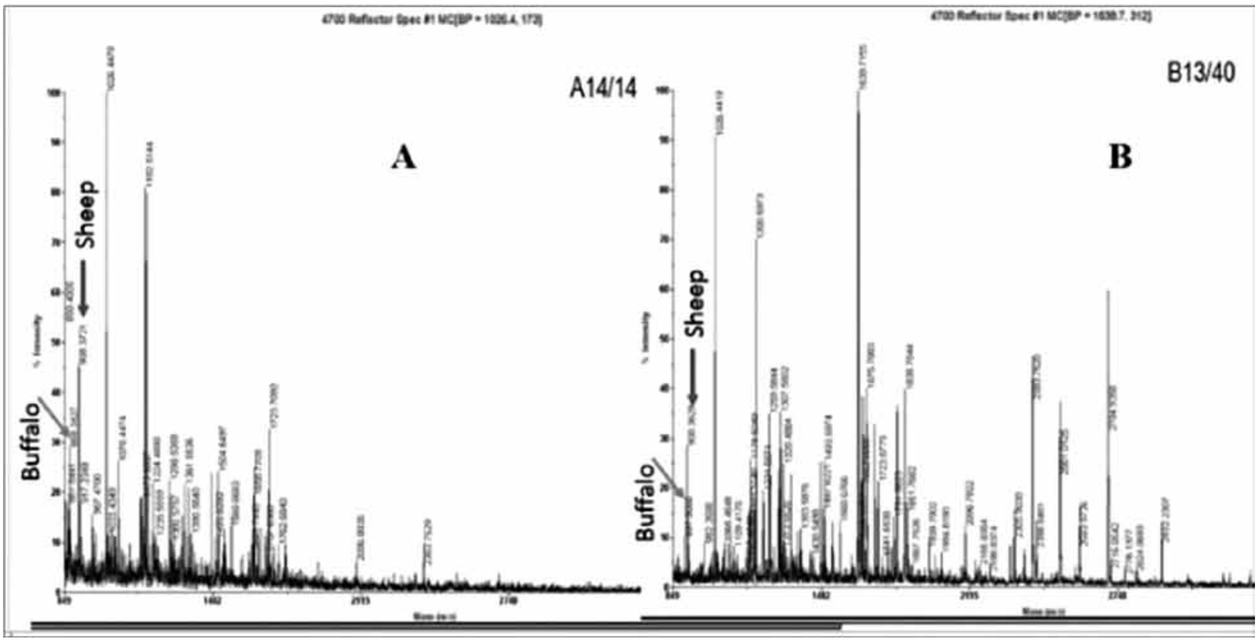


Fig. 3 MALDI-TOF MS spectrum of sheep and buffalo meat-specific peptides derived from myosin light chain-2; A-Raw and B-Cooked.

and robustness of OFFGEL-based method over 2DE based in-gel approach. The aforesaid technology envisages the robustness of high throughput proteomic approach coupled with OFFGEL electrophoresis as an alternative to other existing methods for meat speciation and pave the way for future requirements of food safety and authenticity (Naveena et al., 2016; Naveena et al., 2017).

Halal meat authentication through proteomic approach

One common aspect of commercial halal meat production is the slaughter of animals without stunning which is accepted by many organizations around the world, yet it remains extremely controversial from an animal welfare standpoint of view (Grandin, 2017). In general, slaughter of food animals with stunning is referred as humane slaughter, whereas in conventional slaughtering methods viz, Halal and Kosher, stunning of animals before bleeding is not followed in several countries even though stunning before slaughter is a statutory requirement to make the animal unconscious and insensible to pain from the act of bleeding (Terlouw et al., 2008). We hypothesised that, in addition to various physiological changes the electrophoretic mobility of some important proteins will be altered due to pre-slaughter stress which may result in up-regulation or down-regulation and will prompt us to identify the new biomarkers from meat indicating welfare of the animal before slaughtering. Hence, we conducted a study to unravel the effects of pre-slaughter electrical stunning on bleeding efficiency, serum biochemical parameters, physico-chemical quality and understand proteomic changes in Nellore cross-bred sheep.

We subjected Nellore crossbred male sheep to either pre-slaughter electrical stunning followed by slaughter (ST) or traditional halal slaughter without stunning (NST). Higher pH, water holding capacity and Warner-Bratzler shear force was observed in meat from stunned sheep. Quantitative proteomic approach using DIGE was employed to find a panel of protein markers that could differentiate ST and NST muscle proteome. Comparison of muscle proteome of ST and NST samples by 2D-DIGE and MALDI-TOF/TOF MS analysis revealed 46 significant differentially expressed proteins. Our analysis revealed changes in the abundance of proteins involved in catalytic, structural, and stress related process. Variation in the abundance of cytoskeletal proteins viz, myosin, actin and troponin was seen between ST and NST sample. Peroxiredoxin-6, a potential marker of tenderness in meat was detected in NST samples (Naveena *et al.,* 2019).

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FBB–L–04

Predicting small chemical modulators of protein-protein interactions for drug discovery in lung diseases

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Protein-protein interaction (PPI) plays a vital role in cellular functioning both in normal situation and in disease conditions including Asthma and Lung Cancer. Diseases are primarily caused by aberrant PPIs via loss of interaction or establishment of interaction at inappropriate time and/or location [1]. Targeting the whole protein not only disrupts the disease pathway but also inhibits the normal cellular functioning. Thus, disrupting specific PPI involved in disease pathogenesis and that do not obstruct the normal cellular activity is a better strategy [1, 2]. Therefore, targeting PPIs has emerged as an attractive option for the development of novel therapeutics. Biologics and peptides have been targeted against PPI interface for drug discovery in lung diseases. Biologics like monoclonal antibodies (e.g anti-IgE antibody omalizumab and anti-IL-5 monoclonal antibody Mepolizumab) though highly specific and effective, but are difficult to synthesize, store and are costly [3, 4]. Peptides exhibit high bioactivity and specificity but are often associated with immune response. In addition, in peptide based therapy, high concentrations of peptides are required to obtain inhibition of desired protein-protein interaction [5]. On the other hand, small chemical modulators having comparatively smaller size are easy to synthesize and exhibit easy penetration properties [6, 7]. These are required in low concentration and generally do not exhibit adverse immunological response. Hence, small chemical modulators can be seen as a lucrative alternative to biologics and peptides for therapeutic purpose.

Here, we focus on prediction of small chemical modulators of PPIs involved in lung diseases including asthma, chronic obstructive pulmonary disease (COPD) and cancer using bioinformatics and computational approaches. Protein- protein interaction, more precisely, interaction of interleukins with their corresponding receptors drives series of events involved in asthma and COPD. Involvement of different types of interleukins in non atopic and atopic asthma allows targeting a particular disease phenotype by selecting respective IL/IL-R pair. Small chemical modulators targeting ILs and their receptors (IL-Rs) are considered as an alternative to MAbs [7]. Small chemical modulators of IL/IL-R can be screened computationally from small chemical libraries like TIMBAL, 2P2I DB and IPPIDB and further validated experimentally. IL-2/IL-2R interaction was targeted with SP4206, a clinically tested small molecule modulator [8]. Modulators for interleukins in atopic asthma are yet to be explored computationally and validated thereafter. Two phases computational approach based on various tools and techniques can be used to identify the target interface of PPI and discovery of specific small chemical modulator targeting the IL/ILR interface [7]. The first phase includes a) homology modelling of a template in absence of structure in PDB database, b) prediction of 'hotspot' in PPI interface, c) identification of significant binding residues and d) assessing the druggability of the PPI interface. The second phase includes a) virtual high throughput screening (vHTS) of large chemical datasets like NCI chemical database, b) study the structure-activity relationship (SAR) of small chemicals, c) ADMET assessment and parameter optimization of the lead candidates. The obtained lead candidates are required further validation in pre-clinical experiments.

A few online webservers are available of predicting small chemical modulators, targeting PPIs. 2P2I database and 2P2I hunter allow designing PPI-targeted libraries and prediction of small chemical modulators [9, 10]. A Support Vector Machine (SVM) Based Prediction server namely, PPIMpred (http://bicresources.jcbose.ac.in/ssaha4/PPIMpred/) was designed for the prediction of novel modulators of protein-protein interactions involved in cancer [11]. Another webserver named PPIM-IC50Pred has also been designed using supervised machine learning for predicting the IC50 values of the small chemical modulators targeting important protein-protein interactions in cancer. PPIMdb (http://bicresources.jcbose.ac.in/ssaha4/ppimdb/) was developed as a manually curated repository dedicated for both experimentally verified and predicted small chemical modulators of popular PPIs involved in cancer like Mdm2/p53, Bcl2/Bak and c-Myc/Max. Overall the field of predicting and identifying small chemicals targeting protein-protein interaction needs to be explored for better therapeutics.

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FBB–L–05

Discovery of novel drug candidates for inhibition of Soluble Epoxide Hydrolase of arachidonic acid cascade pathway implicated in Atherosclerosis as inferred from Virtual screening, Molecular dynamics simulation and Binding free energy studies

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Cardiovascular diseases (CVDs) are the major cause of deaths globally and one of the underlying mechanisms involved in the disease pathogenesis is atherosclerosis. Soluble epoxide hydrolase (sEH), a key enzyme belonging to cytochrome P450 pathway of arachidonic acid cascade is a novel therapeutic drug target against atherosclerosis. The enzyme breaks down epoxyeicosatrienoic acid (EETs) to dihydroxy-eicosatrienoic acids (DHETs) and reduces beneficial cardiovascular properties of EETs. Thus, the present work is aimed at identification of potential leads as sEH inhibitors which will sustain the beneficial properties of EETs *in vivo*. A total of 2500 chemical compounds were retrieved from PubChem (N=1000) and ZINC databases (N=1500) and were screened for drug-like compounds based on Lipinski's rule of five and *in silico* toxicity filters. The binding potential of the drug-like compounds with sEH was explored using molecular docking. The top ranked lead molecule (from ZINC database) showed higher GOLD score (80.32) compared with that of the control (77.16) and displayed two hydrogen bonds with Tyr383 and His420 and eleven residues involved in hydrophobic interactions with sEH. The apo_sEH and sEH_ZINC lead complex showed stable trajectories during 20 ns time scale of molecular dynamics (MD) simulation. A decreased fluctuation of sEH upon binding of ZINC lead was deduced from Essential dynamics (ED) study. Molecular mechanics Poisson-Boltzmann surface area (MM/PBSA) binding free energy analysis showed that electrostatic energy is the driving energy component for interaction with sEH. These results demonstrates ZINC lead to be a promising drug candidate as sEH inhibitor against atherosclerosis instead of the present urea-based inhibitors.

ORAL PRESENTATION

FBB–O–01

SOFTWARE BASED ADMIXTURE ANALYSES IN CROSSBRED CATTLE USING BOVINE 50K BEADCHIP

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With the upsurge of crossbreeding in India, the admixture levels are highly unpredictable in the composite breeds with intense inter-se mating. Facts of admixture level along with the information on population structure and stratification are important to meet the production levels in various conditions, for dynamic genetic improvement, to assist the utilization along with conservation and to accomplish the breeding objectives. Hence, in the present study 72 Vrindavani animals were assessed for level of admixture from its known ancestors that are Holstein (63), Jersey (28), Brown Swiss (22) and Hariana (10), through three different software namely, STRUCTURE, ADMIXTURE, and frappe. The genotype data for ancestral breeds were obtained from public repository i.e., DRYAD. After quality check a total of 24,449 markers were retained and utilised for further analysis. In STRUCTURE data were subjected to 10,000 burn-in and 20,000 MCMC runs for all the 10 iterations while in *frappe* 20,000 expectation-maximization iterations along with 200 intermediate steps for $K=4$ in all software. The Vrindavani population was found to consist of on average of 37.40, 42.16, and 42.07% of Holstein, 25.81, 22.39, and 22.35% of Jersey, 9.85, 10.67, and 10.69% of Brown Swiss and 26.82, 24.78, and 24.79% of Hariana blood estimated through STRUCTURE, ADMIXTURE and *frappe*, respectively. The noted variation observed between speeds of the operation, total time consumed and results obtained from different software may be due to different models implemented. ADMIXTURE provides a chance to find out K value by cross-validation and is computationally proved to be much more fast and reliable than other software for analysis involving large datasets. From this study, it can be concluded that the prediction based on a single software should be validated through the use of different approaches and software.

FBB–O–02

Effect of supplementation of spent grain in Congo Signal grass based diet on energy utilization in mithun

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Twenty one mithun with an average live weight of 331±4.45 kg were randomly divided into 4 groups fed on Congo signal based diet. In group I animals were fed Congo signal grass without supplementation of concentrate mixture. However, in group II animals were supplemented with concentrate mixture composed of spent grain 3 parts and wheat bran 1 part on as such basis; in group III spent grain 3 parts and rice bran 1 part; and in group IV traditional concentrate mixture composed of crushed maize 50% mustard cake 30% and wheat bran 20% were fed to determine energy utilization pattern in mithun maintained in intensive system

The dry matter intake per kg metabolic body size was 49.98, 95.59, 94.39 and 89.87 g and DM digestibility was 49.46, 65.60, 64.34 and 65.50 percent in groups I, II, III and IV, respectively. The DM intake digestibility in group II, III and IV were comparable but significantly ($P< 0.05$) higher than group I. The gross energy intake per kg metabolic body size per day was 203, 404, 402 and 366 kcal and digestible energy was 106, 271, 274 and 247 kcal in groups I, II, III and IV, respectively. The gross energy as well as digestible energy intake in groups II, III and IV was significantly ($P< 0.05$) higher than group I. The digestible energy as percent of gross energy was 51.9, 67.1, 67.8 and 67.8 in groups I, II, III and IV, respectively where it was significantly ($P< 0.05$) higher in groups II, II and IV and there was no significant difference between groups II, II and IV. It was concluded that supplementation of spent grain and wheat bran / rice bran in the diet of mithun increased dry matter and gross energy intake and digestibility. And supplementation of spent grain and wheat bran / rice bran in the diet of mithun can replace traditional concentrate mixture.

FBB–O–03

Comparative study of physiocochemical and milk protein characterization of Mithun and cattle

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The present study is the first ever comparative study of milk constituents as well as milk proteins (Casein and Whey) of Mithun (Bos frontalis) and cattle (Holstein Friesian breed). The findings of the study suggested that the pH, specific gravity, corrected lactometer reading (CLR), Fat %, SNF %, total solids, total protein, ash and casein in Mithun milk were higher (p<_0.05) than cattle whereas lactose content and acidity did not show any significant variation. The characterization of caseins (α-, β-, K-caseins) and whey (lactoferrin, β- lactoglobulin, α-lactalbumin) proteins using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) and Urea-SDS-PAGE revealed the absence of any observable differences in the milk proteins of two species. The finding of the study establishes the nutritional superiority of Mithun milk in comparison to cattle.

FBB–O–04

In silico Identification and Analysis of Putative Early Promoter Motifs in Sheep Pox Virus Genome

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In this present study, identified the conserved sequences of the early gene promoter region of the sheeppox virus genome (SPPV), which is located at the upstream of the transcription initiation sites of the specific genes. The complete genome sequences of the SPPV and Vaccinia virus (VACV) were retrieved in both FASTA format and GB file format from NCBI nucleotide database. The SPPV gene sequences were annotated as compared with VACV genome using Genome Annotation Transfer Utility (GATU) online software (https://virology.uvic.ca/virology-ca-tools/gatu). The genes were classified as Early or Late as per published literature of poxviruses. We selected only the early genes of SPPV for identification of appropriate consensus/ conserved sequence of the early promoters and analyzed the spacer regions between the conserved sequences up to the start codon of the genes. The region 200 nucleotides upstream of the translation initiation site of a total 41 early genes were chosen and retrieved in FASTA format from the NCBI database. Multiple Em for Motif Elicitation (MEME) Suite 5.0.2 online website programme (http://meme-suite.org/) used for identification of the conserved sequences (Motif) of early promoter region of SPPV. The result expressed by MEME Suite was a 16 nucleotides consensus sequences (ARAAAATRAAAAWRAW where, R=A/G, W=A/T) that corresponds to the core of early genes promoter of SPPV genes. The length of the spacer between coding region of the gene and its regulatory early promoter motif is different in case of all early genes which might have effect on the gene expression.

MD–O–05

Cloning and expression of *Babesia gibsoni* Secreted Antigen 3 (BgSA3) in prokaryotic system

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Babesia gibsoni is an intraerythrocytic parasite belongs to phylum apicomplexa, and causes piroplasmosis in dogs. It is naturally transmitted by ticks however, there are reports of transmissions *via* the transplacental route, blood transfusion and dog bites has been documented. The parasite has been found in Africa, Asia, United States and Europe. The acute form of infection is clinically characterized by fever, anaemia, lethargy and splenomegaly. Recently this disease has become a serious problem from a clinical viewpoint with increasing prevalence. In India, limited attempts were made to develop immuno diagnostics by immunodominant antigen of *B. gibsoni.* The present study was aimed to amplify and overexpress the BgSA3 antigen for development of recombinant antigen based indirect ELISA. The DNA was isolated from the microscopically confirmed case of canine babesiosis and was further confirmed by species specific primers as *Babesia gibsoni*. From the same sample amplification of truncated BgSA3 gene (1921bp) from cDNA. Amplified product was cloned in pET28a vector at EcoR1 and Not1 site and the legated product was transformed to Top'10 *E.coli* cells. The insert orientation was confirmed by colony PCR and restriction enzymes analysis. Confirmed plasmid was transformed to BL21 codon plus *E.coli* cells for expression. Colony PCR positive colonies were induced for protein expression by IPTG at 37°C. The time depending protein expression kinetics were carried out and showed maximum expression at four hours post induction. The protein is under evaluation for its immunogenic and diagnostic efficiency.

MD–O–06

Ancestry Informative Markers Give an Insight to the Breed Composition in Vrindavani Crossbred Cattle

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Ancestry Informative Markers (AIMs) are the subset of genetic markers that differ in allele frequencies across different populations of the world. So far, there are so many approaches have been practiced to obtain AIMs for breed clustering and many of them successfully infer the admixture level in the population. The present study emphasizes deriving a panel of markers from 50K SNP BeadChip data for predicting the breed composition of a crossbred (combination of four breeds) population namely "Vrindavani". Hence, Holstein (63), Brown Swiss (22), Jersey (28) and Hariana (10) cattle were included in the current study along with Vrindavani (72). Vrindavani individuals were genotyped using 50K SNP BeadChip and for other individuals, the data were retrieved from public repositories. After applying quality check parameters, i.e., MAF >0.01, genotyping rate >95%, 24,449 biallelic markers in Hardy-Weinberg equilibrium were retained out of 54,451 total markers in the merged dataset. Three panels containing 500, 1000 and 2000 markers were selected through delta (δ), informativeness for assignment, F_{ST} and FIFS method. A new method i.e., Discriminant Analysis of Principal Components (DAPC) was also used for the first time to derive AIMs. Vrindavani population along with its ancestral breeds were clustered first by 24,449 markers through STRUCTURE v2.3.4 and then by all the panel of markers for comparison. The breed composition estimated by 24,449 markers was, 37.5 ± 4.4%, 24.9 ± 3.7%, 10.7 ± 2% and 26.9 ± 4.6 % from Holstein, Jersey, Brown Swiss and

Hariana, respectively. The proportion of Holstein, Jersey, Brown Swiss and Hariana estimated through 1000 and 2000 markers selected by DAPC closely resembled the proportion estimated through 24,449 markers. So, the novel method developed can be of much potential for selecting AIMs in the crossbred population.

SESSION – IV

POSTER SESSION

PP–01
Isolation and characterization of *Salmonella* from pygmy Hogs (*Sus salvanius*)

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The pygmy hog (*Sus salvanius*) is the rarest and smallest wild suid which is about 10-20 times smaller than the wild boar (*Sus scrofa*). This species is on the verge of extinction and the World Conservation Union (IUCN) has rated it among the most endangered of all mammals. The present study was undertaken to isolate and characterized *Salmonella* from pygmy hog. A total of 15 numbers of post-mortem samples were received from Research and Breeding Centre of the Pygmy hog Conservation Programme, Guwahati, Assam, India. Out of 15 samples, a total of 3 (20%) *Salmonella* isolates were recovered. The isolates were also put for antibiotic sensitivity test against different antibiotics and were sensitive to Cefotaxime, Gentamicin, Enrofloxacin and Ceftriaxone, while resistant to Amoxyclav, Ofloxacin, Ampicillin and Vancomycin. All isolates were positive for the presence of *inv*A gene of *Salmonella* with amplification of 284 bp fragments and presence of *inv*A gene in all isolates shows that these is the specific targets for *Salmonella* identification and are capable of producing gastroenteritis illness to animals.

PP–02
Molecular characterization of cattle and human rotavirus

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Rotaviruses are members of the *Reoviridae* family and one of the major etiological agents responsible for causing acute, severely dehydrating watery diarrhea, In the present study, 31 cattle faecal and 34 human stool samples were screened for rotavirus by RNA-PAGE and RNA PAGE positive samples were confirmed positive using RT-PCR. Four cattle samples and 11 human stool samples were found positive by RNA-PAGE with a typical 4:2:3:2 electropherotype migration pattern of group A rota virus. In case of human stool samples 11(28.9%) were found positive for rotavirus by RNA-PAGE that too belonged to group A rotavirus. Out of the 15 PAGE positive samples, 2 cattle and 3 human samples were confirmed positive for Rotavirus by RT-PCR.

PP–03
Incidence of Methicillin Resistance *Staphylococcus aureus* in milk samples of south Assam region.

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The antimicrobial resistance is a worldwide problem particularly in developing countries and the diverse use of antimicrobials is a key concern for the emergence of resistant zoonotic bacterial pathogens in food producing veterinary animals. The Coagulase positive (CPS) strains are opportunistic pathogens, commonly residing on outer epidermal layer of humans and animals and cause wide range of infections as well as food poisoning in both humans and animals. Hence, the present study was undertaken to detect the presence of methicillin resistant *Staphylococcus aureus* (MRSA) in a total of 60 raw milk samples collected from different point of sale in Silchar of south Assam region, India. Milk samples were processed for isolation and identification of *S. aureus* using standard bacteriological procedures.

Among all the samples, 33 isolates were confirmed as *Staphylococcus aureus*. The overall incidence of *S. aureus* in the sample was 55.00%. Drug sensitivity revealed the 100% resistance against penicillins, while eight isolates (24.24%) were found to be methicillin-resistant (MRSA), and the remaining (14, 42.42%) were methicillin-susceptible (MSSA). However, 11(33.33%) isolates showed intermediate resistance with methicillin. Thus, the study reveals presence of antibiotic resistance among *S. aureus* in veterinary which may cause hazard for human health via food chain or through direct transmission of resistant pathogens between humans and animals. The findings are useful for spotting the source of infection and therefore formulating specific control programs for bovine mastitis caused by *S. aureus* in this region.

PP–04

Characterization of *Leptospira putative* lipoprotein LIC11966 and its serological diagnostic application in diverse hosts including humans

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Leptospirosis is an emerging infectious disease caused by pathogenic species of *Leptospira*. In bacterial pathogens, cell surface-exposed outer membrane proteins play crucial role in pathogenesis and host-pathogen interaction. We report that *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 genome harbors a gene *LIC11966*, which is predicted to encode conserved outer membrane surface lipoprotein exclusively in pathogenic strains. The gene *LIC11966* was cloned into expression vector pET28a and the recombinant protein (r-LIC11966) was purified using nickel affinity chromatography from *Escherichia coli* BL21 (DE3). Polyclonal anti-LIC11966 antibodies raised against r-LIC11966 in BALB/c mice, detected LIC11966 expression in the *Leptospira* lysates suggesting that antigen LIC11966 is immunogenic. Subcellular localization using phase separation and protease assays demonstrates LIC11966 is a surface-exposed outer membrane protein of *Leptospira*. The recombinant protein showed its ability to attach diverse host extracellular matrix (ECM) components with relatively higher affinity towards fibrinogen suggesting LIC11966 may interfere with the host fibrin based blood clot. *Leptospira* spp. may use these interactions as possible mechanism during the establishment of infection. The enzyme-linked immunosorbent assay performed using human (n=50)/bovine (n=50) and canine (n=18) serum positive for leptospirosis by microscopic agglutination test (MAT +ve) recognized r-LIC11966 with 100% sensitivity and specificity, suggesting that LIC11966 is expressed in diverse hosts during *Leptospira* infection. Thus, the present finding suggests that the *Leptospira* LIC11966 antigen is an outer membrane adhesin of diagnostic importance.

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PP–05

Molecular Characterization and Phylogenetic Analysis of Porcine and Human Rotavirus

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Rotavirus is a dsRNA virus and due to segmented genome, evolution of new Rotaviral strains and interspecies transmission is possible. Considering the zoonotic implications, a total of 94 pig faecal samples and 30 human stool samples collected from different regions of Maharashtra were screened for Rotavirus by RNA-PAGE and RT-PCR. Out of 94 pig faecal samples, 7 were positive by RNA-PAGE and 2 were found positive by RT-PCR. Out of 30 human stool samples, 10 samples were positive by RNA-PAGE and 2 by RT-PCR. All RNA-PAGE positive samples of both pigs and humans showed typical 4:2:3:2 electropherotype migration pattern that belonged to group A Rotavirus. Two samples were subjected to VP7 gene sequencing and one sample was subjected to whole genome sequencing by Next Generation Sequencing. Based on sequencing results and deduced amino acid sequences, the viruses were confirmed to be of group A Rotavirus of Genotype G3 and all the three sequences were closely related to group A Rotavirus strains of Eastern state of India. The time to the most recent common ancestors (tMRCA) for Rotavirus estimated to originate during the middle of 2011 for upper cluster and 1982 for lower cluster with High Posterior Density (HPD) of 95% (2010.1-2013.4) and 1977.3-1987.4), respectively. The Splits Tree neighbour-net network confirmed that there was interspecies transmission of group A Rotavirus from human to pig. The three Rotavirus sequences studied from Parbhani and Jalna districts indicated that all three Rotaviruses (P_Kiran/2018/India), (Q_VP7_KIRAN_HUMAN/2018/India) and VP7/NAG/13/2018/India showed close identity with each other and other group A Rotaviruses of India.

PP–06

Molecular Epidemiology of Rotavirus of Buffalo and Human

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Rotavirus infection is important both in human and buffaloes because of its economic and zoonotic significance. In the present study 49 faecal samples from buffalo and 55 stool samples from human suffering from diarrhea were collected. It was observed that out of 49 faecal samples from buffalos, 10 samples and out of 55 human stool samples, 21 samples were found positive for RNA-PAGE. Representative RNA-PAGE positive fecal and stool samples, 6 each were selected for RT-PCR and out of 6 fecal samples, 3 samples were found positive and out of six stool samples, 3 samples were confirmed positive for Rotavirus infection. One sample from buffalo and one from human were sent for sequencing and one human sample was sent for next generation sequencing. The phylogenetic analysis was carried out and it was confirmed that the positive samples were showing relatedness with the Rotavirus sequences taken from GenBank. The sequence comparison from the NCBI database confirmed that the virus was originating from eastern part of India (West Bengal).

PP–07

Pathological study of *Mecistocirrus digitatus* infection in naturally infested Phere (Cross of Mithun and cattle)

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A histopathological study was carried out of a Phere (cross of mithun and cattle) calf which died of *Mecistocirrus digitatus* infection. Pathologically, the lungs were emphysematous and showed pronounced interstitial pneumonia followed by severe thickening of alveolar septa and bronchial wall associated with hemorrhages, connective tissue proliferation and infiltration of leucocytes around the bronchial wall. Bulla formation was also evident in some places. Liver showed mild hemorrhages of hepatocytes and congestion in sinusoidal space. In the small intestine, there was congestion, edema with thickening of submucosa as well as erosion of intestinal villi in certain areas, adhesion and fibrous tissue proliferation. In abomasums, adult parasites were embedded in mucosa and sections of adult parasites was seen full of eggs. In the large intestine, there was necrosis and degeneration of colonic villi and loss of normal architecture. There was also evidence of hyperactivity of acinar cells, fibrous tissue proliferation with polymorphonuclear cell infiltration. An apical tip shows degenerative changes, adhesion with mild fibrous tissue proliferation. In spleen, there was depletion of splenic venule, congestion of splenic pulp, hemorrhages surrounding the spleenic venule

PP–08

Resiquimod induces proinflammatory cytokines and inhibits Newcastle disease virus replication in chicken embryo fibroblast cells

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Newcastle disease virus (NDV) is an etiological agent of Newcastle disease, which infects wild and domestic birds with high mortality and morbidity worldwide. Although this disease is mainly controlled through NDV vaccines but alternative use of antiviral compounds is increasingly under study. Resiquimod (R-848) is an animidazoquinoline compound and is a potent synthetic agonist of Toll-like receptor 7 (TLR7). Until now reports regarding adjuvant potential of R-848 is well established against human viruses but has been less explored against avian viruses. In the present study, the effect of R-848 was examined on NDV-replication in chicken embryonic cells (DF-1). The treatment of R-848 in DF-1 showed considerable reduction of NDV replication. The results were further confirmed through viral mRNA and western blot analyses against NDV HN protein. About 70% reduction of NDV virion was observed post 48 and 72 hrs of resiquimod treatment on DF-1 cells. In addition, the differential genes expression were observed through real-time PCR using cDNA from DF1 cells and PBMCs. Among all the selected genes significant up-regulation of IFN**α,** IFNγ, IL1β, TNF**α, IL18 mRNA were observed.** The result suggests that the modulation of host innate immunity genes by resiquimod could modulate the NDV replication in DF-1 cells. The result of the study could be explored further to establish R-848 as an alternative anti-viral compound against NDV.

PP–09

Analysis of codon usage pattern in the viral proteins of chicken anemia virus and its possible biological relevance

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Chicken anemia virus (CAV) is an important poultry pathogen. CAV infection can lead to severe immunosuppression and mortality in birds. The viral proteins (VP1, VP2 and VP3) are major protective immunogens that determine the pathotype of CAV strains. The factors influencing the synonymous codon usage bias and the nucleotide composition of the viral protein genes of CAV have not been studied. In the present study, we have analyzed the synonymous codon usage pattern in VP1, VP2 and VP3 genes of CAV. Our results showed that all the genes have a low codon usage bias; however, this bias was slightly higher in the VP2 gene as compared to VP1 and VP3. Furthermore, the major contributing factor is mutational pressure followed by the host factors. The conclusion was drawn based on codon usage, correspondence analysis, ENC-GC3 plot and correlation analyses among different indices. The study will help us to understand the codon usage bias of CAV and related single-stranded DNA viruses which could further be used to explore their biology

PP–10

Cloning and expression of sporozoite and macroschizont (spm2) protein from genomic DNA of *Theileria annulata* isolated from the cattle blood sample

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Tropical theileriosis is a tick-borne protozoan disease of *Theileria* spp. causing fatal leukoproliferative disorder in cattle. The present study was conducted to investigate the reliable serological methods for theileriosis diagnosis in cattle blood samples (n = 61 serum samples) collected from bovine populations of Bihar region. Microscopic examination of blood smears from the suspected animals' revealed *Theileria* infection. Molecular detection using genomic DNA PCR and sequencing of 18s rRNA of the field samples (blood genomic DNA) confirmed *Theileria annulata* as the causative agent of theileriosis in cattle of Bihar region. The 18s rRNA gene were amplified from genomic DNA isolated from infected bovine blood samples, cloned in TA vector, sequenced and were submitted in GenBank (accession no. MH737680, MH737681 and MH737682). To refine the process of epidemiological investigation and for large-scale screening, sporozoite and macroschizont (*spm2*) partial gene from *T. annulata was* cloned in pET28a expression vector and expressed in *E. coli* cells. Recombinant spm2 protein (43kDa) was purified by Ni-NTA affinity chromatography and was used as an antigen for diagnosis of theileriosis in bovine samples using western blot. Recombinant spm2 protein was detected using the sera collected from *Theileria* infected cattle and the detection was specific as serum samples obtained from uninfected bovine did not show any detection of spm2 protein. This suggests that spm2 can be used as a potential diagnostic antigen for tropical theileriosis.

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PP–11

Development and testing of a lateral flow assay kit for the diagnosis of subclinical bovine mastitis

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Subclinical and clinical mastitis is recognized as one of the most important diseases affecting the dairy industry. Acute phase proteins such as serum amyloid A (SAA) is a potential markers of inflammation. SAA is a non-glycosylated apolipoprotein and its molecular weight varies between 11 and 14 kDa depending on species. It is also produced extra-hepatically, e.g. by the mammary gland epithelial cells and a mammary-associated form of SAA has been identified in milk. SAA are major acute phase reactants which increase many fold during such inflammation. SAA3 which appears as a specific intra-mammary biomarker in subclinical bovine mastitis was used to develop a kit by applying modern technology *viz.*, lateral flow assay (LFA) using SAA antibody and nanoparticles. In the present study, the structure and composition of SAA3 antigen was determined using bioinformatic tools and the peptide was custom synthesized, followed by antibody production. Gold nano particle (GNP) synthesis and optimization was done for the conjugation of antibody with GNPs. The antibody conjugated GNPs, primary antibody and secondary antibody were coated on the nitrocellulose membrane and the components of LFA kit were assembled. Experiments were done to optimize the detection of SAA antigen present in bovine milk during subclinical mastitis.

PP–12

Kiss1 and *GPR54* mRNA expression, endocrine profile and follicular development in post-partum anestrus mithun cows following exogenous administration of kisspeptin (*kp-10*)

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Present study was undertaken to find out the effect of kisspeptin (kp-10) administration in post-partum anestrus mithun cows. Animals failing to resume cyclicity even after 90-100 days of parturition were examined through rectal palpation and ultrasonography to ascertain the anestrous status were taken in the study. Expression pattern of *kiss1* and *GPR54* mRNA, plasma endocrine profile and follicular development were also recorded. A total of 12 post-partum anestrous mithun cows, age between 5-6 years, were selected and divided randomly in two groups (n=6). Animals in group A (treatment) were injected with kisspeptin (kp-10) @ 1.30 µg/kg body wt. at every 3 days interval till commencement of estrus. While in group B (control), normal saline was injected as placebo. Blood samples were collected on the days of injection and also during estrus in treatment as well as control groups. Transrectal ultrasonography was also conducted at every 3 days interval till onset of estrus and ovulation to study the follicular development. Results revealed that kisspeptin administration causes early resumption of cyclicity in treatment group as compared to control (24.64 ± 10.43 vs 66.56±14.66 days). There was a significant increase in *kiss1* and *GPR54* mRNA expression in treatment group as compared to control on the day of estrus (1.943±0.29 vs 0.424 ± 0.062 and 1.84±0.31 vs 0.416±0.082, respectively).There was a gradual increase in the circulating levels of Estradiol and FSH after treatment which reached at peak level on the day of estrus in treatment group (25.36 ±1.27 pg/ml and 15.65 ± 1.22 ng/ml, respectively) whereas no significant difference was reported in control group except on the day of estrus (11.29 ± 1.76 pg/ml and 9.86 ± 1.06 ng/ml, respectively). Increased number of medium and large follicles was recorded in treatment group while control group showed only small follicles. It may be concluded from the present study that exogenous administration of kisspeptin (kp-10) resulted in early resumption of cyclicity in post-partum anestrous mithun.

PP–13

Genetic divergence of Mithun and Tho-tho cattle using PCR-RAPD

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Genetic diversity study between Mithun and Tho-tho cattle was done using random amplification of polymorphic DNA – PCR technique. Forty (40) randomly selected decamer was employed to generate polymorphic DNA fingerprints. According to the breed, the pool genomic DNA from 20 Tho-tho cattle and 10 Mithun of each strains were prepared and amplified. The number of RAPD bands with the different primers in Tho-tho and Mithun were found in the range of 3 to 17 and 8 to 26, respectively. Size of the PCR products varied from 200bp to 10000 bp in Tho-tho cattle and 200bp to 10000 bp in Mithun. The genetic similarity and the genetic distance between four strains of Mithun and Tho-tho cattle was calculated by using Popgene software version 1.31. Genetic Distance was found to be highest between Tho-tho Cattle and Arunachal strains of Mithun and also with Mizoram strain Mithun (D= 0.5170). Genetic Distance was lowest

between Nagaland Mithun and Mizoram Mithun (D= 0.0862). The genetic identity was found highest (I= 0.9174) between Nagaland and Mizoram strains of Mithun. The number of polymorphic loci was 117 (53.67%).

RAPD analysis of the present study shows that Mithun and Tho-tho cattle are genetically divergent which is justifiable from their divergent evolutionary patterns. Among the four strains of Mithun, Nagaland strain and Mizoram strain are genetically closer and Nagaland strain and Arunachal strain are genetically distant. Present study will help to plan the fruitful breeding programme among the four different strains of Mithun belonging to Arunachal, Manipur, Nagaland and Mizoram.

PP–14

Inhibitory effect of nitazoxanide on Newcastle disease virus infection: A possible repurposing of drug for poultry.

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Newcastle disease virus (NDV) infection is prevalent throughout India among the domesticated and the wild birds. It is commonly known to affect chicken, pheasant, ostrich, pigeon and waterfowl. NDV belongs to family *Paramyxoviridae* and classified under the genus *Avulavirus*. Depending on the virulence, NDV strains are further classified into lentogenic (less virulent), mesogenic (moderate virulent) and velogenic (high virulent). The velogenic strains cause severe respiratory and nervous disorders with high mortality rate. The average incubation time of the virus is 15-21 days and can be transmitted easily causing significant economic loss to the poultry industry. The live and killed vaccines are available for the prevention of infection in the market but the drug for the treatment is not available. Nitazoxanide (NTZ), a member of thiazolides, is an FDA approved antiparasitic drug. In the present study, we investigated the effect of NTZ on the NDV replication. The experiments were conducted in chicken fibroblast DF1 cells and in embryonated chicken eggs. The inhibition of the NDV production was observed upon treatment of NTZ at a concentration of ~12.5µM. Significant reduction in viral load was observed post-treatment of NTZ upon infection. The mechanism of action by which NTZ causes inhibition of NDV is not known. The study will help us to repurpose a drug for the treatment of viral infection in poultry. This will also pave a way towards understanding of similar effect in other animal pathogens.

PP–15

Prevalence and Pathological detection of Bluetongue virus in Mithun in North East India

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Bluetongue (BT) is an arthropod born, non-contagious viral diseases affecting many species of domestic as well as wild ruminants. In India, BT is endemic all over the country. however, the clinical outbreak of the diseases occurs only in small ruminants mainly sheep more frequently in the southern states of the country. BT often occurs as asymptomatic form of the diseases in other ruminants. In the present study, prevalence and molecular detection of BT was carried out in Mithun (Bos frontalis) in the north eastern region of the country. A total of 297 apparently healthy mithuns from all the Mithun rearing state of the country i.e. Arunachal Pradesh, Nagaland, Nagaland, Manipur and Mizoram representing different agroclimatic conditions were screened for the presence of BTV specific antibodies using competitive enzymes-linked immunosorbent assay (c-ELISA). Very high prevalence of 93.27 % (277/297) was absorbed in free ranging mithuns. Animals showing high titre of BT antibodies were also screened for the presence of BT genome in RBCs using published primers for VP7 genes for RT-PCR AND 26.4% (33/125) of seropositive and.1% (33/297) of total mithuns were observed positive for BT specific products of 222bp were amplified and sequenced. Real time PCR analysis revealed moderate to high Ct values ranging from 22 to 30 using specific primers for NS3 gene. Further, tissues samples (lings, spleen, and lymph nodes) of the necropsied mithuns were also screened for the detection of BT genome by RT-PCR and specific products of 222bp were amplified in 20% (11/55) necropsied mithuns. Histopathological examination of the tissues revealed edema, congestion and haemorrhages and immunohistochemical examination using anti rabbit anti BTV polyclonal antibody revealed positive signals for BTV antigen in the lungs, spleen and lymph nodes. From the study, it can be concluded that BTV is highly prevalent in the north eastern region of the country. Therefore, it is required to carry out systematic investigation of the diseases in different species of domestic and free ranging animals in the north eastern region of the country to establish the prevalence and dynamics of the spread of the disease. It will great help to understand the epidemiology of BTV in North East region and to formulate effective control and preventive strategies.

PP–16

Isolation of a novel Ribosome Inactivating Protein from Cucurbitaceae species

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Ribosome inactivating proteins (RIPs) are protein toxins distributed widely in plant kingdom. Many RIPs have shown practical applications in different fields, such as in agriculture and in medicine. These proteins have the ability to inhibit protein synthesis by inactivating ribosomes. Many plants of Cucurbitaceae family have shown to possess these proteins which have shown promising results against microbes, viruses and are also being explored for anti-cancer activities. In the present study some species were explored for the presence of novel RIP(s) which may inactivate ribosome and in turn may be for their medicinal property in cancer treatment etc. A novel protein was isolated showing ribosome inactivating property. Upon the structure related study it was found that the protein is predominant of alpha helix. This property of inactivating ribosome may further be studied against some animal viruses, anticancer activity etc. and hence with further study of the structure, and understanding the mechanism of its action this protein in the future may be used for the benefit of both human and animal as antiviral, anticancer drug or as immunotoxins.

SESSION – V

EMERGING DISEASE

LEAD PAPERS

ED–L–01
Translational Research and Entrepreneurship Development - two sides of the same coin of future

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Translational Research Platform for Veterinary Biologicals (TRPVB) is a unique partnership initiative in the naïve field of translational research between Department of Biotechnology (DBT), GoI and TANUVAS. The Vision of TRPVB is "To converge the presently distant research, industry and regulatory requirements to foster 'productization' in the field of Veterinary Vaccines and Diagnostics". TRPVB has been established to facilitate product development, provide regulatory-compliant biotechnological services and also to serve as a knowledge resource in veterinary biologicals. TRPVB has established a 'state of the art' research laboratory housing all the major sophisticated equipment's needed for research and product development. It has also mobilized expertise from academia, industry and regulatory fields to work under a single 'roof'. TRPVB has also established a clean room facility that will provide for seamless transfer of products developed at TRPVB to industry without undue delays.

The approaches taken by TRPVB to bridge the translational gap include

- Product development – market to mind strategy
- Novel innovative unique products
- Creating a participatory ecosystem
- Hand holding SME – guidance and lab space for initial batches
- Regulatory compliant validation services
- Collaboration with strategic partners
- Enable seamless transfer of technologies
- Technology incubator
- Technology Acquisition and Licensing

The infrastructural facilities at TRPVB / TANUVAS include

- Clean room facility
- Biosafety laboratory III
- Biosafety laboratory II
- Repository for storage of cell lines, viruses, clones etc.
- Analytical Instrumentation
- Animal experimentation facilities
- Certified cell banks

Entrepreneurship is a French word which means "between-taker" or "go-between". An entrepreneur is an "individual who takes risks and starts something new"

Entrepreneurship is the process of "creating something new with value by devoting the necessary time and effort, assuming the accompanying financial, psychic, and social risks, and receiving the resulting rewards in monetary terms, personal satisfaction and independence.

Thus it involves

- Creation Process (for entrepreneur & audiences)
- Devotion of time & effort
- Assuming Risks (Financial, Psychological & Social)
- Rewards (Independence, satisfaction & monetary rewards)

An entrepreneur traverses the roles of the inventor, the opportunity spotter and the project champion. The process involves the

- Idea
- Identifying the opportunity
- Matching the idea to the opportunity and
- Exploiting the opportunity to build something of value

The advantages of entrepreneurship include but not limited to the following

- Catalyzing innovation
- Creation of job opportunities
- Societal and community development
- Social and economic integration
- Enhances standard of living
- Promotes more research and development

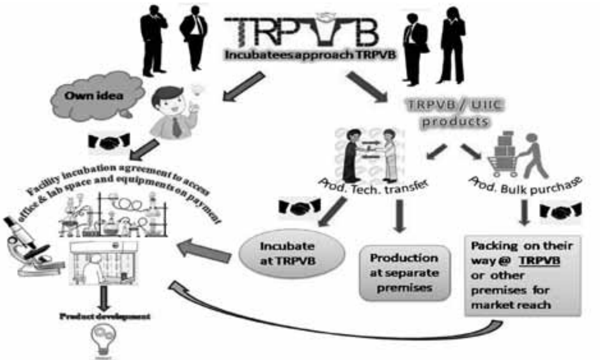
In the present times, entrepreneurship is endorsed by educational institutions, governmental units, society and individuals. Various government agencies are taking an increased interest in promoting the growth of entrepreneurship. The society's is also supporting by providing both motivation and public support. Government sectors and large companies are evincing a keen interest in the special form of entrepreneurship – intrapreneurship for the future. They are increasingly interested in capitalizing on their research and development capability in the hyper competitive translational environment today.

The purpose of establishing TRPVB was to bridge the translational disconnect between academia and industry. During the last 5 years, various multi-faceted approaches have been taken to bridge this disconnect and promote entrepreneurship in the cost-starved Animal Sciences sector. TRPVB has already incubated two Biotechnology Ignition Grants and is presently incubating two companies in its clean room facility. Understanding that the animal sciences sector has case-specific issues that needs tailor-made solutions, TANUVAS has created a non-profit Section 8 company named Veterinary incubation Foundation @TANUVAS which is the first dedicated veterinary incubator in India

The interested industry, students, entrepreneurs, start-up companies, individuals etc. can incubate here and would be given office space and in addition they would have access to the following

- Priority for tech transfer of TRPVB products and services
- Priority to access GMP compliant Clean room laboratory and cell storage facilities at nominal cost
- Priority to produce TRPVB products through technology transfer
- Priority to access infrastructure facilities available
- Priority to services offered at TRPVB
- Right of first refusal for technologies developed in-house at TRPVB
- Preference in applying for joint collaborative projects
- Priority in conduct of required studies / validation
- Preference in workshop / training / conferences organized by TRPVB
- Customized training for industry-sponsored personnel in their required areas
- Leveraging expertise in regulation, animal experiments, Biosafety, Nano technology, etc.,
- The proposed VIF@TANUVAS aims at
- Creation of start-ups for innovative veterinary products including diagnostics, biologicals, farm equipments etc developed by TANUVAS or independently by the incubatee
- Diagnostic & Clinical services established by TANUVAS to be outsourced for potential incubatees for scaling-up the activity as an agreeable royalty basis (through vendor reach-out)

The proposed models for VIF @TRPVB is as follows



Sum up….

All public funded research institutions and universities should ensure that their research and development activities help their stake holders and contribute to society. Their research leads should traverse the entire path of discovery, translation to societal benefit. This is a path full of 'bumps' but to reach the destination this path needs to be traversed. To help in our journey the concepts of translational research coupled with entrepreneurship are very helpful. A typical academician is made to think that commercialization is not his 'problem' or even his 'forte'. This paradigm needs to change. Persons with dual expertise in translating their own technology innovation are needed. Translational research requirements and entrepreneurial skills are essential requirements of the researcher of tomorrow. Or else he gets outdated and becomes a non-performing asset of this country.

Let us all strive not to become one and let us translate at least one technology in to a product during our research careers!

ED–L–02

Antibiotic Resistance in Animal Pathogens–Strategies for Control

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Anti-Microbial Resistance, at a global level, is a major threat to human and animal health. It endangers modern human and veterinary medicine and undermines the safety of our food and the environment. Antimicrobials including antibiotics play a critical role in the treatment of diseases of farm animals (aquatic and terrestrial). Their use is essential to food security, to human health, and to animal welfare. However, the misuse of antimicrobials, in both human and veterinary medicine, is associated with the emergence and spread of antimicrobial-resistant organisms including bacteria and has been identified as a risk factor for human infection. The risk is potentially higher in countries where legislation, regulatory surveillance and monitoring systems on the use of antimicrobials, and the prevention and control of AMR, are weak or inadequate (FAO, 2016). With AMR on the rise, communities in developing countries are highly impacted by the increase of infectious disease outbreaks, and loss in livestock productivity, which ultimately endangers food security and disrupts international trade. In animal agriculture, antimicrobials can be used therapeutically (treating disease, preventing or controlling infection) or non-therapeutically (growth promotion).

The relentless march of antimicrobial resistance (AMR) has emerged as a public health concern, especially in the light of the fact that newer antibiotic classes have been slow to develop and investments in novel antimicrobial drug classes have been receding. The AMR problem transcends international borders and is not limited to nations with limited capacity to manage pathogen emergence or antibiotic stewardship. In keeping with this, the Sixty-eighth World Health Assembly (WHA) came up with the Global Action Plan to contain AMR (GAP-AMR) in May 2015. Aligning national interests with the global context, member states have been tasked to develop a National Action Plan to contain AMR (NAP-AMR), aligned with the GAP-AMR, by May 2017.

Use of antibiotics in chickens in particular, is expected to triple in India by 2030 (Van Boeckal*et al.*, 2015). Resistant bacteria can spread to humans through contaminated food, or by environmental spread. Genes coding for antimicrobial resistance can be transferred from microbes carried by animals to microbes causing disease in humans. There is need to promote national surveillance of antimicrobial use for formulation of appropriate strategies to combat antibiotic resistance to facilitate proper use of antibiotics in animals.

Drivers of AMR

Antimicrobial consumption, agricultural use of antibiotics, environmental contamination, healthcare transmission, suboptimal diagnostics, suboptimal vaccination, suboptimal dosing, including from substandard and falsified medicine, mass drug administration, transmission by food and increasing global trade and travel are contributors for the development of AMR.

AMR in human

In human, very high level from 50-70% resistance is observed for fluoroquinolones, third generation cephalosporin, Carbapenem in *E. coli* (Gandra*et al.,* 2017). Colistin, the last resort antibiotic in human medicine for treatment of carbapenem-resistant gram-negative bacterial infections, have been showing resistance in gram negative bacteria (Kaur *et al.*, 2017; Pragasam *et al.,* 2016; Manohar *et al.,* 2017). The figures are alarming and if we see the veterinary side of it than from Livestock sector, NDM-1 (Ghatak *et al.*, 2013) and ESBL gram-negative bacteria (Das *et al.*, 2017); vancomycin-resistant *Staphylococcus aureus* (VRSA) (Bhattacharyya *et al.*, 2016) have been reported form mastitic cow milk. From healthy pigs, ESBL-producing *E. coli* from fecal samples were isolated (Samanta *et al.,* 2015). From poultry sector, AMR reports are there on ESBL producing *E. coli* (Brower *et al.,* 2017; Shrivastav *et al.,* 2016) and on drug resistant *Salmonella* (Adesiji*et al.,* 2017). Singh *et al.* (2016) reported doxycycline to be the major used antibiotic at poultry farms in Jabalpur, MP.

AMR in animal pathogens

Poultry is one of the most widespread food industries worldwide, and chicken is the most commonly farmed species, with over 90 billion tons of chicken meat produced per year (FAO, 2017). A large diversity of antimicrobials are used to raise poultry in most countries (Agunos*et al.*, 2012; Landoni and Albarellos, 2015), mostly through the oral route, with the aim to prevent and to treat disease, but also to enhance growth and productivity (Page and Gautier, 2012). A large number of such antimicrobials are considered to be of critical and high importance for human medicine (WHO, 2017).

Several strains of from mastitis case have been reported to show resistance against multiple antimicrobials such as penicillin-G, gentamicin, streptomycin, ampicillin, ciprofloxacin,oxytetracycline (Kumar et al., 2011). Chandrasekar et al. (2015) tested 401 mastitis milk samples from cows and found that 235 samples were found to be predominantly antibiotic resistant mastitis due to E.coli (50.64 %), Staphylococcus aureus (44.25 %) and MRSA (5.11 %). These organisms were resistant to penicillin, amoxicillin, oxytetracycline and methicillin. It has been seen that for certain groups of antibiotics, *Staphylococcus* and *Pasteurella multocida* isolated from poultry may have up to 100% resistance (Global Antibiotic Resistance Partnership; 2016*)*.

In Tamil Nadu during 2016 to 2018, a total of 170 suspected mastitis milk samples (66.67 % were sub-clinical and 33.3 % were from clinical cases) were collected for cultural isolation and antibiotic sensitivity testing. From these samples, 93 isolates (81 – Staphylococcus spp., 3-E.coli, 7-Pseudomonas spp., and 2 Klebsiella spp) causing bovine mastitis were obtained. Antimicrobial Susceptibility test was done as per method of Kirby-Bauer using Five drug classes: β-lactams- resistant by Penicillin-G (70.37%), Methicillin (8.64%),Oxacillin-(6.17%). Both MET and OX (14.81%). **Amino-glycosides**-Streptomycin (66.66%), Erythromycin(862.9%) Intermediary & Gentamicin-(85.19 %) sensitive in action. **Fluoroquinolones**-being Highly sensitive in the descending order as Enrofloxacin-(93.83%), Norfloxacin-(91.36%), Ofloxacin (87.7%), Lomefloxacin- (80.25%) and Ciprofloxacin (70.3%) .**Cephalospoirns**-Ceftrioxone (72.84%) and Cefixime (59.3%) as being sensitive, and Cefipime –(88.89%) being Resistant. Among **Tetracyclines**-_Oxytetracycline was (86.42%) resistant & Doxycycline Hcl was (80.25%) sensitive. All *Pseudomonas spp* were sensitive to Methicillin (100%).

In another study, 200 Nos. of intestinal contents of animals and birds including soil samples from different places of Tamil Nadu were collected for isolation and characterization of *C. perfringens*. Of the 200 samples collected, 13 samples were found to be positive by cultural isolation and upon further testing, only 6 isolates were confirmed to be *C. perfringens*. Among the six isolates, two were enterotoxin type and four were classified into major toxin type E strain by PCR method. The antibiotic sensitivity testing of the 6 isolates showed that most of the isolates were highly sensitive to kanamycin, tetracycline, cephalosporin, gentamicin, amikacin and chloramphenicol moderately sensitive to azithromycin, and vancomycin and resistant to penicillin, metronidazole, methicillin, erythromycin and oxacillin.

Economic impact

Globally, by 2050, AMR is estimated to lead to lost outputs worth US$100 trillion. Research by the World Bank also shows that AMR could cause low-income countries to lose more than 5 per cent of their GDP and push up to 28 million people, mostly in developing countries, into poverty by 2050. India is among the nations with the highest burden of bacterial infections. An estimated 410 000 children aged 5 years or less die from pneumonia in India annually; accounting for almost 25% of all child deaths in India. The crude mortality from infectious diseases in India today is 417 per 100 000 persons. Consequently, the impact of AMR is likely to be higher in the Indian setting. AMR is a major public health concern in India. The burden of AMR in livestock and food animals has been poorly documented in India. Shrestha et al. (2018) assessed the economic costs of AMR per antibiotic consumed were considerable; often exceeding their purchase cost based patient, healthcare and societal perspectives. Notwithstanding their limitations, use of these estimates in economic evaluations can make better-informed policy recommendations regarding interventions that affect antimicrobial consumption and those aimed specifically at reducing the burden of AMR.

Strategies to combat AMR

Alternative products to antimicrobials can play a crucial role in reducing the need, and hence misuse, of antimicrobials in animal agriculture (e.g. preventing infectious diseases altogether). Vaccines are among the most promising and widely used of these alternatives. However, other innovative products are in use or currently being investigated and offer additional options to producers (e.g. pre- and pro-biotics or the use of products to enhance the innate immune system). While alternative products have great potential to reduce the emergence and spread of AMR, it should be noted that these solutions reach their full potential when considered as one part of a comprehensive animal management program aimed at ensuring healthy and disease-free animals. Plasmid Curing is the process by which plasmids are removed from bacterial populations. This is an attractive strategy to combat AMR as it has the potential to remove ARGs from a population while leaving the bacterial community intact. Plasmid curing agents could also be taken by international travelers to reduce the global spread of AMR. Unfortunately, at the moment no such treatment options are in use.

ED–L–03

Species Resistance to Select Virus Diseases - An Enigma?

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Certain viruses cause disease in only some species of animals. Other species of animals though present in contact with the disease affected animals do not develop the disease. Experimental inoculation of virulent virus is also not able to cause the disease in the resistant species. Though there is replication of the virus in the resistant species indicated by seroconversion the disease pathology is not produced. Different approaches have been adopted to find out the basis for the species resistance.

In the case of peste des petits ruminants (PPR), the species resistance has been studied at the level of virus receptor, TLR expression and host cytokine expression. The receptor for the virus, SLAM, was found to be more expressed in goats compared to sheep, cattle, and buffalo peripheral blood mononuclear cells (PBMCs). SLAM mRNA expression was found to correlate with PPR virus (PPRV) replication in the PBMCs of different species. This indicated that receptor expression could play a role in modulating the viral replication differently in these animals (Pawar *et al.,* 2008). There was also differential expression of TLR3 and TLR7 in goats and buffaloes. species. Increased levels of TLR7 have been correlated to the release of increased levels IFN- γ which in turn plays a role in decreased replication of PPRV in buffalo tissues (Dhanasekaran *et al.,* 2014)

Expression of different cytokines following in vitro infection of PBMCs from goat and cattle was studied. Interferons (IFN-α, IFN-β, IFNγ) were upregulated more in cattle compared to goats following PPRV infection. The interleulkins IL-4, IL-6, IL-10, and IL-12A were upregulated in goats more compared to cattle. IL -4 and IL -6 are proinflammatory cytokines. Higher upregulation of proinflammatory cytokines IL-4 and IL-6 in goats in may be one of the reasons for the disease development in goats.

The upregulation of expression of TGFβ is higher in cattle when compared to goats. TGFβ is an apoptotic factor. The higher expression of TGFβ may restrict virus multiplication by inducing apoptosis in the infected cells. TNFα was found upregulaed in goats and down regulated in cattle. The resistance of cattle against PPR could be due to the higher expression of INFs and lower expression of proinflammatory cytokines in cattle (Masdooq, 2016).

The host species for bluetongue virus includes sheep, goat, cattle and buffaloes. Among them sheep are affected by severe clinical disease. The other species generally develop an inapparent infection. Though prolonged viremia and seroconversion occurs in cattle clinical disease is rare. They act as asymptomatic reservoir hosts. Studies have focused on infection of endothelial cells to find out the differences in replication and tissue damage in both species. Infection of umbilical vein endothelial cells showed differences in the cytolysis and interferon expression (Russel *et al.,* 1996). Differences were observed in cell death mechanism, infection kinetics and prostacyclin in the infection of pulmonary artery and lung microvascular endothelilail cells of sheep and cattle (DeMaula *et al.,* 2001). In a study with bovine and ovine lung microvascular endothelial cells BTV infection induced a higher prostocycline in cattle when compared to sheep. In vivo infection of sheep and cattle also showed higher prostocycline level in cattle. This suggested that the lower sensitivity of cattle to microvascular injury and thrombosis which in turn may be a reason for the resistance of cattle against clinical bluetongue disease (DeMaula *et al.,* 2002).

The susceptibility differences of chicken and ducks to avian influenza disease were studied by analyzing virus- cell interaction and different host responses. Avian influenza viruses replicate in ducks but the infection is usually asymptomatic. In ducks highly pathogenic avian influenza virus infection was found cause rapid induction of apoptosis while apoptosis was delayed in chicken. The rapid apoptosis was considered advantageous for ducks (Kuchipudi *et al.,* 2012). Differences in the induction of MDA-5, TLR7 and IFN-α following in vivo infection of influenza virus were observed in chicken and ducks. Interferon α and β were up regulated in chicken. In duck IFN- γ was upregulated. There was more induction of TLR 7 and melanoma differentiation-associated protein (MDA)-5 in lungs of chicken (Cornelissen et al., 2012). In another study, interferon-induced transmembrane protein (IFITM) 1, 2 and 3 were shown to be upregulated following infection with highly pathogenic avian influenza. The IFITM123 protein family and RIG-I were considered to play a role in the resistance of ducks to influenza (Smith *et al.,* 2015).

Research on the molecular mechanisms behind species resistance to certain virus infections will help to understand the molecular pathogenesis of the diseases and develop novel methods for prevention and treatment of viral diseases.

ED–L–04

Battling the bewildering bugs

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Discovery of antibiotic, the magic bullet of 21st century which started its journey way back in 1928 with the path-breaking findings of Sir Alexander Fleming considerably changed the modern medical science and its impact over public and animal health is undisputable. However, the rapid and world-wide emergence of resistant bacteria (badbugs) substantially endangered the efficacy of the antibiotics which has remained the saviour of millions of lives. In line with development of antibiotics, bacteria also evolve their own mechanism to prevent the antibiotics to work against them and thus the drugs which were once successfully used to treat infections become no longer effective. This can cause persisting infection with chances of dissemination to the in-contact human beings or animals. There is no doubt that the impact of antimicrobial resistance (AMR) is ominous and far-reaching. According to an estimate by UK's O'Neill Commission, currently, around 7,00,000 human deaths each year can be attributed to antimicrobial resistance. Moreover, the death toll may rise up to 10 million by 2050 which is more than the death due to any other common causes like road accidents, cancer, diabetes and diarrheal diseases, if sufficient measures are not taken. Given such magnitude, the problem of AMR gained political commitment from the world leaders and the matter was discussed in the United Nations General Assembly in 2016 with a political declaration regarding the problem. In April 2015, World Health Organization (WHO) published a document on current global situation of AMR which pointed out several loopholes in the AMR containment policy of the most of the countries. Subsequently, both the Food and Agriculture Organization (FAO) and WHO published some fundamental documents on AMR to address the problem, and the global action plan against AMR has been developed.

Antimicrobial Usage in Livestock Sector: Global Scenario

With rapid globalization and population explosion, there is increased demand for quality animal protein which is acting as a catalyst or stimulating force for rapid intensification for animal farming system. Subsequently, value of the veterinary drugs in the international market is expected to rise by two times in 2018 (42.9 billion USD) from 2010 (20 billion USD) within a span of 8 years. Presently, the global average annual consumption of antimicrobials is about 45 mg/kg of animal product produced in cattle. However, the same is remarkably higher in poultry (145 mg/kg) and pigs (172 mg/kg). By 2030, the global antimicrobial use in animals is expected to increase by 67%. In USA only, about 80% of the annual consumption of antimicrobials is attributed to antimicrobial usage (AMU) in food animals. In about 26 EU countries, average consumption of antimicrobials is about 116.4 mg/kg of estimated biomass in human beings while for animals it is about 144 mg/kg.

Antimicrobial Resistance: An Appraisal

Truly speaking, antimicrobials are equally important for treatment of critically ailing animals infected with life-threatening diseases like anthrax, black quarter and hemorrhagic septicemia, so as in human patients. The use of antimicrobials is not limited for treatment purpose; there are evidences that the antimicrobials are also used for prevention and control of diseases and as growth promoter. Ideally, appropriate therapeutic regimen for any infected / ill animals can only be determined and prescribed based on the results of antibiotic susceptibility test. Moreover, various factors like age, immune status of animals, existing renal or liver function status, pharmacodynamics, pharmacokinetics, tissue distribution of the drugs and approval of the drugs in respective species should be taken into account for determining appropriate therapy. However, such ideal condition is very difficult to follow practically in field or farm conditions due to the lack of veterinary services in far-off or remote places. In the event of any fresh outbreaks of the formidable diseases, many of the animals in flock are also treated with antibiotics even though there is little clinical evidence of infection in the in-contact animals - metaphylaxis. Moreover, prophylactic use of antibiotics is also done by administration of drugs in water or food usually in low doses for a long duration. In many circumstances, the drugs are periodically administered by the owners or quacks and in most of the cases, animals are exposed to only sub-therapeutic dosage of drugs which may maintain sub-optimal concentration for a prolonged period.

Therefore, such exposure may act an important factor to exert selection pressure for the reserve microbiome for acquiring the resistance gene(s) and animals to be an important reservoir of bugs. As many of the challenging and omnipresent resistance gene(s) are carried on the mobile genetic elements (like plasmid, phages, transposon or integron), it is not impossible that the resistance gene(s) or the bugs will not remain restricted within in animal ecosystem; rather these gene(s) or bugs with such resistance determinants may be transmitted to contaminate the environment and infect human beings, via direct and or indirect means. Such transfer of resistance factor may compromise the efficacy of the antimicrobials and preservation of the potentiality of antimicrobials may not be possible.

It is worth mentioning that the spectrum of antimicrobials used in agriculture, particularly in food and pet animals, also include many of "critically important" antimicrobials for human medicine as defined by the WHO, like the compounds that belong to classes of aminoglycosides, quinolones, β-lactam compounds including higher generation cephalosporins. Thus the possibility of the role of animal reservoir to transfer resistance to these compounds to human beings cannot be undermined and resistance against these substances can affect therapeutic options available for life-threatening diseases. In such circumstances, the main objective to preserve the efficacy of available therapeutic armamentarium in human and animal medicine is at stake. This is further complicated with a discovery void for a considerable period of time when no new antimicrobial has been developed and become available clinical use.

There are many of the formidable superbugs especially the vancomycin-resistant enterococci (VRE), the extended-spectrum β-lactamase (ESBL) or metallo-β-lactamase producing *Enterobacteriaceae* and the recently detected bacteria with plasmid-mediated colistin resistant (*mcr-1*) gene which gained attention from the scientific community across the globe for their ubiquitous nature and association with a spectrum of life-threatening diseases. Such bugs were reported to take a heavy toll of human life in recent past and have become a persisting problem for the clinicians.

Antimicrobial resistance: Human-Animal Interface

Considering the intimate contact of the human beings with the livestock and common ecological niches they share, these formidable pathogens are no longer restricted within human beings or animals. Thus, it is not impossible to find an animal pathogen entering human ecology or flora via food chain or other routes or to detect human pathogens causing serious infection in animals. This fact is further substantiated by recent observations in the research works carried out at the ICAR institutes where metallo-β-lactamase (NDM) producing carbapenem resistant E*nterobacteriaceae* and glycopeptide resistant *Staphylococcus aureus* (VRSA) in animals were detected. Interestingly, neither carbapenem (imipenem) nor glycopeptide (vancomycin) are commonly used in veterinary medicine in India and these drugs are mostly reserved for treating serious infection of hospitalized human patients. Studies also revealed the presence of extended spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* and methicillin resistant *S. aureus* (MRSA) in various food animals. Such pathogens are not only responsible for animal diseases but also can cause serious nosocomial infection in human beings, especially in those admitted in ICU/CCU (intensive care unit/ critical care unit) or having post-surgical infection. Moreover, healthcare premises are not the only units where such infections are noticed. It is also possible that human patients in the community set-up beyond the hospital premises with certain risk factors like long term chemotherapy, immune-compromised status, chronic renal failure or frequent renal tract and respiratory tract infections, diabetes and burn injuries can contract such infections. The most complicated fact about the MDR pathogens recorded in human or animals is that these pathogens usually carry several antibiotic resistant genes in their plasmid, transposons, integrons or other mobile genetic elements which possibly play a crucial role in rapid dissemination of these drug resistant genes via horizontal gene transfer.

Strategies for Control and Containment of AMR

For appropriate control and containment of AMR the essential steps include optimization of usage of antibiotics in livestock so as to minimize the interaction between pathogenic microorganism and antibiotics, preventing the dissemination of resistant isolates and judicious use of antimicrobials for treatment of infection and the most importantly adequate IPC (infection prevention control) and or biosafety measures. Taking these points into account, WHO, FAO, and OIE formed a Tripartite Alliance with One Health Approach concept. The National Action Plan on AMR has come into force since then which reiterates the need for integrated surveillance, proper infection control measures, optimal and rational use of antibiotics and research and development initiatives for development of newer antibiotics or antimicrobial compounds. The discovery of newer novel small-molecule antibacterial drugs is underway, but in much slower pace. There are few alternative approaches, like vaccine, bacteriocins, antimicrobial peptides, phytobiotics, immuno-modulators and cytokines, quorum sensing inhibitors, CRISPR-Cas technology, phage therapy and chicken egg yolk antibody, which are also being explored for their therapeutic or prophylactic effect to extenuate the problem of infectious diseases.

Antimicrobial Resistance: Indian Scenario

India is one of the leading antibiotic consumers in the world and occupies a position among top five countries as far as animal health sector is concerned, although the quantity may not be too high in respect of its animal population. However, the exact quantum of antibiotics used in different sectors viz. human, animal health and agriculture remain unknown. Availability of such information is an essential pre-requisite for developing appropriate guidelines for use of antimicrobials. Besides, reliable epidemiological data on antimicrobial resistance of important pathogens of public health importance is very important. But in India, the research on antimicrobial resistance is mainly focused on burden and patterns of resistance and unfortunately, we do not have any national database on AMR for different pathogens, particularly with regard to the livestock sector. Recently, Indian Council of Agricultural Research established a national network - Indian Network for Fisheries and Animals Antimicrobial Resistance (INFAAR) to conduct a nationwide survey of AMR pathogens in livestock and fishery sector using the uniform sampling frame and standard operating protocol. The network will initiate nation-wide surveillance on AMR, produce national database on AMR in livestock as committed in the NAP on AMR.

The Way Ahead

AMR in true sense is a multi-sectorial problem. In spite of this, relatively little attention has been paid to know the degree and impact of the use of antibiotics in farm animals to the overall problem of antibiotic resistance and public health. It is, therefore, necessary to make an integrated effort involving people from human health, animal husbandry, agriculture, pharmacy, industry, environment, policy makers and most importantly, the end users to identify the key areas in which action is needed to minimize and contain antimicrobial resistance. Focus on development of newer generation antibiotics and rapid diagnostic tests, research work on national surveillance of resistant pathogens must be given priority. Besides, it is equally important to update the human physicians and veterinary officers on the appropriate or standard treatment guidelines to avoid unnecessary usage of antibiotics. Stringent regulations enforcing control of antibiotics and making their availability over the counter punishable, is the need of the hour. Because, if there is "**no action today on antimicrobial resistance, no cure tomorrow**."

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ED–L–05

Present status of Foot and Mouth Disease vaccine research in India

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Foot-and-Mouth Disease (FMD) is one of the most contagious diseases of cloven hoofed livestock, with serious socio-economic implications. Among the seven serotypes identified worldwide, outbreaks due to serotypes O, A and Asia 1 have been encountered in India, with predominance of serotype O. Vaccination is recognized as the only available tool in control of FMD. Therefore, in order to control FMD in the country, national level control program is in place, as per the progressive control pathway for FMD, laid down by OIE/FAO. The control programme is implemented since 2003-04, initially in selected 54 districts. As of now the programme is being executed in entire country since 2017-18. The control program focuses on regular vaccination of all the eligible cattle and buffaloes twice a year with inactivated vaccine to maintain herd immunity with regular sero-surveillance/monitoring of animal population.

Currently used inactivated vaccine produces short duration of immunity. Therefore, research emphasis is on to induce a durable virus neutralizing antibody response. In this direction, research and development efforts are underway to develop new generation vaccines with proven efficacy for use in Indian cattle. Further, research on development of highly effective inactivated vaccines that can induce long duration immunity and development of thermotolerant FMD vaccine are current priority. Cattle challenge based potency test to ensure quality of FMD vaccine being used in the country is becoming difficult due to non-availability of large number of FMD sero-negative animals to test more number of vaccine batches. In that direction, research has been taken up in the areas of serology-based alternate test(s) to cattle challenge experiment and use of laboratory animal as an alternate to cattle.

In the years to come, it is expected that the FMD control programme in India will be fruitful and help Indian livestock to be free from this dreaded disease.

ED–L–06

Pandemic Influenza A(H1N1)pdm09 virus (pH1N1): The unseen and unsaid

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Introduction

Influenza A(H1N1)pdm09 virus (pH1N1) was causal factor of the recent flu pandemic which took several hundreds of lives. Influenza virus leads to respiratory disease that result in nasal secretions, barking cough, decreased appetite etc, and human beings serve to be the dead end hosts for the virus. Seasonal reassortment and reemergence of the virus, leads to severe and untreatable infections and introduces drug resistant strain generation and antigenic shift which plays role in viral entry, transmission and tissue tropism. In order to combat with the virus, several drugs and vaccines are under clinical trials. Of those only few antiviral drugs like Zanamivir and Oseltamivir, have been reported to help prevent effects of swine flu, if at all they are administered in the patients within 48 hours of the onset of symptoms. Search for alternative vaccination which can efficiently fight with the reassorting virus is the hot topic nowadays. Hence our article highlights the specific natural bioactive compounds, vaccines and antivirals, present in the market and their fates against the viral attack.

Introduction and origin of the influenza viruses

Influenza A virus is around 80-120 nm in diameter belonging to the *Orthomyxoviridae* family. The genome size of virus is ~13.5 kb. Influenza RNA genome is segmented into 8 different regions which encodes 11 different proteins i.e. envelope proteins HA and NA, viral RNA polymerases PB2, PB1, PB1-F2,PB and PA, Matrix proteins M1and M2 and non structural protein NS1 and NS2 (NEP) essential for efficient pathogenesis and viral replication. Influenza viral infections result due to emergence of new viral strains resulting from reassortment of viral genes HA and NA. These genes are major surface glycoprotein's responsible for viral attachment, replication, pathogenesis in the host. In order to cause severe infection, virus needs to adapt to the conditions of the host and acquire modifications in its genome especially in HA and NA. The major viral adaptations depend upon the host cellular tropism, transmission and viral infectivity in the new host systems.

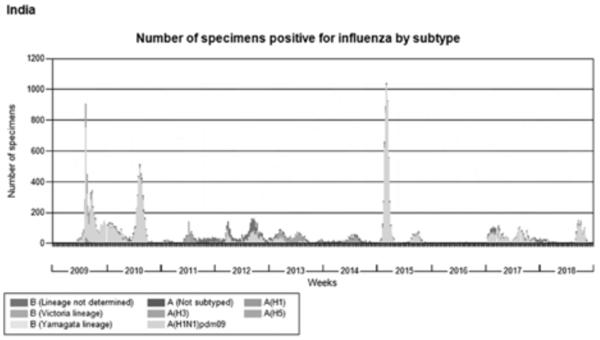

Fig. 1: Influenza laboratory surveillance information of India 2009-2018 as on 22 Nov. 2018 (source WHO 2018)

H1N1 Epidemiology

H1N1 pandemic was first observed in the year 1918 in Spain which caused millions of deaths. Later several outbreaks were reported due to influenza virus in 1968, 1998, 2009, etc. Recently, the World Health Organization (WHO) GISRS/NIC laboratories tested more than 84,313 specimens during 15 to 28 October 2018 (data as of 09-11-2018); for influenza virus detection. The WHO GISRS laboratories tested more than 84313 specimens during that time period. Out of those 2,145 were positive for influenza viruses, 1,845 (86%) were typed as influenza A and 300

(14%) as influenza B. Of the sub-typed influenza A viruses, 905 (64.5%) were influenza A(H1N1) pdm09 and 499 (35.5%) were influenza A(H3N2). Of the characterized B viruses, 54 (52.4%) belonged to the B-Yamagata lineage and 49 (47.6%) to the B-Victoria lineage. Summary of Influenza virus detections from influenza laboratory surveillance information of India during 2009-2018 (as on 22 Nov. 2018; source WHO) has been revealed in Fig. 1. Antigenic drift results in mutation in the genome of virus making it efficient in pathogenesis. 90% of deaths are more prevalent in adult population indicating that the dysregulation of immune system increases along with age. Influenza virus infection leads to symptoms like fever, body pains, sore throat, loss of appetite, headache, cough, aching muscles and joints, weakness, fatigue, pneumatoceles, mediastinal emphysema, and secondary renal failure leading to fatality in extreme cases.

Reservoir, vector and host for the virus

Pigs are the most important reservoirs for influenza A viruses, acting as intermediary host for both interspecies transmission and genetic reassortment of virus. This is due to high susceptibility of pigs to human and avian influenza viruses that makes virus more pandemic to host. Co-infection was observed in pigs by both avian and human influenza viruses. Waterfowls and wild boars are also important reservoirs for influenza viruses. The distinctive alleles of influenza viruses may lead to its transmission to the new mammalian hosts like recently influenza emergence had been found in bats in Central America.

Molecular mechanisms and ramification

The antigenic properties of different influenza viruses are different for binding to the sialyl moiety of host. Some strains have capacity to bind to both glycan linkages, making them more virulent, resulting in various intestinal illnesses. Mutations in the HA region of virus may alter the binding affinity of virus to host through antigenic shift leading to differential pathogenesis by different strains. Several studies have been done on molecular mechanisms of virus attacks. HA helps the virus to attach to sialic acid receptor of the host cell and is followed by endocytosis. Viral RNA polymerase transcribes the viral genome and releases the mRNA for further processing. Viral genomic ssRNA synthesis takes place after mRNA release by exploiting host cellular machinery. It is followed by assembly, maturation and ultimately in production of progeny virions by budding from infected host cell membrane.

Fig. 2: Virus structure, showing structure of one of the anticipated antiviral against influenza virus (inside) and areas (shaded background) on the world map with pH1N1.

Treatment and management

Several antiviral drugs are available for the treatment of influenza disease. One of the important actions of these antivirals is inhibiting the neuraminidase which interferes with release of progeny virions from the surface of the infected host cell. These antivirals include drugs like Zanamivir, Peramivir, Favipiravir which are administrated by intravenous route and Oseltamivir which is administrated orally showing their efficiency against influenza viruses except Amantadine because H1N1 is Amantadine resistant. Peramivir and Oseltamivir inhibit the action of NA by binding to NA site, leading to the inhibition of progeny virion production. Also combination drugs are introduced like in addition to Oseltamivir and Zanamivir, one more drug called favipiravir is administered, which treats inhibits the RNA polymerase present in influenza viruses, more efficiently. Favipiravir has a significant role in inhibiting the replication of 2009 H1N1, drug resistant viruses, swine triple assortments strains. Also current studies showed the role of interferon inducible trans-membrane protein family membranes 3 (IFITM3), as potential candidate for curbing the influenza infections. We analyzed more than 5,00,000 compounds and conferred 4 compounds may prove quite promising novel antivirals [Fig. 2] against influenza virus [Saxena SK *et al.*, Pers. Commun.].

Vaccines

Vaccination is the most effective way to prevent infection and severe outcomes caused by influenza viruses. Development and production of influenza vaccines, planning for their supplies and use as well as provision of other respective health care resources are essential components of a comprehensive seasonal and pandemic influenza response. In order to design potential vaccine candidates against the H1N1 sequence based targets approach is needed. Available H1N1 vaccines which have been developed based on NA and HA do not provide long term protection due to the antigenic shift and reassortment of virus. Viral nucleoproteins can be used as potent epitopes for targeting as universal vaccine. A new and more promising approach is micro-needle based vaccines which involve micro needles coated with inactivated influenza virus inducing humoral and cellular immune responses which provide long time protection. Adjuvants also play an important role in association with the subunit vaccines which can induce appropriate immune response. DC-Chol/DPPC (Cationic liposomes containing a cationic compound neutral phospholipid) has shown strong immunogenicity against H1N1 experimentally suggesting that physiochemical properties of cationic liposomes are necessary for efficient adjuvanticity for subunit vaccines. Also a vaccine, like MF59-adjuvanted vaccine is more immunogenic, and provides persistent protection against virus. Adjuvanted monovalent vaccines are based on nanoparticle having Toll like receptor (TLR) ligands in combination with poly (d, l-lactic-co-glycolic acid) (PLGA) and provide efficient protection against H1N1. Flu vaccination is the best way to prevent flu and its potentially serious complications and prevents millions of flu illnesses and related doctors' visits and tens of thousands of hospitalizations. As per the Advisory Committee on Immunization Practices on 20 June 2018, the overall vaccine effectiveness the 2017-2018 flu vaccine against both influenza A and B viruses is estimated to be 40%. The data revealed 25% protection against A(H3N2), 65% protection against A(H1N1) and 49% protection against influenza B viruses (CDC, 2018).

Control measures

Control measures include simple practices like avoiding the crowd gathering from infected people, protection from infected aerosols by using hygienic masks or a simple nose cover. Suspected individuals should be kept under medical supervision and should be isolated from the young children and the immune compromised individuals. Travelers from the pandemic or endemic regions should be checked before and after their journey, quarantine measures are importantly needed at airports. Few cases are reported from the prisons also, so it is advisable to keep limited prisoners in single cell and affected prisoner should be under medical supervision. Awareness programs should be conducted on swine influenza virus infection and infection process using the media. The transmission of influenza infection occurs through bioaerosols hence personal hygiene is important.

Future perspectives

Influenza outbreaks are reported throughout the world due to emergence of various influenza strains due to reassortment of various strains, climatic changes and rapid globalization; hence it is extremely necessary to have a global alert system, which can predict the future probability of pandemics by its statistical analysis. Need of the hour is to device best practices in prevention, diagnosis, patient care, treatment and use of antiviral medicines. Drugs and vaccines need to be modified with sequence based drug targeting approaches, which may prove to be more efficient. A combined approach from local population, virologists, researchers, doctors, drug designers and policy makers is needed in curbing the emerging and re-emerging viral infections. This knowledge has to be gathered and disseminated to the medical community and newer areas have to be identified that require research and collaboration. Hence to device new strategies are the need of the hour and it should be addressed with a sense of urgency.

ORAL PRESENTATION

ED–O–01

Molecular characterization of *Toxocara* of Canine and Bovine origin

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Toxocara is an important ascaridoid genus containing species of human and animal health significance such as *T. canis*, *T. cati* and *T.vitulorum*. Although adult stage of the parasites canbe identified on the basis of morphological characters but larval and/or egg stages of different species of *Toxocara* are indistinguishable from each other. Considering this fact during the present investigation three species of *Toxocara* such as *T. canis* of dogs from Arunachal Pradesh and Tamil Nadu, *T. cati* of cat from Arunachal Pradesh and *T.vitulorum* of cattle, yak and mithun were collected respectively from West Bengal, Arunachal Pradesh and Nagaland were characterized on the basis of DNA fingerprinting and comparative analysis of sequence information of two mitochondrial genes and two nuclear sequences. By RAPD fingerprinting a total of 14 arbitrary primers of 10 mers could differentiate the three species of *Toxocara*. By PCR based RFLP (using *Rsa*I) *T. canis* could be clustered in one group whereas *T. cati* and *T.vitulorum* were clustered in another group. Phylogenetic analysis of *atp*6, 12S and transcribed sequences could differentiate the three species and sequence information generated during the present study was comparable to the sequence information published earlier in centralized repositories of public data base. Within *atp*6 gene number of preferred and non preferred codons also varied within *T. canis, T. cati* and *T.vitulorum* which has been previously described in different insect species. Amplification of NUMTs was ruled out on the basis of absence of premature stop codons (for coding gene) and topology of phylogenetic tree (for noncoding sequence). Finally ITS sequences were identified as useful sequences for development of diagnostics and molecular prospecting of *Toxocara* due to less evolutionary distances compared to mitochondrial genes and also due to availability of universal primer which can amplify any species of nematodes.

ED–O–02

Outbreak of *Goatpox virus* infection in Wild Serow (*Capricornis rubidus*) in Mizoram, India

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Goatpox virus (GTPV) belongs to the genus *Capripoxvirus* associated with characteristic clinical lesions in fully susceptible breeds of sheep and goats. Till date there is no report of outbreak of GTPV infection in any wild ruminants. The present study reported the outbreak of GTPV infection in wild serow (*Capornicus rubidus*) in Mizoram, India. A total of 113 wild serow carcasses recovered from seven districts of Mizoram during May 2015 to October, 2016. Post mortem exanimation revealed presumptive pox like lesions. Clinical specimens (lung, skin, trachea) were examined for causative agents. GTPV could be isolated and identified from all the animals examined in the laboratory. All the isolates were confirmed by specific PCR assays (RPO30 and GTPV p32 genes). Phylogeneitc analysis exhibited 99.8% sequence homology with GTPV from India and other parts of the world. To the best of the knowledge of the authors this is the first ever report of GTPV infection in any wild ruminants in the world.

ED–O–03
Ehrlichiosis: An Emerging Threat to Livestock and Pet Population in India in the Climate Change Scenario

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Infectious diseases are major concern in livestock production and general well beings of pets. Emergence and reemergence of various vector borne diseases is considered as a prominent health issue for humans and animals in climate change scenario. Ehrlichiosis is a tick borne rickettsial disease caused by various *Ehrlichia* species. *Ehrlichia* is an obligate intracellular pathogen and various species are reported to infect ruminants, dogs and humans. Some of the species like *E. ewingii* and *E. chaffeensis* are of zoonotic importance. In India, information about the epidemiology and genetic diversity of prevalent *Ehrlichia* species is lacking. In the present study, we tested samples collected from 302 locations from 11 districts of Himachal Pradesh and 12 places from Punjab and Haryana. Samples from cows (n=260), buffaloes (n=10), goats (n=5), horse (n=1) and dogs (n=38) including blood (n=127) and ticks from body (n=187) were collected. Samples were screened for ehlichiosis using family and species specific PCR tests. Overall 39.1% (123/314) prevalence was detected in all samples. It includes 37.3% in cow, 40.0% in buffaloes, 59.9% in goats and 50% in dog samples. Total 29.1% (37/127) blood and 46% (86/187) tick samples were positive for ehrlichiosis. Cytological examination of blood smear from cows and dogs showed 13% positivity. Among tick samples, *Riphecephalus microplus*, *Rhipicephalus sanguineus*, *Rhipicephalus annulatus*, *Haemaphysalis bispinosa*, and *Hyalomma anatolicum* were found carrying *Ehrlichia* spp. The 16S rRNA gene sequence analysis of representative samples showed the prevalence of different strains of *E. canis*, *E. platys* and still unidentified species of *Ehrlichia*. These results are indicative of high prevalence of ehrlichiosis among livestock and pets, which may be a serious health hazard in case of climate change induced conducive conditions for multiplication and spread of tick vectors.

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ED–O–04
OMP profile of *Pasteurella multocida* isolates of Buffalo

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The OMPs from 16 isolates (14 Cap A isolates and two Cap B isolates) of *Pasteurella multocida* were extracted using HEPES-Sodium Sarcosyl method and OMP profile was analyzed on SDS-PAGE. Among these isolates Cap A isolates yielded 6 polypeptide bands of approximate molecular weights of 19-76 kDa, whereas the Cap B isolates showed 6 polypeptide bands of 14.7-80 kDa. Based on stain intensity, 38 kDa and 32.5 kDa were considered as major polypeptide bands in Cap A and Cap B isolates, respectively.

ED–O–05

Isolation and characterization of lytic phages against causative agents of bovine mastitis in North-Western Himalayas

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Bovine mastitis is one of the major diseases causing heavy economic loss. Continuous use of antibiotics to treat such infections has generated the emergence of antibiotic resistance among the common causal organisms like *Staphylococcus aureus* and *Escherichia coli*. As an alternative to antibiotic therapy against such infections, lytic bacteriophages are being investigated as therapeutic agents. In total, twenty (ten against each bacteria) lytic phages were isolated from sewage as well as infected milk samples with an aim to develop a therapeutic cocktail to treat mastitis. Out of twenty phages, four phages designated as PS5, B57PHI(L) against *S. aureus;* and PE9 and B57SCI(E) against *E. coli* were selected on the basis of their host range, phage burst size and stability at different temperatures. Comparative *in vitro* lytic efficacy of these phages at 10^7 PFU/mL were at par with tested antibiotics. At the same concentration (10^7 PFU/mL), no significant loss of phage host-specific lytic activity was observed when tested against their host in milk containing medium. When different combinations of the phages were tested together neither cross host-activity nor cross-interference was observed and combinations were equally effective as a single phage. Further, studies in animal modal are required to establish these phages as ideal therapeutic agents for the treatment of bovine mastitis in the region.

SESSION – VI

PRODUCTION AND REPRODUCTION BIOTECHNOLOGY

LEAD PAPERS

PRB–L–01
New vistas in Genomics research for livestock improvement

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India is fortunate to have wide variety of indigenous farm animal germplasm distributed throughout its agro climatic zones and is considered as one of the several mega-biodiversity centres of the world. The domesticated animals of India are represented by a broad spectrum of native breeds of cattle, buffalo, sheep, goat, poultry, camel, equines besides species including yak and mithun. Among them, cattle and buffaloes are considered to be important livestock species contributing to greaterr output value of the livestock sector. Most of our indigenous breeds have evolved over the years for their utility under a certain set of agro climatic conditions and possess some unique traits, which not only distinguish them from others but also have special economic role to play.

India is home for the largest milch animal population in the World and is the world's largest milk producer. The domestic requirement of milk in the next 10 years would be about 200-210 million tonnes. In this context, increasing per animal productivity would be a major focus. Livestock genomics rides on the wave of the genome projects, and there have been massive advances in the field in the last decade. Hence it becomes imperative to review the efforts being made in the area of animal genomics so as to define a road map for future systematic and meaningful exploitation of livestock genome as a major milk and meat source through genomics and functional genomics approaches. Livestock genomics can be sub-divided into: structural genomics, comparative genomics, and functional genomics. Through structural and comparative genomics progressive changes in the genome between species assist also in the study of the evolution of genomes and to the identification of alleles, and their origin, thus contributing to genetic and population studies. Through functional genomics it is becoming clear that phenotypic variation does not only rely on the gene alone, variation in gene expression, but on a whole range of external factors. This is evidenced by the fact that a specific phenotype might be associated with a large number of QTLs, but only a few genes play a major role.

These advances have also opened up new vistas in the field of animal science by identifying the genes/markers governing complex traits. The genome is being explored to identify genes/markers that explain the observed variation in the complex traits. The molecular markers can be used to select the animals at a very early age for specific desired characters. A majority of the traits of economic importance in farm animals are polygenic in nature and are influenced by a variety of environmental and developmental factors, which makes them difficult to isolate and identify at the genome level. Although, screening and identification of some of the quantitative trait loci (QTL) using molecular markers has been successfully carried out in cattle, pigs and other livestock species, most of the genes affecting a polygenic trait such as growth, milk yield, reproduction, diseases resistance are still unknown and identification of genes/alleles underlying these QTL effects remains a challenging task.

Genomic selection(GS) was proposed by Meuwissen et al. (2001) and the advantage of this approach is that DNA markers covering the whole genome are used so that potentially all the genetic variance is explained by the markers. Traditional marker-assisted selection (MAS) focuses only on those regions which are relatively certain to influence the trait of interest and leaves most of the genome and much of the genetic variation unaccounted. Genomic selection puts the emphasis on regions with the largest effects, while also accounting for the other genetic variation in the remainder of the genome. This approach holds importance as genes affecting most economically important traits are distributed throughout the genome and there are relatively few that have large effects with many more genes with progressively smaller effects. Next-generation sequencing (NGS) technologies are widely used for detection of millions of Single Nucleotide Polymorphisms (SNPs) and also provide a means of assessing their variation. This information is useful for composing subsets of highly informative SNPs for region-specific or genome-wide analysis and to identify mutations regulating phenotypic differences within or between populations. SNP identification through NGS led to the development of Genome-wide panels of SNPs. The genetic merit of animals can be estimated by genomic selection, which uses genome-wide SNP panels as markers and statistical methods that capture the effects of large numbers of SNPs simultaneously.The primary requirement for GS is the 'reference population' (discovery dataset) with moderate number of animals for which phenotypes are recorded for all the relevant traits. This dataset is genotyped with large number of SNPs. The marker data is used as input for the prediction equation to estimate breeding value (EBV). The second requirement is validation sample consisting of large number of animals with phenotypes recorded for the traits. The validation samples are genotyped with markers that are to be tested and used commercially. The prediction equation is tested to assess its accuracy on this independent sample set. The selection candidates are genotyped for the markers and the prediction equation is estimated in the discovery data used to calculate GEBV. One of the most economically important aspects of GS is the possibility to use it for improving selective breeding. Genomic selection approach is particularly attractive for expensive-to-measure traits. It also can be more easily applied to traits where the heritability is low and genetic change is slow, and traits that are difficult to measure. The most likely application of this approach will be in dairy cattle where, in principle, bulls could be selected on the basis of genomic information alone and used for mating long before their progeny test results would be known – considerably reducing generation interval and accelerating progress.

The transcriptome is the complete set of transcripts in a cell, both in terms of type and quantity. Transcriptome analysis is a powerful strategy in dissecting the relationship between genotype and phenotype of a cell. The complete transcriptome profiling of cells is crucial for the characterization of gene expression signatures that contribute in defining biological and physiological processes in the cells. Deep RNA Sequencing (RNA-Seq) uses massively parallel sequencing to allow transcriptome analyses of genomes at a far higher resolution than is available with Sanger sequencing- and/or microarray-based methods.

Phenomics, the study of the phenome, is a rapidly emerging area of science, which seeks to characterize phenotypes in a rigorous and formal way and link these traits to the associated genes and alleles. Phenomics is the science of large-scale phenotypic data collection and analysis, while the phenome is the actual catalog of measurements. The phenotypic data and metadata descriptions of the experimental conditions are captured in formats that allow detailed data analysis. These analyses would ideally identify relationships between genotype and phenotype as well as reveal correlations between different phenotypes.

PRB–L–02
Regenerative potential of canine mesenchymal stem cells: Basics to implications

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Therapeutic applications of the stem cells is indeed one of the powerful technologies, but despite having witnessed substantial research with remarkable outputs of adult MSC populations for the therapeutic potential, much remains unknown about their growth regulation and markers for isolation. Mesenchymal stem cells secrete immunomodulatory molecules that establish a regenerative microenvironment for injured tissues or organs to reduce the damage and to augment self-regulated tissue regeneration. Autologous or allogenic MSCs administered via blood stream increase the concentration of MSCs that are drawn to tissue injury sites, where they accelerate the tissue repair process. Therapeutic effects of MSCs are also mediated by paracrine factors secreted by these cells. To enhance the therapeutic efficiency of exogenously administered MSCs, a clear understanding of the biological concepts underlyingstem cell homing remains a crucial preamble. Major challenge in MSCs research is validation of *in vitro* data by finding a suitable animal model.

We have conducted systematic research with an objective to characterize *in vitro* expanded canine BMSCs, demonstrated homing mechanism by luciferase reporter aided *in vivo* tracking in mice and therapeutic evaluation in canine clinical cases of spinal cord injury in allogenic mode. While presenting the work conducted for canine stem cell profiling, I will highlight and elaborate the work done using bone marrow derived canine mesenchymal stem cells in the experimental animals, namely rat, rabbit and guinea pigs and also the work conducted in the canine. As the growth and differentiation are tightly linked processes, the pathways involved in differentiation into adipogenic, chondrogenic and osteogenic lineages remain very important. The differentiation of MSCs into these lineages is affected when these pathways are perturbed by inhibitors of cell surface receptor function. A comparative evaluation of therapeutic potential of undifferentiated and osteogenic differentiated canine and guinea pig BM MSCs in guinea pig model was conducted and the results of comparative therapeutic potential in fracture healing using undifferentiated and osteogenic differentiated MSCs demonstrating no major difference in therapeutic potential between differentiated and undifferentiated BMSCs will be detailed out. During the talk, it will also be highlighting the importance of the preclinical studies using small experimental animals, especially when one has to understand the *in vivo* functions of these cells, post injection in the ailing animals, through live cell tracking. The major considerations for the preclinical animal model study would also be detailed out, such as selection of animal model, general considerations including the delivery mode, dose, route etc. Presentation shall be highlighting our group's effort on the preclinical and clinical data and the *in vitro* and *in vivo* data generated from our laboratory.

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PRB–L–03
Cellular reprogramming approaches in domestic animals

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Summary

The birth of Dolly, first cloned animal produced from differentiated somatic cells, has abolished the old dogma of developmental biology that terminal cellular differentiation is an irreversible process and can't be reversed back to its primordial stage. The process that overturns the old dogma called somatic cell nuclear transfer (SCNT), in which oocyte reprogrammes an adult somatic cell into a totipotent embryo stage that can further develop a new life or embryonic stem cells. Later, the discovery of induced pluripotent stem cells (iPSC) by ectopic gene expression has revolutionized the cellular reprogramming approaches, and now has become common practice in many laboratories. This lead paper highlights the cellular reprogramming approaches in general and some examples to this field, particularly in farm animals, including buffalo- An animal that brings India's white and pink revolution.

Cellular reprogramming by SCNT

SCNT popularly known as an animal cloning technique, in which nucleus is removed from matured oocyte and a nucleus or complete cell (example, skin cell) is transplanted into an enucleated oocyte. The merged somatic cell and oocyte called one-cell stage embryo that further developed into blastocyst stage embryo (please see figure 1A). The generated blastocyst can allow producing live cloned animal or harvesting of embryonic stem cells (ESC). The cloned animal is genetically identical to its donor nucleus or somatic cell. ESC are pluripotent and harbor defined characteristics such as 1) self-renewal ability, 2) proliferation for an indefinite and undifferentiated state, 3) differentiate into different cell types, 4) teratoma formation in nude mice, 5) germline transmission.

The first report of SCNT-based cellular reprogramming in mammals was reported by Campbell et al. 1996. In this study, the culture cells from day 13 ovine fetus were used as nuclear donors and successfully produced two cloned sheep, named Megan and Morag. Just after one year, the same group reported the birth of Dolly, the first mammal cloned from an adult body cell (Wilmut et al., 1997). In the last 20 years, this technology has well established and has been widely used important tool for agricultural and biomedical research applications. At present, most species of domestic animals, including cattle, sheep, goat, pig, horse, dog, and buffalo, have been cloned using SCNT.

Potential applications of SCNT are 1) to make multiple copies of elite animals, such as high milk producing females or proven breeding bulls; 2) to create transgenic animals that have human genes inserted into their genome to serve as bioreactors to produce therapeutic proteins like insulin and clotting factors, preferably in milk; 3) to create disease model animals that are designed to express certain human diseases; 4) xenotransplantation represents organs developed in the animal body for transplantation to humans. The pig is a preferred animal to support the development of human organs for xenotransplantation; 5) conservation of wild animals or endangered species. Despite multiple applications, SCNT is still an inefficient process and on average only <5% of the transferred cloned embryos could produce healthy offspring. The faulty or improper reprogramming of differentiated somatic cells is considered a major problem of the low success of SCNT. Research efforts are on-going worldwide to unravel the exact cause of faculty reprogramming and to improve SCNT outcome.

Cellular reprogramming by transcription factors

The first report of transcription factors based cellular reprogramming in mammals was reported by Takahashi and Yamanaka in 2006. In this study, fully differentiated mouse fibroblast cells were reprogrammed to a pluripotent state by ectopic expression of four transcription factors, namely OCT4, SOX2, c-MYC, and KLF4 (please see figure 1B). This approach of cellular reprogramming called induced reprogramming and generated specialized cells called induced pluripotent stem cells. This report has ignited worldwide intensive research on iPSC, and iPSC has been generated from many cell types such as skin cells, liver cells, organ cells, brain cells, and blood cells, both in human and farm animals. The iPSC possess identical characteristics with embryo-derived ES cells, which includes morphology, infinite proliferation, differentiation to cells of three germ layers, and germline transmission. This approach of cellular reprogramming paves the way for the generation of genetically identical stem cells and animal-specific cells for cell-based therapy, without the use of oocytes that require for SCNT. This approach is extremely useful in those farm animals, in which the pre-implantation embryos and oocytes not available for manipulation. iPSC approach is useful for cell-based therapies and they can also serve as donors to produce transgenic animals for agriculture and biomedical application.

Many researchers used human and mice reprogramming factors to reprogram the differentiated somatic cells of various mammalian species as well as non-mammalian species and successfully generate iPSC, which indicate that these reprogramming factors are highly conserved across the wide phylogenetic range. In the very first report, viral-based reprogramming factors were used to effectively integrate into the somatic cell genome. Viral particle-based reprogramming has been widely used to generate iPSC; however, due to chance to revert of mutagens, alternative methods are being studied and extensively developed. Newer methods include the use of non-integrating rival particles (adenoviruses, adeno-associated virus), piggyback and sleeping beauty transposons, direct reprogramming using small molecules, mRNA or proteins that bypass genome insertions. At present, the aim of any approach is to develop integration and feeder-free procedure to avoid the occurrence of insertional mutagenesis and to avoid contamination of feeder cells, particularly for cell-based therapies and SCNT.

SCNT-based cellular reprogramming attempts in buffalo

Buffalo, a highly valued farm animal, has originated from the Indian subcontinent and widely distributed in many countries, of which the majority of population is located in India, China, and Pakistan. Since the decades, buffalo has been contributing to the growth of Indian agriculture and has played a major role to achieve the white and pink revolution. India has considerably been contributing to various research and development programmes in buffalo, including breeding strategies, reproduction and production management, and advanced research like SCNT and iPSC. Through extensive research for the last 10 years, India has now become a leading country in the field of buffalo cloning research and produced the highest number of buffalo clones in the world.

In comparison to other farm animals, the limited studies were done in the field of cellular reprogramming in buffalo worldwide. In India, a very first attempt was made by researchers at the National Dairy Research Institute (NDRI) during 1995-97, in which cells from fertilized or in vivo embryos were injected into enucleated oocytes to generate blastocyst stage embryos. The method was similar to a method that produced the Dolly. No blastocyst was produced using this approach. The possible reasons could be 1) lack of technical advances at that time, since, highly skilled micromanipulation methods were used; 2) improper culture conditions for SCNT embryos; 3) not much research was done in SCNT during the late nineties; therefore, researchers do not have much access to SCNT literature that may prevent further experiments. Later in 2007, a simplified method of SCNT, named handmade cloning, was used to reprogramme the differential somatic cells such as fibroblasts from adult and fetus, and cumulus cells, and succeed to produce blastocyst stage embryos and finally produced India's first cloned animal. In addition to India, other researchers from China, Thailand, and Italy have also reported SCNT-based reprogramming of a various type somatic cells of buffalo. Since the efficiency of SCNT-based reprogramming is extremely low in buffalo, < 2% live birth rate of a total number of recipients used for embryo transfer, the several approaches and modifications were attempted to improve the reprogramming process, the details about these approaches have extensively been discussed in the review, which is recently published in cellular reprogramming (Selokar et al., 2018).

Buffalo oocytes were also explored for its ability to support the reprogramming of differentiated somatic cells of other species like cattle, goat, and rat (Selokar et al., 2011). This approach of reprogramming called interspecies SCNT and preliminary results suggested that buffalo oocytes, like those from other farm animals, are capable of reprogramming the somatic cells from other species. However, the developmental competence of interspecies embryos was significantly lower than that of intra-species embryos. Recently, we have attempted to reprogramme the somatic cells of horse by transferring it into enucleated buffalo oocytes, but fail to generate blastocyst stage embryos, and all hybrid embryos were arrested at 32-cell stage. This indicates that as the taxonomic distance between the somatic cell and recipient oocyte species increases, the reprogramming ability decreases.

Transcription factors-based cellular reprogramming attempts in buffalo

Human or mouse transcription factors are often used for reprogramming of farm animal cells because the genome of farm animals are not fully annotated and/or many commercial companies provide plasmids containing human or mouse reprogramming factors. For cell type, any type of somatic cells can be used for reprogramming; however, skin fibroblasts are commonly used, simply because skin cells are easy to establish and maintain in the culture, and these cell type has previously validated in many reprogramming experiments.

In buffalo, first attempt to generate iPSC was reported by Chinese researchers in 2012, they reprogramed the buffalo fetal fibroblasts into pluripotent stem cells using retrovirus-based buffalo defined factors; include OCT4, SOX2, c-MYC, and KLF4. These cells displayed similar characteristics of the ESCs, such as morphology, AP staining, and expression of pluripotent markers, the ability to differentiate into 3 germ layers and develop to blastocysts following nuclear transfer. Major disadvantages of retrovirus-based reprogramming are that they may cause insertional mutagenesis and increase the risk of tumor formation. Therefore, we have used an alternative method, transposon-based reprogramming, to avoid these mutagenic effects. Transposons are non-viral elements that are integrated effectively into a host genome with help of their transposase enzymes. There are several advantages of transposon-based reprogramming, including no chance of mutagenesis, large insert capacity, and simple design and easy to multiply in the laboratory. We have used the transposon-based reprogramming method and mouse reprogramming factors to induce pluripotency in buffalo fetal fibroblast cells. The generated iPSC displayed similar characteristics with other iPSC of farm animals, including those were generated using the retrovirus-based method in buffalo.

Conclusions

This lead paper covers the cellular reprogramming approaches, SCNT and iPSC, in farm animals, particularly buffalo studies. Based on current status of SCNT and iPSC, we conclude that there are tremendous opportunities in buffalo to explore more about SCNT and iPSC, and buffalo could also be used as a model to solve many unsolved questions in the field of cellular reprogramming. It will be a major challenge for us to utilize current knowledge, particularly iPSC studies, to generate stable iPSC of buffalo.

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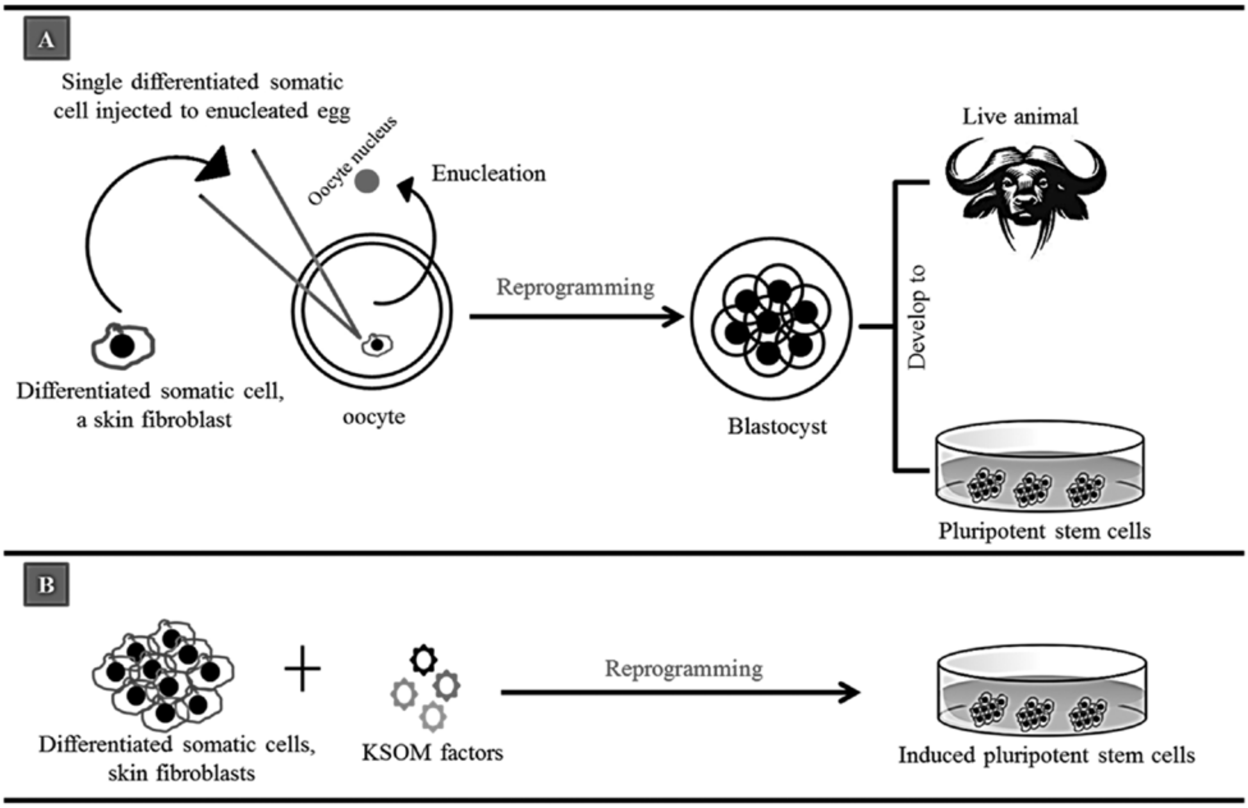

Figure 1. Two most commonly used approaches for studying nuclear reprogramming in buffalo. The first approach called SCNT-based reprogramming, in which differentiated somatic cell is transferred into an enucleated oocyte that develops to blastocyst embryo, which upon transfer to foster animal can give birth to a clone, or, upon the culture of ICM cells can generate nuclear transfer-derived embryonic stem cells (A). The second approach called transcription factors-based reprogramming, in which differentiated somatic cells are transduced with the transcription factors, such as Oct3⁄4, Sox2, Klf4, and c-Myc, that initiates early biochemical events of reprogramming which leads a generation of specialized cells called iPSC, which are similar to embryonic stem cells.

PRB–L–04

CRISPR/Cas: An efficient genome editing tool for functional characterization of genes

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Genome editing refers to the process of making targeted modifications to the genome, its contexts (e.g., epigenetic marks), or its outputs (e.g., transcripts). With the development of recombinant DNA technology, 1970s marked the beginning of a new era for biology. For the first time, molecular biologists manipulated DNA molecules, making it possible to study genes and develop novel medicine and biotechnology. Recent advances in genome engineering technologies are sparking a new revolution in biological research. Rather than studying DNA taken out of the context of the genome, researchers can now directly edit or modulate the function of DNA sequences in any organism.

Of the current generation of genome editing technologies, the most rapidly developing is the class of RNA-guided endonucleases known as Cas9 from the microbial adaptive immune system CRISPR (clustered regularly interspaced short palindromic repeats). Prior to CRISPR/Cas9, genome engineering approaches, like zinc finger nucleases (ZFNs) or transcription activator like effector nucleases (TALENs), relied upon the use of customizable DNA binding protein nucleases that required scientists to design and generate a new nuclease pair for every genomic target. Largely due to its simplicity and adaptability, CRISPR has rapidly become one of the most popular approaches for genome editing.

In general, a CRISPR system works in three stages to carry out a full immune response to invading foreign DNA (Garneau et al., 2010; Hsu et al., 2014). In the first stage, or acquisition stage, DNAfragments of invading plasmids or phages (termed protospacers) are incorporated into the host CRISPR locus as spacers between palindromic repeats. In the second stage, Cas proteins are expressed, the CRISPR array containing acquired spacers is transcribed into pre-crRNA, and the pre-crRNAis cleaved and processed into mature crRNAs by Cas proteins and host factors (Deltcheva et al., 2011). The fully processed crRNA is a guide that contains a spacer sequence responsible for targeting it to the invading genome, as well as all or part of the crRNA repeat sequence, which allows for recognition of the crRNA by Cas proteins and other RNA components. In type II CRISPR systems, the presence of a noncoding *trans*-activating CRISPR RNA (tracrRNA) that hybridizes with the crRNA repeat sequence is critical for crRNA processing, Cas9 binding, and Cas9-mediated target cleavage (Jinek et al., 2012; Deltcheva et al., 2011). In the third stage, Cas proteins recognize the appropriate target with the guidanceof the crRNA and mediate the cleavage of the invading genome, thus protecting the host cells from infection. The action of many CRISPR systems depends on the presence of a sequence-specific PAM that is adjacent to the crRNA target site in the invading genome (Bolotine et al., 2005; Mojica et al., 2009). Cas9 contains two nuclease domains: an HNH nuclease domain that cleaves the target strand of DNA (complementary to the guide RNA) and a RuvC-like nuclease domain that cleaves the non-target strand (Jinek et al., 2012). The resulting DSB is then repaired by one of two general repair pathways- The efficient but error prone Non Homologous End Joining (NHEJ) pathway; and the less efficient but high fidelity Homology Directed Repair (HDR) pathway. A single chimeric guide RNA (sgRNA), which is a fused form of crRNA and tracrRNA (Jinek et al., 2012) simplifies RNA guided engineered nucleases components further.

The use of the Cas9 platform has greatly increased the efficiency of generating transgenic organism, disease models for genetic disorders and cancer, which aids our understanding of the molecular mechanisms of these pathological processes. CRISPR techniques allow scientists to modify specific genes while sparing all others, thus clarifying the association between a given gene and its consequence to the organism. In addition to its nuclease activity, Cas9 can serve as a unique platform to recruit protein and RNA factors to a targeted DNA site, and it has been engineered into powerful tools for sequence-specific gene regulation (Gilbert et al., 2013). To achieve this, transcriptional activators and repressors are fused to dCas9; dCas9 maintains its ability to bind both the sgRNA and targeted DNA, but it lacks nuclease activity and, thus, can serve as a sequence-specific RNA-guided DNA-binding platform. dCas9 alone can efficiently inhibit the transcription of targeted genes through steric hindrance of transcriptional machinery. This novel technique termed CRISPR interference (CRISPRi), as it interferes with the transcription of RNA. CRISPR activation (CRISPRa) has been created by fusing dCas9 to transcriptional activators, such as VP64 and p65AD in mammalian cells.

Therefore, the use of CRISPR/Cas9 allows one to perform studies on the gene expression regulation, which may yield important information about the physiology and biochemistry of different types of cells and could help decipher the molecular mechanisms of disease development and identify potential targets for drug and gene therapy. With the use of a CRISPR/Cas9-based technology in our lab at IVRI, a research work was undertaken to investigate the functional characterization of Thrombospondins (TSPs) and their receptors in ovarian corpus luteum during different stages of development in buffalo.

The thrombospondins (TSPs) are a family of extracellular proteins that participate in cell-to-cell and cell-to-matrix communication and TSP-1 was identified to be the first naturally occurring protein inhibitor of angiogenesis (Lawler, 2000). TSPs mediate their action through interaction with cell-surface receptors CD36 and CD47. In qPCR analysis study, we have observed that mRNA of both TSPs and their receptors were significantly ($p<0.05$) upregulated in late (days 11–16) and regressed (days >17) stage of corpus luteum in water buffalo. The findings of relative expressions were consistent with both immunoblot and immunohistochemistry results in CL. In second experiment, we have demonstrated the effect of exogenous TSP1 on culture luteal cells *in vitro.* In this investigation, we have observed that TSP1 dose and time dependently abrogated both VEGF and FGF2 expression, attenuated luteal cell viability, decreased progesterone concentration in spent media and also induced upregulation of Caspase 3 in cultured luteal cells.

In the next experiment, to validate the transcriptional and functional regulation of thrombospondins we have attempted to knockout TSP1 in luteal cells of buffalo by CRISPR/Cas9 genome editing tool. The experiment was conducted by transfecting 70% confluent culture luteal cells with TSP1 specific SgRNA and Cas9 followed by detection of the knock-out through T7E1 assay. The findings revealed that the viability of TSP1 knocked out luteal cells was improved and the concentration of progesterone in spent media was comparable to that of wild group. Further, mRNA expression of vWF, VEGF and FGF2 expression were significantly elevated in TSP1 knocked out cells. Therefore, it was concluded that thrombospondin regulate luteolytic mechanism by modulating the viability of luteal cells and bioavailability of local angiogenic growth factors in CL of water buffalo.

Thus, the rapid progress in developing Cas9 into a set of tools for cellular and molecular biology research has been remarkable, likely due to the simplicity, high efficiency and versatility of the system. The CRISPR/Cas system is by far the most user friendly. It is now also clear that Cas9 potential reaches beyond DNA cleavage and has brought forth revolutionary changes in genomic research, including genome editing, regulation and imaging.

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PRB–L–05
Dynamics of transcriptome and proteome in embryonic development

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A successful fertilization results in formation of an embryo, which develops according to a predefined genetic programme. It was not until recently much was known about the underlying molecular mechanisms and their regulation. The development of embryo from ova after fertilization results in sequential dynamic changes in transcriptome (Godini and Fallahi 2018) and proteome profile, modulation of chromatin dynamics and other several epigenetic changes. The sequence and pattern of changes in these profiles depend upon the species, even though a general pattern of development can be observed. The elucidation of the transcriptome and its regulation is of central to understanding the biological programs that control mammalian embryogenesis (Lindsay and Copp 2005) Developmental</keyword><keyword>*Genome</keyword><keyword>Genome,Human</keyword><keyword>Humans</keyword><keyword>Information Storage and Retrieval</keyword><keyword>Sequence Analysis, DNA</keyword><keyword>*Tissue Banks</keyword></keywords><dates><year>2005</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0168-9525 (Print. The dynamics of transcriptome during embryonic development is essential for clinical application of in vitro fertilization and early diagnosis of genetic disorders.

In mammals, early embryo development consists of sequential maturation events of the oocyte, fertilization followed by growth of embryo (4-cell, 8-cell, morula and blastocyst). Even though oocytes and spermatozoa are highly specialized cell types as compared to somatic cells, fertilization results in an ultimate totipotent zygote which can give rise to all cell types and a complete offspring. Totipotency of cells persists for the very first cell doublings, from zygote to at least the 4-cell pre-embryo (Assou et al. 2011).

In most of the mammalian species, earliest stages of development are regulated by maternally inherited information. The expression of the embryonic genome can be detected from the later-2-cell stage in the mouse, 4-cell stage in the pig, and 8-cell stage in the sheep. It was reported that the cleavage is not subject to transcriptional inhibition until after the 4-cell stage, suggesting the stage of activation of (Braude et al. 1988).A significant process in the development is the maternal-to-zygotic transition (MZT). During this transition process the maternal mRNAs are degraded and transcription from zygotic genome is initiated. During the MZT, the embryo takes charge of gene expression to control cell differentiation and further development (Jukam etal. 2017). The remarkable transition and switching "on" of embryonic genome requires nuclear reprogramming and binding of RNA polymerase II at thousands of gene promoters. It is also reported by the same authors that during zygotic genome activation (ZGA) the cell cycle, chromatin state, and nuclear-to-cytoplasmic component ratios are well coordinated (Jukam et al. 2017).

Recently, in birds, it was found that 1544 single nucleotide polymorphisms across 424 transcripts derived from parents were expressed in offspring during the early embryonic stages. Only the maternal genome was activated in the zygote, until the second-wave, when the paternal genome was activated. This was irrespective to the presence of a male pronucleus or supernumerary sperm in the egg. Subsequently, the maternal genes involved in cleavage were replaced by bi-allelic gene expression (Hwang et al. 2018).

In Chinese mitten crabs, expression of a total of 566 maternal genes and 1165 zygotic genes were identified, among which 103 and 266 genes were predicted to be conserved maternal transcripts and zygotic transcripts, respectively. It was reported that 87, 76 and 117 differentially expressed genes were associated with the MZT, morphogenesis and immunity were identified (Ning 2018). This study also underlines, the clearance of maternal transcripts in ZGA, the underling mechanisms of which are currently unknown.

Several studies (Braude et al. 1988; Lindsay and Copp 2005; Yi et al. 2010; Assou et al. 2011; Jukam et al. 2017; Godini and Fallahi 2018; Hwang et al. 2018) including that of ours indicate that the development of an embryo from zygote, to multicellular morulae and further transformation to blastocyst requires activation of hundreds of genes. Several genes and proteins involved in the progression of the zygote to blastocyst stages have been identified by multiple studies (Giraldez et al. 2006; Dominguez et al. 2009; Katz-Jaffe et al. 2009; Yi et al. 2010; Assou et al. 2011; Lee et al. 2013; Deutsch et al. 2014; Demant et al. 2015; Dang et al. 2016; Owens et al. 2016; Jukam et al. 2017; Munne and Wells 2017; Palfy et al. 2017). These studies conclude that the number of genes expressed gradually increase as the embryonic stage advances, corresponding to activation of embryonic genome. A detailed report on activation of embryonic genome through fine mapping of transcriptome in germinal vesicle and metaphase II oocytes, and in embryos at the 4-cell, 8-cell, 16-cell, and blastocyst stages has been catalogued (Graf et al. 2014b) and has been reviewed (Graf et al. 2014a) A recent study has reported expression of eleven transcription factors (NR2F2, ATF3, EN1, IFI16, KLF3, RUNX1, SOX2, IKZF3, ZBTB20, NPAS3, and ZSCAN4) were common to various stages of embryonic development (Godini and Fallahi 2018). Studies have shown that transcription factors express in form of waves, some are upregulated or downregulated stage specifically to provide a stage specific transcriptome or proteome for further progression of embryonic development. It was reported that expression of HNF4A, FOXA2, and EP300 genes were essential for the initial division of zygote (Godini and Fallahi 2018).

In a study, 10,032 circRNAs from 2974 hosting genes were identified and most of these circRNAs are stage-specific and dynamically regulated in the embryos. Further, many of them are maternally expressed, were predicted with possible role in oogenesis and totipotent zygotes formation (Dang et al. 2016). In bovine embryos, 87 out of total 1072 proteins identified were found to be different between the zygote and 2-cell and 4-cell stage with MII oocytes (Deutsch et al. 2014). The abundance of 140 proteins was significantly different between morulae and blastocysts, The expression of nucleophosmin (NPM1), eukaryotic translation initiation factor 5A-1 (EIF5A), receptor of activated protein kinase C 1, and annexin A6 (ANXA6) expressions were increased while the levels of glutathione S-transferase mu 3 (GSTM3), peroxiredoxin 2 (PRDX2), and aldo-keto reductase family 1 B1 (AKR1B1) were decreased in blastocysts (Demant et al. 2015). The increase in protein expression, thus appears to correspond to the increased transcription and translation occurred during the activation of embryonic genome.

The members of double homeodomain DUX protein family are reported to be key initiators of activation of zygotic genome (De Iaco et al. 2017). Maternal Nanog, Pou5f1 and SoxB1 are essential to initiate the zygotic development and clearance of the maternal program through activation of the microRNA, miR-430 expression in zebra fish (Lee et al. 2013). The microRNA, miR-430 is reported to facilitate the de-adenylation and clearance of maternal mRNAs during early embryogenesis (Giraldez et al. 2006). Stage specific expression of several microRNAs has been reported (Mondou et al. 2012).

Several epigenetic mechanisms in the regulation of embryonic development and maintenance of epigenetic memory has been described (Calvanese and Fraga 2012; Darbo et al. 2013; Cheedipudi et al. 2014; Canovas and Ross 2016). A transcription factor, Trithorax-like (Trl) is known to act as chromatin remodeling factor suggests that epigenetic regulation plays an important role in zygotic genome activation in drosophila (Darbo et al. 2013). Binding sites for factors such as Zelda, CBP, Trl binding sites, as well as for histone marks associated with active enhancers (H3K4me1) and for open chromatin regions were described by the same authors. Chromatin architecture and spatial organization of the genome is also been identified as a key factor that helps triggering embryonic genome activation (De Iaco et al. 2017). One of the proposed mechanism is the facilitation by proteins that serve as nucleation sites for topologically associating domain (TAD) boundaries, produced through translation of early transcribed genes and requires transcription factor Zelda in mammals for locus-specific TAD boundary insulation (Ostrup et al. 2013; Hug et al. 2017).

Conclusions

The development of embryo is a step-wise process well-coordinated by stage specific expression of few to hundreds of genes in a wave like fashion. The transcription and translation are regulated through both epigenetic and non-epigenetic mechanisms. The embryonic genome is activated subsequent to degradation of maternal RNAs, further control progression of development, cell lineages and tissues. Molecular biology of embryonic development is quite known due to advancement of technologies related to RNA sequencing using next generation sequencing and proteomics. The species specific differences and various regulatory mechanisms involved in the embryonic development are still poorly understood and needs further studies.

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PRB–L–06

Development of Frieswal cattle for improving for milk production

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Introduction

Since selection and grading up of large number of non-descript cattle could not bring rapid increase at production level to meet the requirements of growing population in India, crossbreeding of local cattle with exotic dairy breeds was adapted as an alternative. Cross breeding has played its role well and made a contribution in significantly increasing milk production in India. The crossbreds constitute only 16.6% of the total cattle population but contribute 25.3 million tons (53%) of cow milk. In comparison the indigenous cattle (83.4% population) contributed only 22.5 million tons (47% of cow milk). The population of crossbreds in rural and urban India is steadily increasing. So the contribution of crossbred cattle in making India the world leader in milk production cannot be ignored.

With the growth in human population, annual income and purchasing power, the requirement of milk and milk products will be increasing in the coming years. The awareness on complete nutritional food, interest on high protein diets, change in food habits, and export opportunities further augment the demand for milk and milk products. The country is expected to produce 186.2 million tonnes of milk in 2050 to meet the demand of per capita availability at the present rate and based on the prevailing 3.4 per cent annual rate of increase in milk production, the country can be able to produce 400 million tons in 2050. Even though there is an urge for shift in breeding policy, giving more weightage to well adapted indigenous animals, the crossbreeding will continue as an alternative, especially in commercial herds where the resources are available in plenty, to meet the nutritional security of the nation.

Crossbreeding in India

The history of crossbreeding in India can be traced back to 1856 when the Taylor breed was formed by crossing zebu with the Jersey breed. From there the efforts have been continuing to develop crossbred strains by crossing *Bos indicus* breeds with highly developed temperate breeds. Experimental cross breeding trials using various exotic dairy breeds were initiated mostly at institutional farms. Various research hypotheses such as which breeds were to be employed, the level of exotic inheritance to which the local nondescript zebu animals were to be upgraded and the selection policies for crossbreds to be adopted were discussed in due course. A number of dairy cattle Strains/breeds like Taylor, Jersind, Karan Swiss, Karan Fries and Sunandini have been evolved from these crossbred foundations in various parts of the country. Most of these crossbred strains have existed only in small numbers in organized farms except Sunandini. Crossbreds with exotic inheritance around 50% were suitable in most environments and proved to have 2-3 times more milk production than indigenous cattle breeds. Holstein crossbreds outperformed other crossbreds. Amble and Jain (1966), Katpatal (1979), Bhat (1988), Arora *et al.* (1993) and Taneja *et al.*(2002) have extensively reviewed crossbreeding work in cattle in India. By the late 1970s, the National Commission for Agriculture had issued general guidelines to maintain the level of exotic inheritance at approximately 50 percent in cross-breeding programme and the attempts have been intensified when Indian Council of Agricultural Research (ICAR) established a major project on crossbreeding of cattle.

Frieswal-Its Development

By 1980s, due to the sustained cross breeding, about 8 million crossbred cattle were born using various temperate exotic breeds. In order to stabilize this National milk herd, around 50% temperate inheritance; proven crossbred bulls of high genetic merit were required. To produce the required number of-crossbred bulls it was necessary to fix the genetic value of the resultant new strain. In order to meet these twin objectives, a major programme utilizing the crossbred population with 3/8-7/8 Friesian inheritance at Indian Military Farms was launched in 1984. The Military Farms were the largest source of crossbred cattle in India. They played an important role not only in providing quality milk for defense personnels but also in setting a standard of dairy animal management. Additionally, they served as a rich source of data for the study of dairy animal improvement through the induction of superior exotic germ inheritance.

Initially four and later on two more Military farms were involved in this project to utilize Friesian-Sahiwal crossbred as a base for the evolution of a new crossbred national milch breed-Frieswal through inter se mating, selection and progeny testing. Later on, more military farms were joined in this project. Further, the project Directorate on Cattle was established by the Indian Council of Agricultural Research (ICAR) as a nodal institution to monitor, co-ordinate and support all research and development projects for cattle improvement on 3rd November 1987 at Military Farms School & Research Centre, Meerut, by upgrading the status of All-India Coordinated Research Project (AICRP) on Cattle. The directorate was conceived to take advantage of the achievements made in the AICRP and other crossbreeding experiments at organized farms especially at Military farms and under field conditions for evolution of a national milch breed from a reasonably large crossbred base (Sahiwal x Holstein-Friesian). The directorate has recently been upgraded to Central Institute for Research on Cattle.

The initial breeding policy followed at the military farms was crossbreeding, mainly to maintain 3/8 to 5/8 Friesian inheritances. In 1980, alternative breeding policy was formulated and implemented, involving forward crossing with exotic bulls and backcrossing with Sahiwal bulls. Most of the genetic groups were between 3/8 and 5/8, with a major proportion of 5/8 HF. In the beginning, the crossbred stock with 50 percent or more exotic inheritance (higher cross) was mated with available half-bred Friesian x Sahiwal bull semen. The lower crosses (cows with less than 50 percent exotic inheritance) were mated with imported Friesian semen to raise the exotic inheritance of their progeny to over 50 percent. All animals in successive generations were bred with 5/8 bulls raised from 3/8 dams, yielding over 3000 kg of milk in a lactation period of 300 days, and mated with imported proven Holstein Friesian semen. Testing and ranking of 5/8 HF bulls is based on their daughters' performance. Intense selection based on the standards set out is being carried out to stabilize the breed. The project started in Military Farm, Meerut with 100 heads of crossbred cattle where breeding commenced on 4 September 1984. Military farms at Jalandhar, Bareilly, Dehradun, Ambala and Lucknow further joined and all other Military farms joined the project at a later stage.

The Frieswal project now envisages evolving a National Milch Breed "Frieswal", a Holstein- Sahiwal cross, yielding 4000 kg of milk with 4% butter fat in a mature lactation of 300 days. The evolution of Frieswal breed of cattle has been progressing utilizing the existing crossbred herds available at 37 Military Farms located in various agro-climatic regions of the country. Under broader objective, Frieswal project is being run with the objectives to study the genetic and phenotypic variance in milk production of Holstein-Sahiwal crossbreds and associated characters related to growth, production, reproduction and survival, and covariances among all these characters with a view to develop suitable selection criteria for improving milk production and to undertake progeny testing of a large number of bulls, their selection on the basis of the genetic merit and utilizing them in Military Farms (MFs) and other crossbreeding programmes.

In the Institute various state of the art laboratories have been established such as Semen freezing laboratory, animal nutrition laboratory, animal physiology laboratory and animal breeding and molecular genetics laboratory where research is being carried out to support the project so that objectives may be fulfilled. Central Institute for Research on Cattle is working in collaboration with Directorate on Frieswal Project which is partner agency for rearing the Frieswal cows and maintaining the bull rearing unit (BRU) at Meerut where male calves born out of elite cows mated with ranked Frieswal bulls are received and reared up to the age of maturity for semen collection and pass through rigorous selection processes. Semen is collected and stored only if semen quality is good. Besides this, there are 37 Military Farms which supply the pedigree and performance data of Frieswal cows located in various agro-climatic regions. However, formal Data Recording Units (DRUs) are at Meerut, Lucknow, Jhansi and Bareilly in Uttar Pradesh; Ambala in Haryana; Pathankot and Jalandhar in Punjab; Pimpri in Maharashtra; Secunderabad in Andhra Pradesh; Guwahati in Assam; Bengdubi in West Bengal, Jammu in J & K and Jabalpur in M.P.

Technical Programme

The crossbred females with 5/8 HF inheritances named "Frieswal" are bred with the semen of their own genetic group. Crossbred females having more than 50 % exotic inheritance named "higher crosses" were bred with Frieswal bulls' semen. Lower crosses with 50 % or less HF inheritance were bred with frozen semen of proven HF bulls with sire index of above 9000 kg. The mating of higher and lower crosses as described above produced the Frieswal progeny in subsequent generations.

Sons of 3/8 elite cows bred with proven HF semen and 5/8 elite (Frieswal) cows bred with pedigreed/ranked Frieswal semen were screened and put under the test mating. Male progeny born out of elite cows were reared at the respective farms up to three months of age for the preliminary selection based on their growth rate and conformation traits. After three months, the selected calves were shifted to Bull Rearing Unit, Meerut for further rearing.

Phenotypic Characterization of Frieswal cattle

The main body colour of Frieswal animals, was black and white in different proportions varying from 10 to 90%. Animals with entire black or tan or tan colour mixed with white have been also observed. Skin colour in majority of animals was black followed by black and brown. The prominent colour of tail switch was white. Most of the animals had black muzzle (90% cows and 96% heifers). Hoof colour was black in 57% cows and 59% heifers. Adult Frieswal cows were medium (39%) to large in size (56%) with medium dewlap (87%) and small naval flap (93%). Most of the animals were docile (83%). Udder was symmetrical in most of the cows (90%) and heifers (100%).

Frieswal calves averaged 26.51 kg at birth, 66.69 kg at 3 months and 117.95 kg at 6 months of age. The body weight of the heifers was 257 kg at 18 month and 323 kg at 24 month of age. Weight at first calving averaged 412 kg.

Predominant coat colour of Frieswal bull is black patched with white, sometimes brown colour in combination with either black or white. The main muzzle colour of Frieswal bull was black. Another characteristic feature of Frieswal bulls was presence or absence of white spot on forehead. Nearly 40% of the bulls had prominent poll covered with long hair. At the age of 3 months the mean body weight of Frieswal bulls was 103.03±7.43kg. Body weight continued to increase up to 48 months of age and reached to 631.73±7.43kg. Thereafter the increment showed fluctuating trends. The bulls attained body weight of 700kg at 75 months of age, and there after started declining, which is an indication of senility with age advancement.

Milk Production performance

The overall means of 300 days milk yield and total milk yield based on 48050 lactation records were 3317.53 and 3332.46 kg respectively. The peak yield of the Frieswal herd averaged 15.13 kg. The effects of farm, parity, season and year of calving and regression on AFC were significant on all the traits. Frieswal cows at MF Mhow (3900.31 Kg) produced the highest 300 days milk yield followed by those maintained at Kanpur (3892.94) and Lucknow (3824.54 kg). Frieswal cows at Kanpur (4030.19 kg) had the highest total lactation milk yield followed by those maintained at Allahabad (3896.16 kg) and Mhow (3884.31 kg). The peak yield was highest at MF Allahabad (17.97 kg) followed by at Kanpur (16.80 kg) and Agra (16.76kg). PY ranged from 11.76 kg at MF Panagarh to 17.97 kg at Allahabad. The variation in production performance among farms might be due to varying managemental and feeding practices and location of farm in various agro-climatic zones. Season of calving also affected the production performance. The cows calved in winter season yielded the highest 300 days milk (3384.51 kg) followed by those calved in post monsoon (3376.37 kg), summer (3268.10 kg) and rainy (3241.16 kg) season. Same trend was observed in the case of total lactation milk yield as well as peak yield. The total lactation milk yield and peak yield for those animals which calved in winter was highest (3392.26 and 15.83 kg) followed by those calved in post monsoon (3354.82 and 15.42 kg) and summer (3332.94 and 14.79 kg). The animals which calved during the rainy season had the lowest TLMY and PY (3249.80 and 14.46 kg). There was lot of fluctuation in total lactation milk yield during various years and it ranged from 2633.44 kg to 4030.19 kg. Higher PY was observed in those animals which calved in the year 2003 (16.11 kg) and 1996 (13.64 kg).

The average lactation length of the Frieswal cows was 326.30 days. The effects of farm, parity and season and year of calving and regression of AFC also played a significant effect on lactation length as in the case of other traits. Lactation length was longest at MF Rajouri (352.71 days) followed by at MF Panitola (346.33 days). All the Military Farms had lactation length higher than 300 days. The cows calved during summer season had the longest lactation length (334.71 days).

Reproductive performance

The overall mean of age at first calving was 972.18 days (31.98 months). The effects of farm, season and year of birth were significant on AFC. The animals at MF Dimapur (29.28 months/ 890.28 days) had shorter age at first calving followed by Namkum (30.15 months/916.54 days) and Lucknow (30.32 months/921.63 days). The longest AFC was recorded at MF Rajouri (34.71 months/1055.29 days) followed by Gwalior (33.65 months/ 1023.09 days). Large variation in AFC reflects the scope of its improvement by way of improving general management practices including feeding standards, timely heat detection and artificial insemination. Declining trend in AFC over the years was noticed which indicates a desirable improvement in this economically significant trait.

Service period (SP), Dry period and Calving interval (CI) were also evaluated. The average SP, DP and CI were 159.44, 117.98 and 439.93 days respectively. These traits were also significantly influenced by farm, parity and season and year of calving and regression of AFC. The shortest service period was observed at MF Bengdubi (138.65 days) followed by Secunderabad (144.11 days) and Mhow (145.72 days). Longest service period was observed in cows at MF Missamari (195.65 days). Similar trend was observed in the case of calving interval also, as expected. The shortest calving interval was observed in cows maintained at MF Bengdubi (418.81 days) followed by those kept at Secunderabad (421.92 days) and Mhow (425.33 days). The longest CI was also noticed at MF Misamari (476.09 days). MF Mhow had the shortest dry period (97.28 days) followed by MF Jabalpur (104.90 days) and Bengdubi (105.79 days). Longest DP was found in the cows at MF Missamari (140.10 days). In general, service period and calving interval had no definite trend over the lactations. Frieswal cows calved during post monsoon season were having the shortest SP (140.57 days) followed by those calved in rainy (153.05 days), winter (167.46 days) and summer (176.67 days) season. Seasonal variation in the reproductive traits also showed similar pattern and post monsoon calvers were having shortest DP and CI (109.17 and 421.67 days) and summer calvers the longest DP and CI (125.86 and 456.79 days) too. There was no definite trend in SP and CI over the years. Longest SP was found during 1994 (188.41 days) and shortest during 2014 (96.70 days). The cows calved during 1999 had longest (134.59 days) dry period and thereafter a declining trend in dry period was observed. It reflected that attempts are being made for improving the reproductive traits at Military Farms over the years.

Performance of Frieswal Cow: Frieswal cattle were maintained at various location in the country i.e. Central, Eastern, Northern, Southern and Western region of the country. Result of the milk performance over 15 years indicate that 300 days milk yield varies from 3442.45 kg in central region to 2900.16 kg in Western region. These cows were managed under farm condition under Military farm of Ministry of Defence. Frieswal bulls were also used Frieswal cattle perform well throughout the country and also well in eastern region. With suitable management (housing, feeding) management they may be reared in eastern region.

Impact of Frieswal Project in Farmers' fields

The crossbred cattle in different agro-climatic region of the country are being improved through utilization of high quality germplasm of genetically superior breeding bulls under the Field Progeny Testing programme of the Institute. A total 261 bulls has so far been introduced in 14 different sets and total 3,55,353 inseminations have been done in which 37,308 female progenies were born out of which 1,0234 has reached age at first calving with over all conception rate of 43.5%. Presently the programme is implemented in collaboration with Kerala Veterinary and Animal Sciences University Thrissur, Kerala, (KVASU), Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab, (GADVASU), BAIF Development Research Foundation, Uruli-Kanchan, pune and G B Pant University of Agriculture & Technology, Pantnagar, Uttarakhand (GBPUA&T).

A total of 354619 inseminations were performed in the four field centres (100284 in BAIF, 107294 in KVASU, 133452 in GADVASU and 13589 in GBPUA&T, Pantnagar), of which 284848 inseminations were followed for pregnancy confirmation and 124100 pregnancies were confirmed since inception of the project, with average conception rate as 43.56 per cent. A total of 39893 female progeny were born in four centres. Through the intervention of Field Progeny Testing programme, the average first lactation 305 days milk yield of the Frieswal progenies in the adopted villages of FPT project has increased by 40.6 % at GADVASU, 39.0 % at KVASU, 11 % at BAIF (Fig 10) and 19 % in Pantnagar unit.

ORAL PRESENTATION

PRB–O–01

Investigation of association of SNPs in TLR4 gene with paratuberculosis infection in cattle

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The bovine paratuberculosis, a chronic granulomatous inflammation of the intestine characterized by persistent diarrhea, progressive wasting and death is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Paratuberculosis has been reported to be heritable and genes with vital roles in immune response are strong candidates for investigating genetic basis of resistance. In the present investigation, SNPs in TLR4 gene were investigated for association with paratuberculosis in cattle. A total of 549 animals from Kamadhenu Gaushala, Nurmahal, Jalandhar, Punjab and Military Farm, Bareilly were screened using a panel of diagnostics to establish a population of healthy and affected animals. On the basis of these tests a total of 50 paratuberculosis affected and 50 healthy control animals were used for genotyping for 2 SNPs viz rs8193046 and rs8193060 associated with TLR4 gene by PCR-RFLP. The effect of nongenetic factors and SNPs in TLR4 gene on the occurrence of paratuberculosis disease was analyzed by PROCLOGISTIC model of SAS 9.3 software. Both the SNPs were found to be polymorphic in affected and healthy population. SNP rs8193046 yielded three genotypes viz AA, AG and GG while SNP rs8193060 yielded three genotypes viz CC, CT and TT in the studied population. The logistic regression analysis revealed that for SNP rs8193046, genotype frequency differ significantly ($p<0.01$) between affected and healthy population. Three genotypes AA, AG and GG were observed at this locus with ODDs of AA and AG compared with GG were 0.94 and 3.94 respectively *i.e.* probability for getting infection in animals with AG genotype was 3.94 times more as compared to GG genotype. For SNP rs8193060, ODDs ratio revealed that probability for getting infection in animals with CT genotype was 1.35 times more as compared to TT genotype. However, SNP didn't exhibit significant difference ($p>0.05$) in affected and healthy population. Further investigation on larger and diverse population would warrant about strength of association of investigated SNP.

PRB–O–02

Effect of FBS on Growth curve, Population doubling time and Colony forming unit of Bone Marrow Mesenchymal Stem Cells

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Out of many factors which are directly and indirectly influence the growth and proliferation of BmMSCs, serum concentration is one such pivotal factor. The type of serum and its concentration can affect MSCs isolation, expansion and differentiation *in vitro*. The exogenous supplementation of serum impacts on the longevity of MSCs *in vitro* as the source of serum is a determinant of the time till senescence in cultured BmMSCs through telomere-dependent pathways and regulation of cell cycle and the levels of heat shock proteins. It also found that adding serum in the medium increases apoptosis pathways in BmMSCs. The bone marrow was aspirated to a syringe with anticoagulant. The collected bone marrow sample was loaded carefully on histopaque in sterilized centrifuge tube and centrifuge at room temperature at 2800 rpm for 30 min. The re-suspended cells was seeded in to a culture flask and incubated at 37°C in humidified atmosphere with 5% CO_2. The BmMSCs from primary culture were further resuspended after trypsinisation in complete DMEM/F12 media with six different concentrations of FBS i.e. 5%, 10%, 15%, 20%, 25% and 30% to observe their growth curve, PDT and CFU. The growth kinetic study was estimated to analyse the growth kinetic potential of each concentration of melatonin on

MSCs on different days. PDT was calculated after 14 days and for that cells were Trypsinised and counted manually with haemocytometer. To assess the capacity and efficiency of self-renewal, cells were seeded at low density and colonies derived from single cells were counted. The number of colonies was counted under stereozoom microscope (Nikon) with 8× magnifications. The results show that the BmMSCs attained a maximum growth rate in high FBS concentration (30%) initially which differs significantly ($p<0.05$) from other groups. However, the cells attained senescence earlier in high concentration groups and optimum growth was observed in media with 20% serum concentration which differed significantly ($p<0.05$) . The effect of serum, as evident from this experiment, was found to be optimum on cell growth at 20%. Although cell's growth was good at 30% initially, the high conc. impacted harshly on cellular growth as days advanced. Similarly, the population doubling time was lower in 20% signifying the fact that this conc. of serum can be used as an optimal conc. for complete tissue culture media as far as BmMSCs are concerned. The CFU of BmMSCs was highest in 25% FBS concentration initially which differs significantly ($p<0.05$) from 20% FBS. However, the cells attained senescence earlier in high concentration groups and optimum CFU was observed in media with 20% serum concentration which differed significantly ($p<0.05$) .

PRB–O–03

Molecular characterization of TLR1 in camel using RT-PCR

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Toll like receptors (TLR) are a family of pattern recognition receptors (PRR) that function as primary sensors of the innate immune system to recognize microbial pathogens. The number of receptors that recognize pathogen associated molecular patterns (PAMPs) as infectious non self-ligand, in response activate inflammatory cascades that include recruitment of dendritic cells. The camel is an important animal uniquely adapted to hot and arid environments and, therefore, contributes significantly to the food security of the nomadic pastoral households. The camel has less studied for TLR related gene. We Sequenced complete TLR 1 gene and deposited in NCBI (MG655186) which has 2391 base pairs and it encodes an open reading frame translated into 796 amino acids. The camel TLR 1 has the highest similarity of amino acids with pig TLR1. The future prospective include checking the fold expression in TLR 1 from dendritic cells obtained from camel dromedary under different pathogenic conditions using RT-PCR.

PRB–O–04

Genetic Relation of Goat (*Capra hircus*) with other Livestock Species Measured through Different Molecular Techniques

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Goats (Capra hircus) are one of the oldest domesticated species and have been used for their milk, meat, hair and skins. Goats are adapted from the wild version, "*Capra aegargus*". Six individuals, included both sex, in each of goat (*Capra hircus*), cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep *(Ovis aries*) and mithun (*Bos frontalis*) were utilized in this investigation. When RAPD markers were used, the estimate of genetic similarities, based on band sharing (BS) among the livestock species with RAPD markers pooled over the primers ranged from 0.15 to 0.56. Goat and sheep (BS=0.56) followed by goat and buffalo (BS=0.29) showed the maximum genetic similarity. Minimum genetic similarity was observed between goat and cattle (DS=0.20) followed by goat and mithun (BS=0.25).When RAPD-PAGE technique used then the genetic similarities, based on band sharing (BS) among the livestock species with RAPD markers pooled over the primers ranged from 0.295 to 0.452. Goat and sheep (BS=0.452) followed by goat and buffalo (BS=0.318) showed the maximum genetic similarity. Minimum genetic similarity was observed between goat and mithun (BS=0.295) followed by goat and cattle (BS=0.312). Genetic distance estimates, pooled over the primers ranged from 0.795 to 1.221. Goat and mithun (DS=1.221) followed by goat and cattle (DS=1.164) showed the maximum genetic distance. Minimum genetic distance was observed between goat and sheep (DS=0.795) followed by goat and buffalo (DS=1.144). When MASA markers technology used the results revealed the maximum genetic similarity between sheep and goat (0.72) while goat and mithun showed least genetic similarities (0.35). It also showed highest genetic distance with goat and mithun (0.91) than others.

PRB–O–05

Candidates' genes affecting economic traits in Marwari goat populations in North western part of Rajasthan

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Marwari breed of goat is a medium sized dual purpose breed, reared for both mutton and milk, and is well adapted to the harsh environment of the Thar Desert. The birth weight of kid varies from 1.90 to 2.50 kg and producing average milk yield is 1 kg/day. It therefore comprises a unique repository of goat genetic diversity in the north western part of Rajasthan. A total of 271 candidate genes have been detected in goats (Supakorn 2009). However, comprehensive investigations have been carried out on the polymorphism of some genes, involved in the control of economic traits. Candidate genes have an effect on the physiological pathway, metabolism and expression of phenotypes. For growth traits, growth hormone (GH), growth hormone receptor (GHR), insulin like growth factor I (IGF-I), leptin (LEP), caprine pituitary specific transcription factor-1 (POU1F1), caprine myostatin (MSTN) and bone morphogenetic protein (BMP) genes are necessary for bone formation, birth weight, weaning weight, body condition and muscle growth. The functions of these genes on economically important traits are different. Some genes have synergistic or antagonistic effects in nature for expression of phenotypic traits. On the other hand, some genes could control more than one trait. Also, the producers should be concerned with these effects because selection of a single trait by using only a gene could affect other traits. Therefore, the identification of candidate genes and their mutations which cause variations of gene expression and phenotype of economic traits will help breeders to search some genetic markers for these economic traits. It may be used as an aid in the selection of parent stock at an early age in the future.

A novel single nucleotide polymorphism within the Marwari goat population in and around Bikaner district was identified for five genes (BMP-15, GH, GHR, leptin and Myostain). The polymorphism in BMP-15 (Fec X and Fec X in exon-2) gene was investigated by RFLP technique which revealed variant at 153 bp and 141 bp. Leptin and GHR gene yielded 338 and 84-bp, and 160 and 155-bp size fragments with restriction enzyme *Hinf I* and *DdeI I* respectively. The genetic variability of MSTN gene for exon 1, 2 and 3 were analysed. The PCR amplification yielded 458 bp products for exon 1, 375 bp for exon 2 and 438 bp for exon 3. The exon 1 revealed only one conformation pattern 'AA' on 8% non-denaturing polyacrylamide gel. The exon 2 revealed two conformation patterns AA and AB. Similarly exon 3 also produced two conformation patterns AA and AB with frequency of 0.90 and 0.10, respectively

These patterns may be characteristic for Mrawari breed and could be used to differentiate them with other breeds of goat. Information could be utilized in future breeding plan to exploit the unique characteristics of Marwari goat of Rajasthan.

PRB–O–06

Augmenting fertility in post-partum repeat breeding crossbred cows following ovulation synchronization protocols

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Repeat breeding is one of the major frustrating reproductive syndromes among dairy farmers due to extended inter-calving period, lower conception and higher cost of rearing. This study was designed to evaluate the efficacy of G6G treatment on pattern of estrus response and conception rate over Ovsynch treatment initiated on random days of estrous cycle in post-partum repeat breeding crossbred cows. A total of Sixteen (n=16) cows were equally divided into two groups *viz.*, Group 1, which received Ovsynch treatment and Group 2, which received G6G treatment. The onset of estrus in Group 1 and 2 was 48.21±1.91 and 50.12±1.84 hrs respectively. The duration of estrus in Group 1 and 2 was recorded as 19.12±1.07 and 21.47±1.18 hrs respectively. In the present study, the conception rate was 12.50 and 50.00 percent in Group 1 and 2 following Ovsynch and G6G treatments respectively. From the study, it could be concluded that the use of G6G treatment was capable of responding better in terms of estrus response and conception rate than Ovsynch treatment initiated on random days of estrous cycle in post-partum repeat breeding crossbred cows. However, onset and duration of estrus was not significant.

Keywords: Repeat breeding, Ovsynch, G6G, estrus, conception rate.

PRB–O–07

Establishment of selective clonal somatic cell lines from single cells for cellular reprogramming studies in buffalo (*Bubalus bubalis*)

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The aim of this work is to establish the selective clonal cell lines from single fibroblast cells to provide a powerful biomaterial to cellular reprogramming studies, including SCNT, in buffalo-India's valued livestock species. We used buffalo skin fibroblast cell type to optimize cell culture conditions to achieve the proliferation of a single cell. The growing fibroblast cells (70-80% of confluence) were dissociated using trypsin and single cells were picked up and cultured each cell per well in 96-well plate. In each well, 100 μl of culture medium was supplemented and replaced it with in every 72 hr. The culture medium was DMEM with 20% serum, 1X vitamin mix, 1X non-essential amino acid, 1X antibiotic mix, and 10 ng/ml of epidermal growth factor. In addition to culture in 96-well plate, we also attempted to culture individual cells in 20μl of drops of above mentioned culture medium in 35-mm^2 dishes. Cell attachment was observed at day 2 after seeding and cell division at day 4 of culture. We observed cell multiplication in 96-well plate cultures, but no cell multiplication in droplet cultures which was made in 35-mm^2 dishes. We noticed that the cell division was extremely slow in 96-well plate and a very few attached cells were able to proliferate beyond 4 cell multiplications. This indicates that the culture of a single cell is challenging and needs specific requirements, at present, we are trying to improve culture conditions by supplementing the culture medium with a cocktail of growth factors or fibroblast conditioned media to establish clonal cultures of single cells.

PRB–O–08

Lab to Land: Birth of cloned Assamese buffalo at an Indian dairy farm

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India has privileged to possess the largest and best breeds of livestock that have been immensely contributing to Indian economy and rural upliftment since decades. The Buffalo, named India's milk machine and meat factory, plays a pivotal role in India's rural economy. India has been engaging in excellent buffalo science and becomes a leader in the field of buffalo cloning research and development. India is a first country in the world to produce a cloned riverine buffalo, which was produced in 2009. Recently, in December 2017, we added one more milestone in India's buffalo research by producing the world's first cloned buffalo in the field, away from the laboratory. To achieve this, we airlifted tissue biopsy of Assamese buffalo from the college of veterinary science, Assam, which is approximately 2000 km away from our laboratory. The Assamese buffalo somatic cells were fused with enucleated oocytes of Murrah buffalo to produce cloned embryos using the optimized method of our lab (Selokar et al., 2012). The blastocyst stage embryos were transferred into five recipient buffaloes at the Hi-tech Sach Dairy Farm, which is 100 km away from our laboratory. One recipient found pregnant and delivered healthy calf having 53 kg birth weight. This calf has normal blood profile and no chromosomal abnormalities. The cloned calf is growing normally and now 11 months old. This research output could realise the concept of 'Lab-to-Land' to transfer agricultural and allied technologies to the farmer's field. We suggest buffalo cloning technology can be used as a valuable tool to uplift the buffalo germplasm in India.

Keywords: Buffalo, Cloning, Dairy, Lab-to-land.

PRB–O–09

Genotyping of cattle breeding bulls for A1/A2 variants of beta casein gene

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A1/A2 milk types were established in today's dairy industry. A1 milk type is considered hazardous for human consumption because it is responsible for many human diseases such as diabetes type II, Heart diseases, SIDS (Sudden Infant Death Syndrome), schizophrenia etc. Indian cattle breeds are A2 type only. But, for increasing milk production cross breeding strategy is used in India. Due to crossbreeding strategy we may loss our A2 milk cattle for breeding. So, considering this the present study was carried out. In the present study, HF cross-breed cattle breeding bulls were genotyped by using PCR-RFLP and AS-PCR methods. 49 blood samples were collected from Frozen Semen Laboratory. DNA extracted by traditional method and quantity checked on nano-drop spectrophotometer. The purity of DNA was found between 1.7 to 1.8. The isolated DNA then subjected to PCR to amplify 121 bp fragment of beta casein gene. This PCR fragment then digested with the *Dde*I restriction enzyme. This restriction digestion resulted in to two bands pattern and confirmed as A1A2 and A2A2 genotype with estimated frequency 65 per cent and 35per cent, resp. This PCR-RFLP result validated by allele specific PCR with 244 bp fragment of beta casein gene. This validation resulted same genotype with slight difference in frequency i.e. 73 per cent and 27 per cent. The observed cumulative frequency of A1 and A2 allele is as were 0.35 and 0.65, respectively. The data generated from present study on current status of A1 and A2 type breeding bulls may be used in formulating suitable breeding plans in order to minimize undesired A1 allele in future generation.

PRB–O–10

Physiogenomic comparison of native, crossbred and exotic pigs during different seasons

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Piggery in India that contributes substantially to the livelihood and nutritional security especially in north-eastern part, is vulnerable to environmental variables largely because of the backwardness of the production system. The present study was conducted to ascertain the influence of seasonal variation on (re)production in pigs through endocrine, gene expression and oocyte competence study. Blood samples of different genetic (native, crossbreed and exotic) and age (grower, finisher, adult) groups of pigs were collected across the seasons. Records of ambient temperature and relative humidity were collected, and THI was calculated. Hormones and candidate genes involved in stress, reproduction and production were studied. Hormones cortisol, estradiol, progesterone, insulin, T_3 and T_4 were found to be influenced by season[a]. Breed influenced cortisol[b], testosterone[b], insulin[b] and T_3[a], and age influenced cortisol[b], estradiol[a], progesterone[a], testosterone[a], insulin[a] and T_4[a]. Level of LDH, GH and leptin was not influenced by season, breed or age. Lower expression of HSP70, GnRH, LHβ and FSHβ genes were observed in almost all age groups of native pigs in comparison to crossbred and exotic during summer. Native grower pigs also had lower GH expression compared to other breeds. The percentage of oocytes that developed into morula was also low across genetic groups during summer. It is concluded that season in interplay with genetics and age significantly influences (re) production in pigs. Our study generated a baseline data of the levels of hormones and genes relevant to (re)production and adaptability during different seasons. It will act as a knowledge base, and support in suggesting suitable ameliorative measures for pigs to counteract seasonal stress.

([a] denotes P<0.01, and [b] denotes P<0.05).

PRB–O–11

Production Performance of synthetic sheep developed for fine wool production

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Key words: Sheep, Fine wool, Breeding, Temperate climate

The fine wool sheep breeds are largely reared throughout the world for production of apparels and in India, the sheep breeds with fine wool are limited to the Himalayan temperate terrains. A synthetic sheep strain was developed at ICAR-North Temperate Regional Station, Garsa of Central Sheep and Wool Research Institute from Gaddi Synthetic (about 25% inheritance) with Merino and Rambouillet (about 75% inheritance). The breed has been improved over the years by *inter se* mating following mild selection pressure for production, conformational and wool quality traits. The least square mean values of the production parameters of the flock for combined sex in the present generation for birth weight, 3 month BW and 6 month BW is 3.69±0.04kg (n=133), 16.57±0.24kg (n=125) and 21.75±0.39kg (n=98), respectively and exhibiting an increasing trend over the generations. Except for the birth weight, the 3 and 6 month body weights showed significant difference among both the sexes with males weighing heavier than the females. The tupping % also showed improvement generation wise and approaching near 90%. The tupping % during the last generation was 92.48% whereas the lambing % on tupped and on ewe available basis was around 80%. The 6 month greasy fleece yield (GFY) and annual adult GFY was 0.63±0.03 and 2.37±0.05 kgs, respectively. The staple length, fiber diameter and medullation parameters for 1st GFY (6 month clip of lambs) are 3.48±0.05 cm, 19.02±0.12 µ and 0.70 %, respectively. This synthetic sheep is having a good potential and the wool produced is comparable in quality with the finest wool of the apparel industry.

SESSION – VII

VACCINE AND IMMUNO-THERAPEUTICS

LEAD PAPERS

VIT–L–01

Gut mucosal immune response in pig: An interaction of inductive and effector components of GALT

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***Key words :** Gut immunity, Peyer's Patch, T-cell, B-cell, GIT, Pig*

The mucosal surface of the gut is protected by specific and non-specific defence mechanisms. It is considered as the second largest organ after skin in terms of surface area and it contains more immune cells than any other organ, including spleen and liver. This vast mucosal area of the gastro-intestinal tract (GIT) is very much susceptible to the assault of antigens of various origin right form pathogenic microbes, viruses, food toxins. Confronting with vast arrays of antigens, the immune system faces a considerable challenge in its efforts to maintain local tissue homeostasis in the intestinal mucosa. Interactions among the microbiota, the gut epithelium, and the GALT are "trialogue" exists among these components that shape the intestinal ecosystem. The communication within the mucosal immune system is carried out by a large and highly specialized collection of tissues and cells within the GIT. Immune cells within the GIT are highly compartmentalized.

The components of GALT can principally be divided into *inductive sites* and *effector sites*. The inductive site consists the antigen sampling areas of the mucous membrane including the local aggregates of lymphoid tissue in the lamina propria and tunica submucosa. In the gastro intestinal tract, the inductive sites refer to the follicle associated epithelium of the Peyer's patches (PP) and the solitary lymphoid nodules (SLN) of small and large intestine respectively. They are collectively called as gut associated lymphoid tissue (GALT). GALT is the most specified and largest organization of lymphoid tissue protecting the gut mucosa. Peyer's patches were named after Johann Conrad Peyer who described them in 1673 as 'elevated areas composed of lymph nodules in the mucous membrane of the small intestine'. Peyer's patches are considered as the immune sensors of the intestine. PPs are organized into three major regions: a series of large follicles rich in B cell; the overlying follicle associated epithelium (FAE) and associated sub-epithelial dome (SED) that lies between the follicles and the FAE, and; the small T cell zones that are situated adjacent to the B cell follicles designated as interfollicular area. On the other hand, the solitary lymphoid nodules are the aggregates of lymphoid tissue in the wall of the large intestine which are observed grossly on the surface of the large intestine in the form of some minute and round elevations with a central depression. A hallmark of mucosal immunity is the induction of an immune response in Peyer's patches and the subsequent production of secretory IgA by B lymphocytes in the lamina propria. The unique antigen sampling mechanism of the lymphoid tissue components is responsible for immune protection and/or tolerance. The specialized type of epitheloid cells present in the follicle associated epithelium (FAE) of GALT, known as microfold or membranous cell (M cell), are primarily responsible for the uptake and processing of particulate antigen from the gut lumen. The mechanism of M cell, many a times, is utilized by various pathogens for entry into the host system. The lymphoid subsets (T and B-cells) are distributed in the follicle, dome and in the apex of the follicle. Most B-cells are located in corona of the follicle and dome area. Interfollicular area (IFA) is a zone of specific T-cells ($CD4^+$ and $CD8^+$) and traffic area for the circulating lymphocytes. Solitary lymphoid nodules are located anti-mesenteric wall of the small and large intestine. Similar to Peyer's patches, SLNs contain germinal centers with segregated B- and T-cell areas and an overlying follicle-associated epithelium complete with M cells. Isolated lymphoid follicles function as inductive sites for antigen-specific mucosal immune responses.

Subsequent to immune induction, as gut effector site the lamina propria has proven to function as the regulator of immune responses in the intestine. After induction in the Peyer's patch, mature T and B cells travel to the mesenteric lymph nodes via the lymphatic circulation before homing to the lamina propria, where T cells can directly eliminate pathogens and where T and B ells can participate in the production of cytokines and immunoglobulins (e.g., Ig A). Much of the burden on mucosal immunity is shouldered by intestinal epithelial cells (**IEC**). Failure to deal appropriately with antigenic stimuli can result in chronic inflammation, decreased digestive function, and a decreased rate of growth. About 75% lymphoid population in pigs is $CD8^+$ T cells. In addition, IEL can be αβ and γδ T-cell receptor positive. Neonatal pigs are mostly CD2–CD4–CD8–, and CD8+ IEL cannot be recognized until the animal matures. IEC have evolved mechanisms that constitute an effective anatomical and immunologically active barrier. One mechanism of the IEC barrier is the innate recognition and differentiation of commensal and pathogen-associated molecular patterns via pattern recognition receptors such as the Toll like receptor family. The crosstalk among the gut lumen, IEC, and the lamina propria provides the information that directs the mucosal immune system. Besides, the barrier function of IEC, they are prominent sources of soluble protein factors like chemokines and cytokines which initiate both the innate and adaptive immune responses. The porcine IEC line IPEC-J2 expresses mRNA for IL1α, IL6, IL7, IL12p40, IL18, tumor necrosis factor-α, granulocyte-macrophage colony-stimulating factor, CCL20, macrophage migration-inhibiting factor, and osteopontin, and is also a prominent source of highly polarized IL-8 secretion. Antimicrobial peptides are also secreted by IEC. Briefly stated, this role for IEC may be described as a complex bidirectional relationship between IEC and leukocytes that contributes to homeostasis under normal conditions as well as during the development of disease.

Successful development of a mucosal vaccine depends on delivery of the antigen to the proper inductive site. Therefore, understanding the characteristics of the M cells and determining the molecules expressed specifically by them is important for design of efficient mucosal vaccines in different species. After the antigen sampling and capture by the M cells in the FAE, the particulate antigens are presented to the dendritic cells or other types of APC which are present in the sub-epithelial dome and interfollicular areas of Peyer's patch and solitary lymphoid nodules. This enables the lamina propria to function as the regulator of immune responses in the intestine. The lamina propria contains macrophages, dendritic cells, neutrophils, mast cells, and diffused lymphocytes that participate in lamina propria effector functions. The follicles are rich in B cell and follicular dendritic cell; while the interfollicular areas are rich in T cell. After induction in the Peyer's patch, mature T and B cells travel to the mesenteric lymph nodes via the lymphatic circulation before coming back to the lamina propria. The activated T cells can directly eliminate pathogens as well as T and B cells can participate in the production of cytokines and immunoglobulins (e.g., IgA).

In summary, the immune system of the GIT maintains its ability to mount active immune responses against pathogens, and tolerance to food antigens and the commensal biota, by a highly complex network of cellular and molecular interactions. Knowledge regarding the intricacies of mucosal immunity as it applies to the inductive and effector sites is particularly important in pigs because of the development of these sites along with the process of the pig matures. The period from birth through weaning represents a critical time for pigs. During this period, the pig is exposed to and must mount appropriate immune responses toward or be tolerant of dietary and environmental antigens. Mucosal immunity, including the inductive and effector components of GALT, is extremely important in guiding the immune response toward an appropriate and effective immune response that strives to maintain intestinal homeostasis, better health and performance throughout the productive lifetime of the pig.

VIT–L–02

Major aspects of vaccine development: an essential requirement in Integrated Farming Systems- Vistas and Frontiers

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Vaccine Development

The vast majority of virus diseases cannot be treated with the equivalent of antibiotics for bacteria. Once infected, the individual's immune system has to combat disease. Prevention of disease through prophylactic measures is therefore necessary and this is achieved through administration of vaccines. The history of virus vaccines goes back to Jenner's cowpox vaccine for smallpox in 1798. Advances in tissue culture methods and reagents in the sixties and seventies allowed the successful production of vaccines for man and animals. The more recent development of biotechnology coupled with research has offered some alternative strategies and production possibilities (Brown, 1996).

Classical vaccines

Classical vaccines are prepared in one of two ways. Inactivated vaccines are produced by growing virus in large amounts and then chemically inactivating this under conditions where the immunogenicity is retained, e.g. current foot-and-mouth disease vaccines. Attenuated vaccines are produced by reducing or eliminating the pathogenicity of the virus with regard to the target host, through growth in an unnatural host or under special conditions. Such a virus multiplies in the target host but does not cause disease. An excellent example of an attenuated virus is that for rinderpest. Despite the past and present record of success for conventional vaccines, they have several disadvantages. Killed vaccines must be proven free of any live organism and attenuated strains must not demonstrate reversion to virulence. There is also the possibility of contaminating adventitious agents. Attenuated viruses also can be unstable and have to be kept cool under physiological conditions. This factor also affects the vaccination per se once a fully formulated vaccine is constituted in the field. Such stability factors (the virus must be "live"), affect the strategies of a campaign necessitating setting up of cold chains.

Newer strategies

Developments in the human sphere offer the models for approaches in animal virus vaccines. In 1981, the first vaccine against a non-propagable agent and, in fact, the first against human cancer, was derived from antigen purified from the blood of Hepatitis B carriers. The plasma derived sub unit was purified to ensure the destruction of all possible life forms. Once attached to an alum adjuvant, it proved safe and effective. However, the purification was slow and tedious and there were fears about the safety of human blood as a source of antigen.

Molecular biological methods offer ways of inserting genes into vectors so that these are expressed to produce proteins. There are only two ways in which these the many expression systems available can be exploited for vaccine manufacture. In the first, the vector is cultivated in vitro to produce large amounts of protein for use as an inactivated vaccine. This can be regarded as being "safer" than where infectious virus is produced and then possibly not completely inactivated. In the second, the foreign DNA is inserted into a live vector capable of replicating in the host species in the same way as an attenuated strain.

The first recombinant vaccine, also against Hep B, was produced in 1986 was produced in yeast . The vaccine was constructed by excising the S antigen gene of the viral DNA complete with flanking promoter and terminator sequences, and inserting it into the ring structure of a plasrnid vector. Transvectored into yeast cells, the plasmid encoded for production of the antigen which could be readily purified, however, the vaccine offered poor protection. This is now attributed to a deficiency of helper T cell determinants in an outbred population. Moreover, even with adjuvants, multiple doses were required to generate high levels of antibodies and sometimes these levels were short-lived. Nonetheless, research continued to develop other recombinant vaccines which could be expressed in host cells.

The E. coli vectors although easy to use, produce non-glycosylated proteins and have proved to produce poorly immunogenic vaccinating products. The mammalian and yeast cell systems producing glycosylated proteins have been found to produce more immunogenic proteins, particularly against enveloped viruses, presumably since they resemble the surface antigens of lipid containing viruses more closely. Such approaches also include the use of transgenic animals with acquired genes to produce high levels of "antigen" e.g. for porcine reproductive and respiratory syndrome (PRRS), an emerging viral disease of pigs in the United States and other swine producing countries causing losses due to acute and persistent chronic infections in pigs of all ages. Current immunization strategies rely on the use of attenuated live-virus because killed-virus vaccines have been found ineffective against PRRSV infection. The "in vivo" production of antigenic and immunogenic PRRSV glycoproteins in the milk of transgenic animals and the further use of recombinant PRRSV proteins as subunit vaccines is being explored.

Virus vectors have also been used for the expression of other "foreign" virus antigens, including vaccinia virus for the control of rabies in foxes. However, because of safety concerns, application of vaccinia recombinants has been very limited. The use of Sheep and goat pox instead of vaccinia is being actively pursued e.g. genes encoding the H and F proteins of rinderpest have produced successful results. (Giavedoni et al, 1991)

Engineering viruses to delete genes is illustrated well in Pseudorabies vaccine. Here, the virulence of the vaccine strain of Aujeszky's disease was modified by engineering a mutation of the thymidine kinase (TK) gene, so that the vaccine has no detectable TK activity. In addition to a TK deletion, a second deletion removed a gene coding for viral glycoprotein which prevents antibodies being produced against the glycoprotein. This second deletion allows vaccinated pigs to be identified by ELISA from pigs naturally infected and kits have been developed.

A process for producing live, non-pathogenic, vaccines for the pathogens RNA tumour virus utilizes gene-altering technology to produce an altered genome which codes for the antigenic determinants of a pathogen, but has no genes coding for pathogenicity. The vaccine is the phenotypic expression of the altered genome. Specifically, an avian RNA tumor virus env gene is cloned into the non-pathogenic RNA virus RAV-O and the resulting recombinant product is replicated in host cells to provide a recombinant vaccine for the pathogen avian RNA tumor virus.

Vaccines based on nucleic acids show promise, (Dixon, 1995) This began with the observation that in vivo inoculation of purified genetic material can elicit immune responses against the encoded antigens in mice and other animals. Immunisation with purified genetic material allows presentation of the antigen in a native form, synthesised by the host in a similar way to that by which the antigens are synthesised during infection by that pathogen, and so it is possible to code for pathogens that escape the immune system by modifying their antigens. An additional advantage of purified nucleic acid vaccination over viral carrier systems is that only genes coding for antigens are delivered, and not the genetic material of the carrier organism. The disadvantage of this approach is the concern over possible integration of the DNA into the human genome. This could be overcome by using by using RNA, but the short lived molecules may not generate the required long lasting protective response.

Another field of vaccine research is the designed construction of synthetic vaccines, (Lerner, 1983). These have the advantage of being non-infectious and free of nucleic acids. As long as the synthetic antigen has peptide epitopes recognised by helper T cells and by antibodies, the immune system should react. However proteins separated from virus particles are generally much less immunogenic than the intact particle. Protective antibody mediated responses depends on the presentation of the antigen to the immune system and this must mimic the configuration of an intact organism. So far, successful methods include the creation of particles containing many copies of the immunogenic site or linking the protein to an immunostimulating complex (iscom) with activities equivalent to those of the virus particle.

Adjuvants

The main functions of the adjuvant in a vaccine are to keep the antigen at, or near, the injection site and to activate antigen presenting cells to achieve effective antigen processing and interleukin production. There is currently great interest in developing new adjuvants, particularly those which act as 'antigen depots' providing controlled release of antigen over a long time span. Candidates include oil-water emulsions, liposomes, iscoms and biodegradable microspheres.

Cytokines

The immune system is regulated and activated by hormone-like cytokines. Immune responses to vaccines depend on complex cytokine mediated interactions and it is known that injection of certain cytokines can augment responses to vaccines. Recently cytokine genes have been engineered into live virus vectors enabling production of cytokines in a much localised environment. The expression of certain cytokines by vaccinia virus can selectively stimulate particular responses in mice following immunisation.

EdibleVaccines

(Plant and Plant viruses based vaccines)

These are the vaccines produced from transgenic plants in which an active antigen of the target pathogen is expressed and accumulated which can give protective immunity against the particular pathogen when fed to the animals. Edible vaccines are prepared by introducing bacterial or viral genes coding for antigens into desired plants with the help of electric impulses or the particle bombardment or the vaccume infiltration or by direct DNA transfer.

Plants/Vegetables/ Fruits for Edible vaccines

• Banana	May, G.D. *et al.* (1995)
• Tomato	Sandhu, J.S. *et al.* (2000)
• Potato	Tacket, C.O. *et al.* (2000)
• Peanuts	Daniell, H. *et al.* (2001)
• Rice	Daniell, H. *et al.* (2001)
• Wheat	Daniell, H. *et al.* (2002)
• Corn	Kusnadi, A.R. *et al.* (1997)
• Soyabean	Kusnadi, A.R. *et al.* (1997)
• Carrots	Daniell, H. *et al.* (2002)

Advantages of plant system in the development of oral vaccines

- Edible plants are very effective as a delivery vehicle for inducing oral
- immunization
- Adjuvant for immune response is not necessary
- Excellent safety and economic feasibility of oral administration compared to injection
- Easy for separation and purification of vaccines from plant materials
- Effective prevention of pathogenic contamination from animal cells
- Convenience and safety in storing and transporting vaccines
- Effective maintenance of vaccine activity by controlling the temperature in plant cultivation
- Easy for mass production system by breeding compared to an animal system
- Possible production of vaccines with low costs
- Edible means of administration
- Reduced need for medical personnel and sterile injection conditions
- Economical to mass produce and transport
- Reduced dependence on foreign supply
- Storage near the site of use
- Heat stable, eliminating the need for refrigeration
- Antigen protection through bioencapsulation
- Subunit vaccine (not attenuated pathogens) means improved safety
- Seroconversion in the presence of maternal antibodies
- Generation of systemic and mucosal immunity
- Enhanced compliance (especially in children)
- Delivery of multiple antigens

Edible Vaccines against Viral Diseases

- A highly successful example is the hepatitis B vaccine, which uses HBsAg expressed in transgenic yeast.
- HBsAg is the main envelope protein of hepatitis B virus (HBV), and is an integral membrane protein of the endoplasmic reticulum (ER).
- When expressed in transgenic tobacco, HBsAg makes VLPs with an average size of 22 nm.
- The plant-derived HBsAg was structurally similar to VLPs produced in transgenic yeast which is used as hepatitis B vaccine.
- A corn based an oral transmissible gastroenteritis virus vaccine boosts immunity in Swine
- Herpes simplex virus in transgenic soybeans.
- Capsid protein of FMD virus in transgenic *Arabidopsis thaliana*.
- Norwalk virus Capsid protein in transgenic tobacco and potato was successfully demonstrated.
- The other organisms mostly targeted by edible vaccine were Rota virus.
- Plant viruses have also been identified as potential vector for expression of foreign antigens and development of novel vaccines.
- Cowpea mosaic virus (CPMV) is one of them because of several important features of this virus.
- FMDV and HIV epitopes were expressed in CVPs (Chimeric virus particles) of CPMV which produced specific antibody response.
- Induction of a protective antibody response to foot and mouth disease virus in mice following oral or parenteral immunization with alfalfa transgenic plants expressing the viral structural protein VP1.
- A subunit vaccine using the TGEV envelope spike (S) protein was produced in transgenic corn and fed to piglets, resulting in 50% of virus-challenged animals being free of diarrhea.

Oral vaccine uses a plant system

Most vaccine-developing technologies of remixing genes use revealed systems of animal cells and certain conditions of the cultivation of animal cells. Also, a disease virus is various and there are many variants so we cannot develop vaccine without paying a great deal of expenses to research and develop such vaccine. But the workers had already kept producing technology of vaccine including transgenic plant, transgenic vector production, and control of genetic revelation, separation and refinement of remixing protein analysis of transgenic plants, producing an antibody and separating proteins, and examination technology of the cause of an animal immunity and others. We can produce a great deal of safe vaccine from vegetable cells at a low price. The technology of producing vaccine using vegetable systems has many good points and has attracted public attention as the technology of developing effective vaccine a (covering the introduction of producing oral vaccine from plants).

The fruit delivery system: a new way to vaccinate

Vaccines produced in raw foods such as bananas may be a cost-effective alternative for controlling important diseases in developing countries. The proteins targeted for use in subunit vaccines are antigens, which may be thought of as the molecular signature of the pathogen. In viruses, antigens are usually proteins which appear on the surface of the virus and are well recognised by the immune system. If humans are exposed to an antigen, they recognise it as being a foreign molecule and develop an immune response against it, so, if exposed to the real bacteria or virus, their system can mount an effective and protective defence.

Virus diseases of animals pose the most important and continuously evolving threat to economics of both developed and developing countries. Control measures include the use of vaccines and the need for rapid and accurate diagnosis. Conventional methods of tissue culture growth and inactivation or attenuation of viruses are still those most used to prepare vaccines. Molecular biological methods has offered the chance to develop new strategies involving identification and manipulation of genes for expression of protein in vectors to produce large amounts of vaccinating protein or insertion into new “safe” replicating agents. The immunological basis of host/pathogen relationship has been better understood through use of the latest molecular and serological advances. This understanding will improve the chances of designing appropriate vaccines in future.

Diagnostic improvements stem from the identification of relevant “diagnostic” proteins, the availability of defined products in large amounts through gene manipulation and expression and the improvements in methods, particularly those based on MAbs and PCR technologies and developments in instrumentation. These offer potentially highly sensitive and specific methods for unequivocal results concerning disease or immune status.

The exploitation of the newer methods has not been as rapid in the veterinary sphere as in human medicine. The reasons for this come from the highly fragmented nature of the problems encountered in animal science which is not so attractive to commercial interests. There is also a misunderstanding of the damage caused by virus disease in both developed and developing countries. The newer methods do offer a new level of sophistication to all laboratories, including those in developing countries. All rely on the continued research into virus disease at all levels, molecular, serological and epidemiological as well as the applied production of defined reagents, including kits for serology and molecular biology. This is particularly important in developing countries but is essential to all involved in disease control, particularly since virus diseases have to be regarded as trans-boundary in nature.

Conclusion

- The development of vaccines against viral diseases is one of the great achievements of human endeavors and science of Vaccinology has gone under revolutionary changes.
- Efforts have been made for developing more efficacious, safer, thermostable and economically affordable vaccines using molecular approaches.
- Recombinant vaccines have already been commercialized and some are under different stages of development.
- The promising area with future potential is expression of genes encoding immunogenic protein in plants and plant viruses.
- Naked nucleic acid vaccines also hold very promising future.
- Biotechnologically designed vaccines would allow the production of bivalent and even multivalent vaccines that are easier to produce and administer and have increased safety, efficacy, and potency.
- Biotech vaccines are generally expected to be more stable and heat resistant, so that low temperature would not be required.
- Ongoing researches on Edible vaccines will definitely come up with a safest way to immunize children and animals. Get ready to eat a VACCINE BANANA.

VIT–L–03
Reverse genetics for animal vaccine development

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Newcastle disease (ND) is one of the highly pathogenic viral diseases of avian species. ND is economically significant because of mortality and morbidity associated with it. Newcastle disease virus (NDV) belongs to the family *Paramyxoviridae* and the genus *Avulavirus*. Recent advances in recombinant DNA techniques have brought forward to an era of new vaccine technology in modern medicine. One attractive strategy is the application of reverse genetics to make recombinant Newcastle disease virus (rNDV). rNDV can deliver protective antigens of pathogens in host and evoke a protective immune response. The recombinant NDV vaccine offers a pertinent choice for the construction of live attenuated vaccine due to its minimum recombination frequency, modular nature of transcription and lack of DNA phase during its replication. NDV is also explored widely in the field of cancer biology due to its property of replicating selectively in tumor cells. In the recent years, the reverse genetics technology allowed to generate rNDV having high tumor suppressor property. By understanding NDV molecular biology, it is feasible to develop gene-modified recombinant NDV vaccines possessing better safety and immunity for both animals and humans.

Article

Newcastle Disease (ND) is a highly infectious viral disease of avian species. ND infection has been reported from a wide variety of birds with varying degree of susceptibility (Kaleta and Baldauf, 1988). ND was first reported from Indonesia in 1926 and in the New Castle upon Tyne in England in 1927 (Doyle, 1927; Kraneveld, 1926). Based on pathogenic studies ND is categorized into three groups: lentogenic (low virulence), mesogenic (moderate virulence) and velogenic (highly virulent). The velogenic ND can either be viscerotropic or neurotropic depending on its predilection site (Alexander, 2000). Velogenic ND may result in 100% mortality in poultry leading to significant impact on trade restrictions and embargoes in the regions of its outbreak.

The causative agent of ND is a Newcastle disease virus (NDV). NDV is classified as a member of genus *Avulavirus* in the subfamily *Paramyxovirinae* under family *Paramyxoviridae* (Lamb and Parks, 2007). The genus *Avulavirus* is divided into nine serotypes based on haemagglutination inhibition (HI) and neuraminidase inhibition (NI) assays. NDV is a pleomorphic enveloped virus around 200-300 nm in diameter. The genome of NDV is a non-segmented, negative sense, single stranded RNA. The NDV strains isolated from different parts of the world fall into three genome size groups: 15,186 nucleotides (nt) long in the isolates before 1960; 15,192 nt long in the isolates discovered in China, and 15,198 nt long in the avirulent strain from Germany.

NDV is an attractive vaccine vector candidate for both human and animal use (Samal, 2011). It presents a promising candidate for rational design of live attenuated vaccine and vaccine vectors because of its modular nature of transcription, minimum recombination frequency and an absence of DNA phase during replication. The genome of NDV is quite easy to manipulate using reverse genetics system (Bukreyev and Collins, 2008; Huang et al., 2003; Krishnamurthy et al., 2000; Peeters et al., 2001). Live attenuated vaccines and bivalent vaccines are economically very popular for the poultry industry. The lentogenic strain of NDV appears to be a good vaccine. Both live attenuated and recombinant viruses are explored as vaccine and vaccine vectors with various degrees of success. The recombinant NDV expressing foreign protein is explored as a viral vector by many scientists around the globe. Following properties of NDV can be attributed for its credibility as a viral vector:

i. NDV grows with high titres in embryonated chicken eggs, cell culture and respiratory tract of avian and non-avian species.

ii. NDV infects naturally via the respiratory tract and is thus useful to deliver protective antigens derived from respiratory pathogens. In addition, it induces both local and systemic immune responses.

iii. NDV elicits both humoral and cellular immune response.

iv. NDV has a modular genome with only six essential and two accessory genes that is easy to manipulate.

v. NDV does not integrate with host genome as it replicates in host cytoplasm and shows least genetic recombination.

vi. Recombinant NDV expressing the foreign antigen shows quite a high and stable expression of foreign protein after many passages both *in vitro* and *in vivo* (Huang et al., 2003).

vii. NDV can be attenuated using reverse genetics for the development of stable vaccine and vaccine vector. Development of deletion mutants (V, W) and mutants with altered F cleavage site may result in more immunogenic and attenuated recombinant virus at the same time (Huang et al., 2003; Samal et al., 2011).

For construction of vaccine vector, the foreign gene (~ 3.8 kb) must be flanked by NDV specific gene-start and gene-end sequences at the gene junction or intergenic sequence without perturbing the rule of six (Bukreyev et al., 2006; DiNapoli et al., 2007; Huang et al., 2001). The level of foreign gene expression is found to be more in case of its insertion at the 3' end of the genome (Carnero et al., 2009; Huang et al., 2001).

Recombinant NDV acts as a potential vaccine vector for humans because of its attenuation due to natural host range restriction (Samal, 2011). NDV offers an important vaccine candidate in terms of safety, efficacy and cost effectiveness. A number of NDV based vaccines are generated for treating various human viral infections. Recombinant NDV confers immunogenic response against different antigenic challenges like human influenza virus heamagglutinin protein (HA) (DiNapoli et al., 2010; Ge et al., 2007), HIV and SIV Gag protein (Lawrence et al., 2013; Nakaya et al., 2001; Nakaya et al., 2004), HIV glycoproteins (Khattar et al., 2011; Khattar et al., 2013), F glycoprotein of human respiratory syncytial virus (Martinez-Sobrido et al., 2006), HN protein of human parainfluenza virus 3 (Bukreyev et al., 2005), and spike glycoprotein of SARS-CoV (DiNapoli et al., 2007).

NDV acts as an excellent vaccine vector for veterinary pathogens and is successfully marketed. Recombinant NDV expressing VP2 protein is used as a dual vaccine against NDV and infectious Bursal disease infection in chickens (Huang et al., 2004). Recombinant NDV expressing the HA protein of H5N1 (Ge et al., 2007; Nayak et al., 2009; Park et al., 2006; Romer-Oberdorfer et al., 2008) and H7N7 influenza virus (Park et al., 2006; Schroer et al., 2009) protects against both NDV and influenza virus infection in chickens. Recombinant NDV expressing gN and gG glycoprotein of Rift Valley fever virus is shown to protect mice and lambs, against viral infection by eliciting effective antibody response (Kortekaas et al., 2010). Recombinant NDV expressing gD glycoprotein partially protects cattle against bovine herpes virus infection (Khattar et al., 2010). In another study, recombinant NDV expressing the G and F proteins of Nipah virus induces neutralizing antibody responses in mice and pigs (Kong et al., 2012).

Many studies have been conducted around the world to improve the vaccine efficiency of NDV. It has been shown that recombinant NDV expressing the H5N1 HA protein in mule ducks showed sterile protection in the absence of maternally-derived antibodies (Ferreira et al., 2012). Recombinant NDV expressing soluble trimeric HA protein against highly pathogenic H5N1 influenza virus provides better efficacy than the membrane anchored HA suggesting that the type of antigenic foreign protein could be modulated for better protection (Cornelissen et al., 2010).

ND is a major threat to the poultry industry around the globe. The disease is endemic in many developing countries while the disease free countries are prone to accidental outbreaks. NDV strains having varying degree of virulence circulate among avian species. The topographical distribution of NDV is not well understood and regular sporadic cases are reported throughout the years from endemic areas. Research priorities are towards the improved diagnostics and better vaccine development. The reverse genetics system offers the opportunity to engineer NDV as a vaccine vector for human and animal uses.

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VIT–L–04

New Generation Vaccines

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The population of our country is increasing steadily with annual growth rate of 1.4% and has reached to more than 1.27 billion (United States Census Bureau). It is projected that the population of India would be around 1.61 billion by year 2050 surpassing China (People Facts & Figures-www.os-connect.com/pop/p2.ai.htm). There would be great challenge to meet growing demands of food to such huge population as agricultural productivity is declining mainly due to shrinking of cultivable land. Under such conditions, livestock sector may play important role in providing nutritional food security and help in improving socio-economic status by generating employment opportunity. This important sector is also facing many problems which include low availability of feed and fodder and loss due to infectious and non-infectious diseases. To overcome problems faced by livestock sector from the infectious diseases, scientists across the world have aimed for developing effective treatment and prevention strategies including vaccination. The major goals of veterinary vaccines are to prevent loss due to these diseases and increase production of livestock in a cost-effective manner and also improve the health and welfare of companion animals and prevent animal-to-human transmission from animals. The term "vaccine" (from the Latin term "vacca," meaning cow) was first coined by Edward Jenner to describe the inoculation of humans with the cowpox virus to confer protection against the related human smallpox virus. The earlier vaccine (First generation vaccine) included crude whole organism to more defined and pure cell culture (second generation) vaccines.

The advancement in molecular biology, bioinformatics and detailed knowledge about pathogen has led to the development of molecularly defined sub unit vaccines, DNA vaccine, vectored vaccine, multivalent vaccines like chimeric vaccines and vaccines developed through reverse genetics technology. Now with these advancement, it has become possible to develop a vaccine with specific antigenic epitopes that can induce specific types of enhanced immune response (humoral, cell mediated or mucosal) required to confer complete protection. Further, these technologies are very powerful and can be used to develop multivalent vaccines containing antigens from different strains or even different pathogens, thus cover wide range of disease with single inoculation and can also overcome problems of vaccination failure faced in disease where pathogens are evolving. Most of the second generation vaccines are very good, easy to administer and provide solid protection thus careful thinking is to be made before going for these vaccines and it should not be opted indiscriminately. Another issue in vaccine and vaccination is delivery of antigen to the target cells/tissues. Due to non-availability of proper delivery system, large quantity of antigens per dose are required which put unnecessary burden on animal's immunes system and also increase cost of vaccine production. Therefore, research must be focused for developing effective delivery system for eliciting comprehensive immune response in low doses and developing combination vaccines to reduce cost of vaccine and vaccination.

VIT–L–05

Genetic Diversity of Equine Herpesviruses from Clinically and Latently Infected Equines in India

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Equid herpesvirus 1 (EHV1) is one of the most economically important equine viral pathogens causing acute upper respiratory tract disease in young horses, late-term abortion in pregnant mares, neonatal foal mortality and neurological disease termed equine herpesviral myeloencephalopathy (EHM). The global incidence of abortion and rhinonpneumonitis is on decline, while that of EHM is on the rise, due to widespread use of vaccines to control respiratory infections and abortions. Although clinical form of EHM is less frequently observed, it can cause serious economic losses in breeding horses and has very negative impact on equine industry. Following infection, EHV1 establishes a life-long latent infection within the host specific tissues where viral genome persists with very limited transcription. The viral factors that influence EHV1 disease severity and latency are poorly understood, and this has hampered vaccine development. Latent infections are the cornerstone of survival of EHV1 in horse population, as more than 50% animals after infection become latently infected and cannot be readily identified by any currently available laboratory tests. Targeting expression of late structural genes and latency associated transcripts, it was observed that 37-74% horses in India were latently infected with EHV1.

To broaden understanding of EHV1 diversity in India, EHV1 strains isolated in India since 1990 were characterized for prevalence of neuropathogenicity and genetic diversity. Sequencing of ORF30 genes and SNP real-time PCR results have been employed to detect neuropathogenic strains circulating in India. The findings revealed that 96% of EHV1 isolates circulating in India are of non-neuropathogenic genotype (A_{2254}) while 4% had the neuropathogenic marker (G_{2254}). Sequence analysis of the polymorphic region of ORF68 of EHV1 isolates from India indicated that Delhi/1998, Tohana-2/2013, Hisar-2/2014 and Hisar-15/1990 isolates belonged to group 4 while Jind/1996, Rajasthan/1998, Delhi-3/2007 and Tohana-5/1996 isolates clustered within group 5. The complete DNA sequencing of two representative EHV1 isolates was done and phylogenetic analysis showed existence of EHV1 belonging to different clades in India. The findings establish the existence of limited EHV1 genetic diversity in India and its significance in persistence of EHV1 infection and development of control strategies will be discussed.

VIT–L–06
Immune responses to bluetongue vaccines

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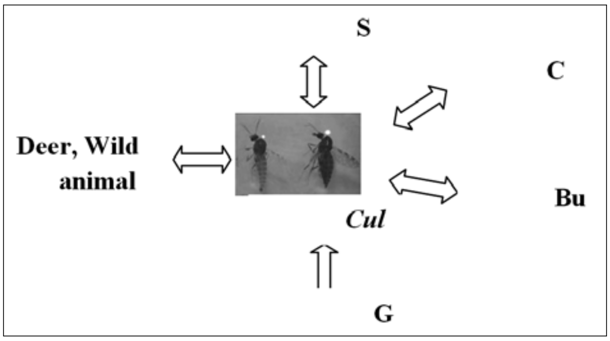
Bluetongue continues to be the disease of major economic importance in sheep since 1964 in India. Though sheep show severe clinical disease, BTV antibodies are regularly being reported from goats, cattle , buffaloes , deer, camels, mithuns and bisons from several parts of India. High seropositivity is usually observed in goats, cattle and buffaloes. BTV is being isolated from sheep and occassionally from cattle and goats. Bluetongue is transmitted by the bite of *Culicoides* and there is no direct transmission from animal to animal.



Full proof vector control methods are difficult to implement because of sheep husbandry practices prevalent in the country. Sheep flocks are migratory in nature and open housing is also being practiced.

Insecticide application on livestock is often used as a first barrier to reduce BTV transmission. However, transmission of BTV has been shown to occur even when deltamethrin has been used. The lack of efficacy of deltamethrin, as well as the local traditional methods, in the Indian field studies suggest that local populations may have developed some form of physiological and behavioural resistance to the compound, possibly due to consistent exposure of populations to pesticides (Rien *et al*., 2017).

Specific strains like BTV-8 caused severe disease in cattle and goats in Europe. During these outbreaks in sheep flock, ewes were predominantly affected. In Netherlands, the bluetongue disease outbreak in herds that recovered from clinical disease was approximately eight times higher for cattle compared to sheep (Elbers *et al.,* 2008b). Experienced farmers in BTV endemic areas of South Africa, observed from time to time mild clinical BT in their cattle, but general impression was that BTV did not produce more than transient and mild clinical signs in cattle (Hourrigan and Klingsporn, 1975).The most prominent clinical signs in BTV-8 affected cattle herds were: crusts/lesions of the nasal mucosa, erosions of lips/crusts in or around the nostrils, erosions of the oral mucosa, salivation, fever, conjunctivitis, muscle necrosis and stiffness of lambs (Elbers *et al.,*2008a). There have been reports of FMD like disease in cattle from Central Iran (Noaman *et al.,*2013) and also from India (Arun *et al.,*2014).

Recently clinical disease in goats and cattle are being reported from Europe and hence cattle and goats were also included besides sheep in the BT vaccination campaigns using inactivated vaccines in Europe. Simultaneous occurrence of BT and PPR in goats and FMD like lesions in cattle is being reported from India. It is a common practice of sheep farmers to keep few goats along with sheep flocks. Cattle and buffaloes are also reared by the farmers.

The problems associated with the live attenuated bluetongue vaccines are the development of viraemia, possibility of transmission by vectors, reversion to virulence and re-assortment with wild-type viruses in vaccinated animals (Veronesi *et al.*, 2005). After vaccination with modified live vaccine, the animals showed some reactions like temperature, Oedema, lameness, hyperaemia and decrease in milk production in some instances in Europe (Savini *et al.*, 2010). Inactivated vaccines can generate a safe and protective immunity. A single vaccination is usually insufficient to provide an immunity that lasts for several months. A strong and long term immunity can be elicited by two injections. They are the best currently available safe/efficacious vaccines (Schwartz-Cornil *et al.*, 2008). BT inactivated vaccines were developed and commercialized for BTV9, BTV1 and BTV8. Most of recent European BT vaccination campaigns have exclusively used inactivated vaccines (Wilson and Mellor, 2009). The use of inactivated /modern vaccines avoid risk associated with the use of live-attenuated vaccines, such as reversion to virulence, reassortment of genes with field strain, teratogenicity and insufficient attenuation leading to clinical disease (Roy *et al.*, 2009). The European Food Safety Authority currently recommends using inactivated vaccines to protect animals from BT. The virus-like particles (VLPs) produced from recombinant baculovirus and live recombinant vaccinia or canarypox virus-vectored vaccines were tested in the laboratory and yet to be commercialized (Niedbalski, 2011).

During 2003, OIE symposium held at Italy has recommended the development and usage of inactivated / recombinant vaccine to control bluetongue. It has recommended to discourage the usage of live attenuated multivalent vaccines in the field because of apprehension that new strains will emerge due to reassortment of genome segments since BTV has 10 double stranded RNA segments.

During 2007, All India Network Programme on Bluetongue (ICAR) has recommended the development of bluetongue multivalent inactivated vaccine to control bluetongue in India. Accordingly guidelines for the development and evaluation of bluetongue multivalent inactivated vaccine (containing BTV serotypes 1, 2, 10, 16 and 23) were prepared (AINP BT, 2010). These five serotypes were isolated and prevalent in India. The vaccine was developed and evaluated under experimental and field conditions and subsequently commercialised.

In the present study, bluetongue pentavalent inactivated vaccine adjuvanted with either oil adjuvant or aluminium hydroxide gel were prepared incorporating BTV serotypes 1, 2, 10, 16 and 23. Humoral and cell mediated immune responses in sheep, goats, calves and buffaloe calves with bluetongue pentavalent inactivated vaccine adjuvanted with either oil adjuvant or aluminium hydroxide gel were assessed. The seronegative animals which were vaccinated on 0 day, were revaccinated on 28 and 365 days post vaccination (DPV). Competitive enzyme linked immunosorbant assay and micro serum neutralization test were employed for assessing humoral immune responses in the serum samples collected on 0, 28 and 42 DPV in case of sheep / goats / calves / buffaloe calves under experimental conditions and 0, 28, 42, 90, 180, 365 and 379 DPV in case of sheep / goats / calves / buffaloe calves under field / farmers holdings. Lymphocyte proliferation assay, gamma interferon assay and flow cytometry analysis of CD4+ and CD8+ lymphocyte subset population were assessed in the blood samples collected on 0, 28 and 42 DPV in case of sheep / goats / calves / buffaloe calves under experimental conditions and 0, 28, 42, 90, 180, 365 and 379 DPV in case of sheep / goats / calves / buffaloe calves under field / farmers holdings.

The vaccinated animals showed both humoral and cell mediated immune responses till 365 DPV and sheep/goats/calves/buffaloe calves vaccinated with BTPIV with oil adjuvant showed higher immune responses. Montanide ISA 206 VG was used as oil adjuvant.

However all the animals showed both humoral and cell mediated immune responses on 379 DPV. This indicates immediate anamnestic response on 14 DPV following revaccination on 365 DPV of the vaccinated animals indicating the booster vaccination with BTPIV oil / BTVPIV Al gel of the sheep /goats /calves / buffaloe calves on 28 DPV will protect the animals till one year. Primary vaccination of the animals above 3 months of the age is to be followed by booster vaccination at 28 DPV. Thereafter annual vaccination is recommended for the protection of the animals against BTV (Sathianarayanan, 2017). Successful vaccination always depends on the herd immunity, hence it is essential to immunize all the susceptible population.

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ORAL PRESENTATION

VIT–O–01

Immunological Characterization of Field Isolates of Classical Swine Fever Virus Adapted in Different Cell Lines

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Classical swine fever is a highly contagious and fatal disease of domestic pigs, wild boar and pygmy hogs. Genogroup 1.1 is prevalent in India along with genogroup 2.1 and 2.2 are also circulating in India. In the present study , adaptation of selected field isolates of representative genogroups 1.1 , 1.2 and 2.2 along with vaccine virus as refrence virus in PK-15, RK-13, Vero. All the field isolates were passaged upto 10th passage level and could be adapted in PK-15 and RK-13 cell line except genogroup 1.2. Virus isolates could not get adapted in vero cell line . The presence of CSF virus was detected by nRT-PCR and FAT. The log TCID50 of adapted vaccine virus in PK-15 cell line upto 7th passage was found to be 6.25 followed by the field isolates. Similarly in RK-13 cell line, the vaccine virus showed the highest log TCID50 value followed by the field isolates. Antigenic characterization of the selected isolates of representative genogroup 1.1 and 2.2 proapagated in PK-15 cell line was done. Immunogenic epitopes were retained in all genogroups even after several passages in heterologous cell lines. The B-cell and T-cell epitope prediction of specific E2 gene of respective isolates were conducted using suitable bioinformatics tool viz. BepiPred Linear Epitope Prediction and IEDB 2.9 tool. It was deduced that there were large number of immunogenic region which has higher binding capacity with antibodies in respect to B-cell epitope and with MHC-I and MHC-II molecules in respect to T-cell epitopes.

VIT–O–02

Development and evaluation of combined fowl cholera and avian mycoplasmosis vaccine in layer birds

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Fowl cholera is a common avian contagious bacterial disease which may affect all types of birds throughout the world. Fowl cholera caused by Pasteurella multocida leads to mortality of 25 to 35% in chickens. Avian Mycoplasmosis is another important infection to poultry farming caused by *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) which are involved in chronic respiratory disease and heavy economic loss through embryo mortality (5-20%), chick mortality (5-10%), slow growth (8-25%), condemnation of carcass (5-10%), and production drop (8-20%).

In the present study, the combined vaccine was developed with the locqally prevalent local isolates of *Pasteurella multocida* (capsular type A) and *Mycoplasma gallisepticum*. The inactivated, combined vaccine was prepared as per the Standard Operating Procedure. An experimental study was conducted using the prepared vaccine in 7 weeks old White Leghorn layer birds (20 vaccinates, 10 control birds). Blood samples were collected at 0, 21, 45, 60 days post vaccination randomly. A total of 60 serum samples were collected for assessing vaccine potency by indirect haemaggltination test for Pasteurella multocida and direct haemagglutination test for avian mycoplasmosis for antibody. The results showed that there was an increase in antibody titre from 1:16 to 1:128 and 1:32 to 1:256 respectively. The combined vaccine gave good protective titre for both the pathogens. A limited field trial was also conducted through our university peripheral labs viz. Poultry Disease Diagnostic Laboratory and Avian Disease Laboratory at Namakkal and Thalaivasal respectively with 200 doses of the vaccine in each place. After 21 days post vaccinal blood samples were collected for assessing the antibody response using the above tests. The results revealed that the vaccine is working better at field conditions. It is concluded that the fowl cholera and avian mycoplasmosis combined vaccine is working well for growers and is advocated in layer birds at the age of 8-12 weeks to immunize against the two pathogens before the start of production.

VIT–O–03

Isolation of a novel Ribosome Inactivating Protein from Cucurbitaceae species

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Ribosome inactivating proteins (RIPs) are protein toxins distributed widely in plant kingdom. Many RIPs have shown practical applications in different fields, such as in agriculture and in medicine. These proteins have the ability to inhibit protein synthesis by inactivating ribosomes. Many plants of Cucurbitaceae family have shown to possess these proteins which have shown promising results against microbes, viruses and are also being explored for anti-cancer activities. In the present study some species were explored for the presence of novel RIP(s) which may inactivate ribosome and in turn may be for their medicinal property in cancer treatment etc. A novel protein was isolated showing ribosome inactivating property. Upon the structure related study it was found that the protein is predominant of alpha helix. This property of inactivating ribosome may further be studied against some animal viruses, anticancer activity etc. and hence with further study of the structure, and understanding the mechanism of its action this protein in the future may be used for the benefit of both human and animal as antiviral, anticancer drug or as immunotoxins.

VIT–O–04

Upregulation of small cell lung cancer pathway in response to 2,4-Dichlorophenoxyacetic acid and endotoxin interactions.

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The present investigations included male Swiss albino mice (N=48) aging 6-8 weeks to identify molecular pathways associated with pulmonary damage following exposure to 2,4-D with or without endotoxin. Animals were divided into two treatments and one control group (N=16). Treatment groups were orally administered 2,4-D @1/10th and 1/20th of LD50 dissolved in corn oil for 90 days followed by LPS or NSS challenge @80μl/animal via intranasal route. Blood and BAL fluid were collected and analyzed for TLC and DLC. Lung tissues were subjected to histopathological and microarray analysis. BAL fluid and histopathological analysis revealed lung inflammation following exposure to 2,4-D with or without LPS. Further, 2,4 D exposure alone or in combination with LPS dysregulated 5351 genes ($p<0.05$; minimum cut off of 1.5 log fold change) along with 356 commonly expressed genes. Bioinformatic analysis of differentially expressed genes revealed small cell lung cancer as topmost dysregulated pathway in various groups. The key genes of small cell lung cancer pathway viz *Nfkb1, p53, Cdk6, Itgb1* and *Myc* were significantly upregualed and *Apaf1* was down-regulated. These signature genes are being validated by Real time PCR. The data taken together suggest that exposures to 2,4 D with or without LPS upregualte the expression of small cell lung cancer pathway.

VIT–O–05

Polarization of macrophage towards M1 and M2 phenotype can influence the intracellular survival of *Salmonella* pathogen in chicken

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Two types of macrophage polarization, M1 (classical) and M2 (alternative) is now well established in mouse model. In current study, the role of macrophage polarization in clearance of *msrA* gene deleted *Salmonella* Typhimurium (Δ*msrA*) has been explored in chicken species. Chicken monocytes cultured in presence GM-CSF along with IFNγ or IL-4 polarized towards M1 or M2 phenotype respectively. Blunt cytoplasmic process of typical macrophage phenotype appeared within 48 hours of *in vitro* culture. Occasionally cells progressed towards dendritic cell morphology. However, nonspecific esterase staining has confirmed the macrophage lineage. From L-arginine, enhanced levels of nitric oxide in M1 and urea in M2 cultures as end products have been observed.

Phagocytic ability of M1 and M2 was not differed rather low CFU derived from M1 stage as compare to M2 indicated high susceptibility of *msrA* strain in intracellular environment. Cytokine form M2 was skewed towards IL-6 whereas M1 produced IL-10. During *in vivo* trial birds were sensitized with either M1 or M2 macrophage harboring intracellular *msrA* strain. The antibody response in birds were similar and not been influenced by the M1 or M2 stage rather time dependent difference in magnitude of immune response has been observed in both the groups. Up to 15th day the presence of bacteria was evidenced in liver and spleen but on 35th day both the groups cleared the pathogen from the system. The early removal of bacteria may be due to hyper susceptibility of *msrA* strain towards host derived reactive oxygen radicals.

The overall study has shown that M1 and M2 population of macrophage is not too absolute under *in vitro* culture condition however both the phenotype may appear depending upon exact cytokine available in the micro environment once there is pathogen invasion that can influence the pathogen survival or removal.

VIT–O–06

Molecular characterization of Bifidobacteria isolated from faeces of piglets

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Keywords: *Bifidobacteria, 16sRNA gene sequencing, Phylogenetic analysis.*

The potential health benefits to the host rendered by bifidobacteria of the gut have led to their wide application as probiotics in dairy products and food additives for betterment of animal health. The present study was undertaken with a view to isolate bifidobacteria from various sources, *viz.* fresh bovine milk, faeces of calves and piglets, ruminal content of slaughtered bovines and intestinal/caecal content of chicken, and to characterize the isolates by random amplified polymorphic DNA (RAPD) analysis and sequencing of amplified 16S rRNA gene fragments. A total of 12 (2.52%) out of 477 samples from various sources were found positive for bifidobacteria, which were cultured anaerobically at 37° C for 48-72 hrs by using Lactobacillus Man Ragosa Sharp agar with 0.25% L-cysteine (MRSC agar). All the isolates were recovered from faecal samples of young piglets (6.12%), while samples from other sources examined did not reveal the presence of bifidobacteria. The isolates were characterized and identified based on morphological characteristics, biochemical tests and phosphoketolase (F6PPK) enzyme assay, which showed characteristic features of *Bifidobacterium* spp. All the 12 isolates were also confirmed by genus-specific PCR based on amplification of 16S rRNA gene. Sequencing of the amplified products and subsequent sequence analysis by NCBI-BLAST revealed that out of the 12 isolates, 3 (25%) belonged to *B. breve*, 1 (8.33%) to *B. pseudolongum*, 6 (50%) to *B. animalis* subsp. *Lactis* and 1 (8.33%) to *B. Thermacidophilum* subsp. *porcinum*. Phylogenetic analysis based on the banding patterns could not differentiate the isolates belonging to different species of *Bifidobacterium*.

*Oral/poster will be presented by Dr Bedanta Pathak, MVSc, Department of Animal Biotechnology, CVSc, AAU, Khanapara (bedantapathak.13@gmail.com)

VIT–O–07

The integrated analysis of miRNA and mRNA expression profiles depicts key role of miR-22-5p, miR-27b-5p, miR-146a-3p and miR-1343 in response to classical swine fever vaccine virus

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The classical swine fever (CSF) is one of the most important and highly contagious viral diseases of pig, causing tremendous impact on animal health with widespread economic implications. The present study was undertaken to investigate the differentially expressed miRNAs (DEmiRNAs) in response to CSF vaccination in crossbred pigs. It was observed that the proportion of CD4+CD8- T cells, CD4-CD8- T cells and CD4+/CD8+ were decreased subsequently on 7 and 21 dpv compared to 0 dpv, whereas the proportion of CD4-CD8+ T and CD4+CD8+ T cells were increased subsequently on 7 and 21 dpv compared to 0 dpv. Humoral immune response measured in terms of mean percentage inhibition (PI) values was 86.89% at 21 dpv, indicating protective immune response to CSF vaccination. Here, genome wide miRNAs were sequenced to identify DEmiRNAs on 0, 7 and 21 dpv. A total 46 DEmiRNAs were identified on 7 dpv versus 0 dpv and 41 DEmiRNAs were identified on 21 dpv versus 0 dpv from miRNA-seq data. It was observed that 22 miRNAs were commonly expressed on both the time points (21 dpv versus 0 dpv and 7 dpv versus 0 dpv). Using mRNA-seq data, 378 DEmiRNAs were predicted on 7 dpv *versus*0 dpv and 371 DE miRNAs were predicted on 21 dpv versus 0 dpv from. Of these predicted DEmiRNAs, it was found that 40 and 35 DEmiRNAs were common from miRNA seq and mRNA seq data on both time points. Two DEmiRNAs, miR-22-5p and ssc-miR-27b-5p, were selected on the basis of their fold change and functions in activation and/or inhibition of their target genes in immune process/pathway of viral infections for validation using qRT-PCR. Target genes against each selected DEmiRNAs *viz*CD40 and SWAP70 (target gene of ssc-miR-22- 5p), TLR4 and LYN (target genes of ssc-miR-27b-5p) were selected for qRT- PCR validation which were in concordance with miRNA expression. It was observed that ssc-miR-146a-3p was upregulated and ssc-miR-1343 was downregulated on 7 dpv compared to unvaccinated crossbred pigs. The expression of DE miRNAs in qRT-PCR was in concordance with miRNA Seq analysis. Two set of target genes, *CD86* and *TNFAIP3* (target gene of ssc-miR-146a-3p); *TLR4* and *IFIT1* (target gene of ssc-miR-1343) were validated by qRT-PCR and were also found to be in concordance with the results of RNA seq analysis.

VIT–O–08

Immune responses in buffaloes following bluetongue vaccination

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Immune response to bluetongue pentavalent inactivate vaccine (BTPIV) adjuvanated with either oil adjuvant or aluminium hydroxide gel incorporating bluetongue virus (BTV) serotypes 1, 2, 10, 16 and 23 in buffaloes under experimental and field conditions were examined. The seronegative buffaloes which were vaccinated on 0 day were revaccinated on 28 and 365 days post vaccination (DPV). Lymphocyte proliferation assay, gamma interferon assay and flow cytometry analysis of CD4+ and CD8+ lymphocyte subset population were employed for assessing cell mediated responses in blood samples whereas competitive enzyme linked immunosorbent assay (cELISA) and micro serum neutralisation test (mSNT) were employed for assessing humoral mediated responses on 0, 28 and 42 DPV in experimental buffalo calves and 0, 28, 42, 90, 180, 365 and 379 DPV in field or farmers holdings.

Bluetongue pentavalent inactivated vaccine adjuvanted with Montanide ISA 206 VG / Aluminium hydroxide gel elicited both humoral and cellular immune responses upto one year in buffaloes. Higher humoral and cell mediated immune responses were observed in buffaloes vaccinated with BTPIV with oil adjuvant when compared to BTPIV with Al gel. All the buffaloes showed both humoral and cell mediated responses on 379 DPV, indicating immediate anamnestic response on 14 DPV following revaccination on 365 DPV of the vaccinated buffaloes.

VIT–O–09

ADAPTATION OF FIELD STRAIN OF DUCK PLAGUE VIRUS IN HETEROLOGOUS CELL CULTURE SYSTEM

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Duck plague is an acute, fatal viral infection of ducks, geese, swans and other species of the order Anseriformes. Live attenuated vaccine can be an effective tool to control outbreak of DPV. The present study was undertaken with a view to adapt the wild strain of DPV in heterologous cell system. A total of 6 wild strains of DPV were revived in ducklings and was confirmed by PCR. All the isolates revived were further propagated in duck embryo fibroblast (DEF) up to 5th serial passage. Sequence analysis of all isolates revealed genomic identity to circulating vaccine virus and other Indian isolates of DPV. On the basis of DID_{50} and $TCID_{50}$, a very virulent (VV) strain of DPV was selected for adaptation in chicken embryo fibroblast and other established cell lines. Propagated virus was further adapted in CEF, vero cell line and QT-35 cell line upto 12th passage level. The CEF adapted virus exhibited distinct CPE from second passage onward. TCID50 at 12th passage level was $10^{9.67}$/ml. In case of Vero cell line there was no any distinct appearance of CPE till 12th passage. However, propagation of virus was confirmed by PCR. In QT-35 cell line CPE was recorded from 9th passage onward and the viral nucleic acid was detected by PCR. In this study it was seen that wild strain of DPV adapted well in CEF. However, adaptation of DPV in cell line requires more passages

VIT–O–10

Knowledge and attitude about rabies prophylaxis: a survey amongst the high risk population in Tamil Nadu

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Rabies is an important zoonotic disease in India. Despite the availability of vaccines and anti rabies immunoglobulins, rabies kills over 50000 people a year. Human rabies deaths are entirely preventable through appropriate post-exposure prophylaxis (PEP) to victims of bites by rabid animals. In addition, periodic mass vaccination campaigns (for dogs) that achieve 70% vaccination coverage can bring rabies under control in reservoirs. We investigated the knowledge and attitude about rabies prophylaxis through a cross sectional survey of the high risk population.

The survey was undertaken using a structured questionnaire with both open and closed-ended questions. The respondents were veterinarians (n=46), dog owners (n=92) and animal handlers (n=86). The survey results showed that 100% of the respondents were aware of rabies and knew that the disease is transmitted by dog bite. 97.8% of the dog owners have vaccinated their dogs against rabies. While 61.6% of the respondents knew that rabies can be transmitted by other animals apart from dogs, only 29.4% of the respondents said that any unprovoked animal bite should be suspected for Rabies. The availability of vaccines for rabies was known to all the respondents. However, only 38.4% knew of the post exposure prophylaxis (PEP). The use of anti-rabies immunoglobulins following dog bite was only known to veterinarians (20.5%) and one dog owner. The need for immediate PEP was reported by 13.4% of the respondents and 40.17% responded that the PEP can be done within 24 – 48 hours of dog bite. The survey indicated that the gaps in knowledge about rabies prophylaxis were predominant among the animal handlers who have less formal education. Interventions aimed at addressing these knowledge gaps should therefore be made available to all the individuals who are at a higher risk of coming in to contact with a rabid animal.

VIT–O–11

Immunological Charactrization of Wild Strains of CSF

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Classical swine fever is highly contagious and economically devastating viral disease of domestic pigs, wild boar and pygmy hogs. Classical swine fever outbreak also found in vaccinated pigs. Therefore it is necessary to characterize wild strains of CSFV at nucleotides as well as at amino acids level for any variation among circulating wild strains of CSFV. Immunological characterization of field isolates was done by cross neutralization with CSF vaccine virus specific antibody following the method of NPLA. E2 is the major glycoprotein of CSFV which is highly immunogenic and responsible for neutralization of virus. The sequence TAVSPTTLR is a conserved and neutralizing epitope on E2 glycoprotein of CSFV. Comparision of the CSFV ALFORT/187 E2 sequence around the TAVSPTTLR region with those from other field strains were done. Viruses were propagated in pk-15 cell line up to 5th passage level. Based on virus propagation titre, conserved immunogenic epitope (TAVSPTTLR) of field strains(Domestic pig, pygmy hog and wild hog) remained intact with the established vaccine strain(Viz. ALFORT/187 and IVRI CSFV vaccine strain) .However at amino acid level there was substitution in amino acid sequences of E2 and Erns genes of field strains of CSFV.

VIT–O–12

Immune responses to *Peste des petits ruminants* virus (PPRV) vaccines in goats and sheep in Tamil Nadu

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In India, peste des petits ruminants (PPR) is a major disease of goats and sheep which is endemic throughout the country. Government of India officially reported that PPR accounted for maximum incidence (32.3%) and deaths (41.5%) of all diseases in goats in India. The World Organization for Animal Health has classified PPR as a notifiable and economically important transboundary viral disease of small ruminants. FAO and OIE is contemplating PPR global eradication programme for complete eradication of PPR by proper vaccination and other control measures by the year 2030. Though PPRV vaccine is commercially available, the PPR disease is prevalent throughout the country. Hence the study was taken to evaluate the commercial PPRV live attenuated vaccines available in India for their efficacy in goats and sheep under field conditions.

Two commercial PPRV Vaccines from vaccine manufacturer A and vaccine manufacturer B are available currently. The serum samples collected from the vaccinated goats and sheep on 0 DPV, 28 DPV, 90 DPV, 180 DPV and 365 DPV for the following four categories were tested for PPRV antibodies by cELISA and SNT. Category 1: Goats and sheep with 4 months to 1 year of age were vaccinated with either PPRV vaccine from vaccine manufacturer A or B Category 2: Goats and sheep with more than 1 year of age which were previously vaccinated once with PPRV vaccine were revaccinated with PPRV vaccine from the same manufacturer A or B respectively. Category 3: Goats and sheep with more than 1 year of age revaccinated with PPRV vaccine from manufacturer A, which were previously vaccinated once with PPRV vaccine from different manufacturer B and vice versa. Category 4: Kids and lambs that were seronegative at 45 days old were vaccinated with either PPRV vaccine from vaccine manufacturer A or B. In all the four categories of vaccination studies, the average PI values in cELISA continue to increase from 0 DPV till 90 DPV (21.28±0.54 to 83.88±0.70), thereafter there was decline in the average PI value in cELISA with the presence of PPRV antibody even at 365 DPV (83.88±0.70 to 54.19±0.78). The study has indicated that the currently available live attenuated vaccines used in the study are safe and potent, which can be effectively used for PPR global eradication programme by FAO and OIE.

VIT–O–13

Establishment of Vero Cells Constitutively Expressing Caprine Signaling Lymphocyte Activation Molecule

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Peste des Petits Ruminants (PPR) is a highly contagious transboundary disease of goats, sheep and small wild animals causing significant economic losses due to its high morbidity and mortality. Peste des Petits Ruminants Virus (PPRV), the causative agent of PPR utilizes signaling lymphocyte activation molecule (SLAM) or CD150 as receptor for virus entry. The likelihood of isolating the virus using commonly used cell line like Vero cells is very low as these cells do not express SLAM. The present study describes the establishment of Vero cells constitutively expressing caprine signaling lymphocyte activation molecule for isolation of PPRV. A 954 bp caprine SLAM (gSLAM) gene without the signal peptide was amplified by RT-PCR from the RNA extracted from the phytohaemaglutinin (PHA) stimulated peripheral blood mononuclear cells (PBMCs). An expression module carrying gSLAM gene was cloned in laboratory modified pCI neo vector having neomycin resistant selectable marker. The Vero cells (Vero-gSLAM) were selected against G418 antibiotic following transfection and then the gSLAM expressing cells were positively selected by using immobilized metal ion affinity chromatography (iMAC). The gSLAM expression was detected by immunocytochemistry by using anti-FLAG antibody. These results clearly indicate that the Vero-gSLAM cells are constitutively expressing caprine SLAM on the cell surface and may support easy entry and multiplication of the PPRVs.

VIT–O–14

Pulmonary expression of key genes associated with oxidative stress following exposures to fipronil alone or in combination with LPS

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The present investigations included male Swiss albino mice (N=60) aging 6-8 weeks to identify key genes associated with oxidative stressin response to fipronil with or without LPS. Animals were divided into two treatments and one control group (N=20). Treatment groups were orally administered fipronil @1/10th and 1/20th of LD50 dissolved in corn oil for 90 days followed by LPS or NSS challenge @80µl/animal via intranasal route. Lung tissues were subjected to microarray analysis.Both doses of fipronil individually or in combination with LPS dysregulated5847 genes (p<0.05; minimum cut off of 1.5 log fold change).Treatment with 1/10th of LD50 of fipronil caused the upregulation of 1942 genes and downregulation of 1212 while with same dose combined with LPS up-regulated 1236 genes and downregulated 854 genes. Fipronil at a dose of 1/20th of LD50 up-regulated 2912 genes and down-regulated 1670 genes while with same dose combined with LPS up-regulated 3368 genes and down-regulated 2478 genes. The gene overlap studies between differentially expressed genes in all the groups showed 74 (66 up-regulated and 9 down-regulated) commonly expressed genes.Further, the key genes of oxidative stress viz*Gpx2,Sod1,Sod2 and Cat*were significantly upregulatedwhereas,*Prdx1* wasdown-regulated. These key genes are being validated by Real time PCR. The data taken together suggest that exposures to fipronil with or without LPS altered the pulmonary expression of key genes associated with oxidative stress.

VIT–O–15

Immune responses in buffaloes following bluetongue vaccination

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Immune response to bluetongue pentavalent inactivate vaccine (BTPIV) adjuvanated with either oil adjuvant or aluminium hydroxide gel incorporating bluetongue virus (BTV) serotypes 1, 2, 10, 16 and 23 in buffaloes under experimental and field conditions were examined. The seronegative buffaloes which were vaccinated on 0 day were revaccinated on 28 and 365 days post vaccination (DPV). Lymphocyte proliferation assay, gamma interferon assay and flow cytometry analysis of CD4+ and CD8+ lymphocyte subset population were employed for assessing cell mediated responses in blood samples whereas competitive enzyme linked immunosorbent assay (cELISA) and micro serum neutralisation test (mSNT) were employed for assessing humoral mediated responses on 0, 28 and 42 DPV in experimental buffalo calves and 0, 28, 42, 90, 180, 365 and 379 DPV in field or farmers holdings.

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VIT–O–16

Cytokine Level in Skin Tissue Homogenate of Cattle with Different Coat Colors During Different Seasons

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To examine the effect of heat stress on cytokine production in cattle with different coat colors, ten each of Tharparkar and Karan Fries were selected from NDRI herd, Karnal. Tharparkar has lighter hair and darker skin, whereas Karan Fries has darker hair and lighter skin. Animals were maintained under standard managemental practices followed at the farm. Skin biopsies were aseptically collected from each animal during winter, spring and summer. IL-6, TNF-α and cortisol level was determined in skin tissue homogenate using ELISA. Real-time PCR was performed to examine the expression of skin color genes (*MC1R, TYRP-1*) in skin tissue of both the breeds. Level of cytokines (IL-6, TNF-α) and stress hormone (cortisol) was highest ($P<0.05$) during summer followed by winter and spring in both Tharparkar and Karan Fries. IL-6, TNF-α and cortisol levels during summer heat stress were significantly ($P<0.05$) higher in Karan Fries than Tharparkar. In such cattle breed (Karan Fries) with higher cytokine production and cortisol level during heat stress, the magnitude of expression of skin color genes was significantly ($P<0.05$) lower than Tharparkar. The results demonstrate that cytokine production by skin tissue is influenced by seasonal change in temperature. Furthermore, cytokine production during heat stress is higher in lighter pigmented skin of Karan Fries than Tharparkar.

Keywords: Cytokines; Cattle; Coat color, Heat stress

SESSION – VIII

Improvement of Animal Productivity of North- East Hilly Region

LEAD PAPERS

NES–L–01

Reproductive Biotechnologies for Augmenting Reproductive Efficiency of Farm Animals

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Modern biotechnologies have allowed successful improvement in livestock productivity. Farm animal selection and reproduction are on the threshold of the application of new biotechnologies. advances to be made. Globally, there is active competition for new technologies that may directly or indirectly affect the future of animal production, including breeding. Efficient reproductive performance and its monitoring are imperative for sustainability in any livestock production system, especially for milk, meat, draft and replacement animals. In recent times, there has been increasing challenges for increasing productivity and disease with altering climate. These targets, thought to some extent, can be achieved by conventional reproduction techniques. Advent and use of modern reproductive technologies have opened many avenues to study, treat and manipulate the reproductive phenomenon both in vitro and in vivo to improve reproductive performance in various domestic species of livestock. Artificial insemination, embryo transfer, in vitro fertilization, cloning, transgenics and genomics all are components of the tool box for present and future applications. Individually, these are powerful tools capable of providing significant improvements in productivity. Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile (or sub fertile) animals ((Kharche et al., 1995). Based on the progress in scientific knowledge of endocrinology, reproductive physiology, cell biology and embryology during the last fifty years new biotechniques have been developed for and introduced into animal breeding and husbandry (Goel and Kharche, 2009).Among them are oestrus synchronization/induction, artificial insemination, Multiple Ovulation Induction and Embryo Transfer (MOET), in vitro embryo production (IVP) and cloning by Nuclear Transfer (NT). The aims of these reproductive technologies were initially to speed up the genetic improvements of farm animals by the increase of offspring of selected males and females and the reduction of the generation intervals. Techniques are now available to get genetically improved farm animals in large numbers. This involves collection of the fertilized egg from genetically superior female which ovulate spontaneously or are induced to super ovulate (Kharche et al., 1995, 1996). The above goal can also be achieved by transferring the embryo of genetically improved variety into genetically less desirable females, thus getting many more progeny of superior individuals. The current efficiency for producing transgenic animals particularly farm animals, is low and the cost is high.

1. Artificial Insemination

Artificial Insemination (AI) is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female. The current thinking is to improve this resource through artificial insemination by economic conservation of superior native germ plasm. In order to maintain a continuous supply of superior germ plasm it is necessary to establish frozen semen banks at the national level. Its benefits can increase many folds when AI is practiced in conjunction with oestrus synchronization as a reproductive management tool as compared to the use of AI alone. Farmers normally depend on sharing of available male of unknown genetic merit in the village maintained by a big flock owner of 10-15 animals. These breeding males are seldom of high genetic merit and often sub-fertile. It is therefore a strong need to strengthen the breed improvement/ upgradation of germplasm dissemination programmes by selecting superior breeding males of different breeds of goat for natural mating and artificial insemination (fresh/frozen).

AI with frozen-thawed semen has been successfully employed in India for large scale genetic improvement in cattle and buffalo. However, the commercial use of frozen-thawed semen has been relatively limited in caprine reproduction (Batisa *et al.* 2009). Three methods of semen preservation (fresh, refrigerated and frozen) and three techniques of insemination (vaginal, cervical and intrauterine) are used worldwide in goats (Evans and Maxwell 1987, Chemineau and Cognie 1991, Leboeuf *et al.* 2000). Fresh semen is preferred when a male is maintained in the herd and the use of refrigerated semen can be a common strategy when a particular male is shared among a group of farmers. In such case the semen is stored at 4°C and can be used upto 24hrs from collection. But cryo preservation helps in storing the semen for a longer period allowing it to be marketed over a wide area, used throughout the year and the genetic material can be preserved even after death of the animal (Baldassarre and Karatzas 2004). When appropriately performed, insemination of does with fresh semen yield conception rate comparable to natural mating. But poorer conception rates are observed with frozen-thawed semen as a general rule (Holtz 2005). In France, where AI is an established technique in goat breeding management average conception rate of 60-65% is reported (Leboeuf 1992, Leboeuf *et al.* 1998). A conception rate of 44.4% was obtained in does inseminated with skimmed milk extended semen as compared to 12.5% for Tris-citric acid extended semen (Mehmood *et al.* 2011). Batisa *et al.* (2009) has reported 43.6% of conception rate in Majorera goat breed using frozen thawed semen. The status of AI and conception rate in different countries are given in the table 1.

Country	million sperm	Fertility	Reference
France	120	60-83.5	Corteel, 1974
	120	54.7-59.2	Furstoss et al. (2015)*
Australia	60	46.7	Ritar and Salamon, 1982
Greece	80	66.6	Samoulidis et al. (1982)
South Africa	75	56.0	Loubser et al. (1983)
New Zealand	80	25	Tervit et al. (1984)
Spain	200	43.6	Batista et al. (2009)
Norway	75	73.6	Aamdal, 1982
	200	62.0	Nordstoga et al. (2010)
India	200	36.4	Sharma, 2004
	100-120	71.4	Bhattacharyya et al. (2012)
	80-100	53.0	Kharche et al. (2013)
Thailand	60	53.3	Anakkul et al. (2015
	120	56.7	
Brazil	-	89.6	Maia et al. (2017)

ICAR-CIRG has developed frozen semen technology for AI in goats. Tris-Egg Yolk based extender is used to dilute the neat semen and AI is performed in a specifically fabricated indigenous insemination crate/cradle (Kharche et al. 2013). Frozen semen technology has been standardized at ICAR-CIRG by using different concentration of egg yolk ranging from 0, 5, 10, 15 and 20% with varying percentage of glycerol in the dilutor (Priyadharsani et al. 2011 and Ranjan et al. 2015) and by supplementation of different concentrations of vitamin C (Gangwar et al. 2015) and Chlorpromazine Hydrochloride (Ranjan et al. 2017, Annual Report 2016-17). AI Technology has been standardized on goats of different breeds (Barbari, Jakhrana, Jamunapari and Sirohi). We standardized and optimized the Goat semen diluter composition by altering different ingredient composition (TRIS, Citric acid, Fructose, antibiotics etc.). We reduced the egg yolk and glycerol level in diluents, standardize equilibration period, height of straw for vapor freezing etc. we also tested different types of additives: membrane stabilizer (Chlopromazine hydrochloride), Antioxidant (Vitamin C and Vitamin E, Glutathione reductase), chelating agent (EDTA), cryoprotectants (Glycerol, DMSO, Propelene glycol, Ethylene gycol) The good quality straws having post thaw motility of minimum 50% were stored in LN_2 container for further use/study. The overall AI success rate was 37.57% and 35.32% on actual kidding basis in 2016-17 and 2017-18 respectively by using frozen semen straws at our Institute.

Recently ICAR-CIRG has transfer the technology of dilution, freezing and AI to a company named M/S Aegipan Animal Biocare Pvt Ltd., Hooghly, W.B.

In Future, Frozen semen based AI technology has great potential for improving native goat genetic resources and farmers flocks through the use of elite buck semen from organized farms in future. AI under farmers flocks needs to be initiated/strengthened by state Animal Husbandry functionaries for upgradation and genetic improvement of 75 to 80% small ruminants of non-defined breeds.

2. Oestrous Synchronization

Oestrus synchronization is a valuable management tool that has been successfully employed in enhancing reproductive efficiency in Cattle, buffalo, goats and sheep (Kharche et al., 1996, Kharche and Srivastava 2002, Kharche and Srivastava 2005, Kharche et al., 2002; Goel et al., 2003, Kharche et al, 2017). Although the merits of oestrus synchronization are best realized in large animal breeding establishments. This technique can be used to benefit the small holder producer.

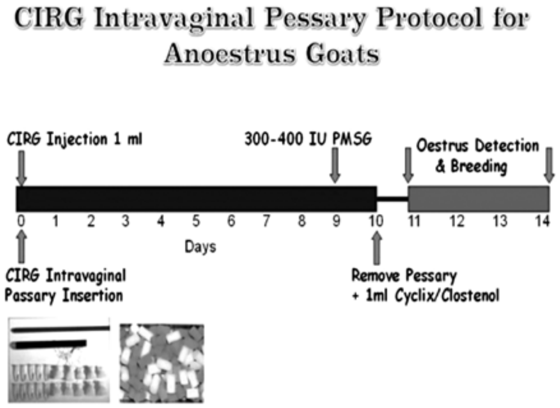
Oestrus synchronization in does is achieved either by reducing the length of the luteal phase of the oestrous cycle with prostaglandin F_2alpha (Lutalyse, Upjohn, USA) and or its analogues, such as Carboprost Tromethamine (Upjohn, UK) or extends it artificially with exogenous progesterone or more potent progestagens (Kharche et al., 2002; Goel et al., 2002). The single injection of 7.5 mg of Lutalyse (Upjohn, USA) during luteal phase (day 10 to 14) of the oestrous cycle effectively synchronizes oestrus in 75% to 81.81% goats within 96 hr of $PGF_2\alpha$ administration (Kharche et al, 2002; Goel and Agrawal, 1998). Administration of two doses of $PGF_2\alpha$ at 11 days interval irrespective of the stage of oestrous cycle in cyclic goats would be advantageous for close synchrony of the oestrus (Goel and Agrawal, 1991; Shabber et al., 2003) but it increases the cost of synchronization techniques. Similarly administration of two does of $PGF_2\alpha$ in anoestrus goat at any stage is not effective for synchronization of oestrus (Goel and Agrawal, 1991).

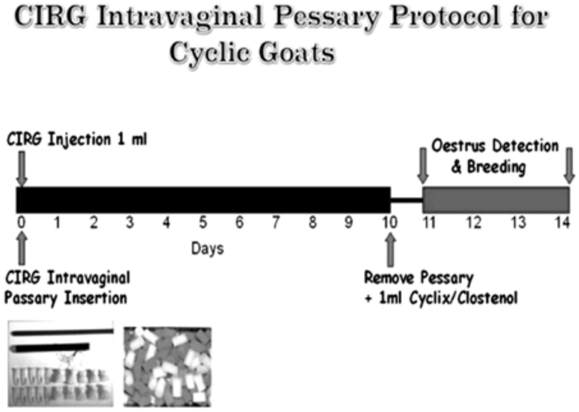
Table 5: Synchronization of Oestrus in Goats

Sr. No.	Name of agent	Ingredients	Dose	Route of administration	References
1.	Lutalyse(Upjohn Co. Kalamazoo M.L.USA)	Dinoprost tromethamine	Double injection at 11 days apart, 7.5mgx2 ,I/M	I/M	Goel et al. (2003)
2.	Lutalyse (Upjohn Co. Kalamazoo M.L. USA)	Dinoprost tromethamine	7.5 mg single inj. ,I/M	I/M	Kharche et al. (2002)
3.	Crestar ear implant (Intervet, Holland)	Norgestomat	3mg ear implant + I/M injection of 1.5mg Noregestomet +2.5mg estradiol vale rate.	Subcutaneous Implant	Kharche et al. (2002)
4.	N Natural Progesterone (S.D. F Fine Chemicals, India)	Progesterone	350 mg for 12 to14 days	Intravaginal sponge	Das et al. (2000)
5.	CIDR (Inter Ag, Hamilton, New Zealand)	Progesterone	330 mg	Intravaginal sponge	Waldron et al. (1999)
6.	Synchromate-B(Sanifi, Animal Health Overland Park, KS)	Norgestomet	3 mg for 9 days	Subcutaneous Implant	Rowe & East, (1996)
7.	Choronogest (Intervet, Angers France)	Flurogestone acetate	45 mg for 9 to 12 days	Intravaginal sponge	Freitas et al. (1996)
8.	Veramix (Pharmacia& Upjohn, Orangeville Canada)	Medroxy progesterone acetate	60mg for 18 days	Intravaginal sponge	Goel and Agrawal (1991)

Multiple injections, subcutaneous implants, feed additives, intravaginal sponges and controlled interval drug releasing devices have been used to administer progesterone in goats. Daily injection of 12.5 mg progesterone for 14 to 16 days synchronizes the oestrus but the onset of oestrus after withdrawal of treatment is significantly longer as compared to the goats treated with progesterone and $PGF_2\alpha$ (Shabber et al., 2003). The use of Crestar ear implant (Interval, Holland) containing 3 mg Norgestomet, and injection of 1.5 mg norgestomet+2.5 estradiol valerate at the time of implant insertion and 500 IU of PMSG at the time of implant removal resulted in 77.78% goats in oestrus within 48 hr to 72 hr of treatment with estrus duration ranging from 24 to 72 hr (Kharche et al., 2002) Similarly, synchromate-B ear implant (Sanofi Animal Health Inc, Overland Park, KS, USA) containing same schedule as mentioned above could result in 85-100% oestrus response (Palanisamy et al., 1996). Similarly controlled interval drug release devices are made up of progesterone impregnated medical silicone elastomers. CIDR are used intravaginal for 9 to 16 days. Waldrone et al. (1999) reported a satisfactory pregnancy rate (61.1%) in goats with 16 day intravaginal treatment of CIDR and 250 IU of PMSG 48 hr before the implant removal. Goel and Agrawal (1991) reported 66.66% cyclic does in oestrus with Veramix vaginal sponge (Upjohn, U.K) containing 60 mg Medroxy progesterone acetate.

A poor goat farmer is unable to afford all these synchronizing agents. The cost of synchronization can be reduced to a great extent by preparing sponges indigenously with the naturally available/ synthetic progesterone. ICAR-CIRG has developed low cost intravaginal sponges for induction and synchronization of oestrus in cyclic and acyclic goats. These sponges were validated at Division of Animal Science, Central Agricultural Research Institute, Port Blair, Andaman and Nicobar islands, Animal Biotechnology Center, NDVSU, Jabalpur, COVSc, Akola, COVSc, Shirwal, Satara and COVSc Nagpur. The oestrus induction and synchronization efficiency was varied from 80 to 100% and conception rate 50 to 70% were reported in goat and sheep by using these CIRG sponges (Kharche et al. 2009, Kharche et al. 2017).





In Future, oestrus synchronization coupled with AI can be used to breed a large number of does over a short period. As a result, management can be concentrated during short periods. Does can be bred at a time that will be suitable for the best marketing opportunities. Synchronization would also allow producers to schedule kidding to take advantage of feed supplies, labor, upward price trends and implementation of breeding technologies such as artificial insemination.

2. Multiple Ovulation and Embryo Transfer (MOET)

MOET is a composite technology and includes super ovulation, estrus synchronization among recipient, Artificial Insemination of donor, embryo recovery from donor, embryo transfer into recipients. The gonadotropins of pituitary origin (FSH and LH) or from the serum of pregnant mares (PMSG) are mostly used as superovulating hormones. Gonadotropins in combination with progestogens and/or prostaglandins have also been successfully used for induction of superovulation in goats. More pure forms of gonadotropins (Folltropin, Super-ov) are very effective superovulating agents. A variety of super ovulation regimes for goat have been reported. (Rosniha et al., 1992 ; Goel and Agrawal, 1993; Majumdar et al., 1997; Goel and Agrawal, 1998; Selvaraju et al., 2003, Kharche et al 2008). The response of does to exogenous gonadotropins is highly variable.The commonly used gonadotropins are pregnant mare's serum gonadotropins (PMSG or eCG), porcine FSH-P and ovine FSH-P. Pregnant mares serum gonadotropin is administered as single injection of 750 to 1000 IU during mid luteal phase, followed by 7.5 mg of prostaglandin F^{2a} (Lutalyse) 48 hr after PMSG injection. Super ovulation with FSH-P requires multiple injections of 16 to 18 mg of FSH-P in the morning and evening at 12 hours interval for 3 days and single injection of 7.5 mg of $PGF^{2}\alpha$ (Lutalyse) 48 hr after first FSH-P injection for better super ovulation and oestrous response (Goel et al., 2002, Kharche et al. 2008). It is greatly recognized that PMSG produces lower response than that of the follicle-stimulating hormone. A major problem often associated with PMSG super ovulation in goats is the presence of large number of un ovulated follicles and premature regression of corpora lutea which is often associated with an early return to oestrus before the normal time of embryo collection.

ICAR-CIRG has standardized the superovulation, embryo collection and embryo transfer in goats and several kids were produce through embryo transfer technology (Kharche et al. 2010, Annual Report)

In addition to artificial insemination which has provided considerable opportunity to improve the genetic potential of the livestock by using semen from proven males, the embryo transfer technique permits exploitation of both male and female genotypes. It is one of the fasted method of propagation of superior germplasm.

The advantage of MOET to obtain more offspring from valuable females, obtaining offspring from infertile females, exporting or importing animals as fresh or frozen embryos, testing for mendelian recessive traits, introducing new genetic material into specific pathogen-free farms, increasing the population of rare or endangered breeds or species. MOET schemes allow genetic progress required for successful breeding programmes. Meuwissen (1991) showed increased in female reproduction rate through the use of MOET. MOET-ONBS was more advantageous over closed system to increase the overall genetic gain. ONBS with MOET was superior to maximum annual genetic improvement by 62 per cent (Dixit and Sadana, 1999). It provides degree of control on genetic changes i.e. intensity of selection, generation interval and rate of inbreeding. If these controlled, then high rate of genetic progress may be achieved.

4. *In vitro* Embryo Production (IVP)

Among the reproductive technologies developed, in vitro embryo production (IVEP) through a combination of in vitro maturation (IVM), fertilization (IVF) and culture (IVC) of oocytes holds a prominent place as it is an integral part of a number of other advanced reproductive technologies. It is a valuable tool to capture vast reserve of oocytes from abattoir material as an excellent source of low cost embryo production for basic research on developmental biology and physiology with commercial application of the emerging technologies such as nuclear transfer, transgenesis, parthenogenesis and rescue of endangered species. Progress in cellular and molecular embryology in farm animals has been difficult in the past due to limited availability of suitable experimental material at a reasonable cost. For such reasons, *in-vitro* embryo production (IVEP) techniques, particularly based on ovaries of abattoir origin have received greater attention in past two decades (Galli and Lazzari 2003). Besides the efficiency of i*n vitro* derived embryo production using conventional technique of multi ovulation and embryo transfer (MOET) is not optional, rather it is inconsistent due to variable superovulatory response and flushing procedures (Cognie *et al.* 2003). Alternatively *in vitro* production of embryos provides cheaper and consistent source for supply of specific developmental stages for biomedical research and emerging biotechnologies viz. cloning, transgenesis through somatic cell nuclear transfer (SCNT), intracytoplasmic sperm injection (Kharche *et al.* 2016) and embryo sexing. *In-vitro* embryo production technology has been established for goats (Chauhan *et al.* 1994, Kharche *et al.* 2008). During the past two decades, considerable advancement has been made in the laboratory production of embryos since the birth of buffalo calves (Chauhan *et al.* 1998) and kids following the transfer of IVP embryos (Kharche *et al.* 2008, Kharche *et al.* 2011).

In future, the use of pre pubertal animals for collection of oocytes at an earlier age would reduce generation interval and accelerate the propagation of the genetically valuable livestock. For such reasons major research is currently focused to factors involved in determining the developmental competence of oocytes from pre pubertal and adult goats (Singh *et al.* 2009).

5. Ovum pick up Technology

The poor availability of superior germplasm is a major problem in the buffalo, which is the principal dairy animal, the major contributor to meat production and generator of draught power in the Southeast Asian countries. Buffaloes, which produce 3500–4000 kg of milk in a 305-day lactation period, constitute only 0.1% of the total buffalo population in India (Misra et al. 1990). There is an urgent need to multiply this superior germplasm as quickly as possible. Although embryo transfer technology offers one of the fastest ways for achieving this objective, its application to buffalo has had a limited success primarily because of poor superovulatory responses. Similarly, Cattle are an important species of our dairy industry. The milk yield of cattle has enabled India to become the world's largest milk producing nation. Application of superovulation and embryo transfer (ET) has given satisfactory results in cattle. However, conventional superovulation and embryo transfer (ET) cannot be applied to animals that are clinically subfertile, infertile or aged or those which do not respond to superovulation but which are otherwise of high genetic merit. Furthermore, the development and refinement of techniques for *in vitro* embryo production (IVEP) has made available a tool for utilization of the female gamete pool for enhancing the maternal contribution to genetic improvement. However, if the source of oocytes is slaughterhouse ovaries, the offspring produced cannot be used for breeding purpose due to lack of information of pedigree of the dam. Ovum Pick-Up (OPU) or ultrasound guided transvaginal oocyte retrieval (TVOR) is the only means available for obtaining oocytes from live animals of known pedigree which enables repeated collection of oocytes from live animals, on a weekly or biweekly basis over long periods of time. The India's first buffalo calf from ovum pick up was born on 28 September, 2008 at G B Pant University of Agric. And Technology, Pantnagar (Maurya et al., 2009) and first female Sahiwal calf named 'Holi' from aged animal was born on 7 March 2012 at NDRI Karnal (Saini et al., 2015).

6. Sex determination of sperm and embryo

There has been great interest in sexing sperm ever since AI was practiced widely (Seidel, 2003). The basic principles are simple; the X-sperm contains more DNA than the Y-sperm. A number of methods for sexing of semen have been attempted. However, the only method of semen sexing that has shown any promise has been the sorting of spermatozoa according to the DNA content, by means of flow cytometry. Flow cytometric technology is widely used for sexing sperm in mammalian species. Sperm sexing by high speed flow cytometry has been one of the most significant new technologies for artificial breeding of livestock developed in the twentieth century (Seidel, 2007). A very recent and most exciting development in this field is the establishment of reverse sex-sorting technology for the utilization of frozen ram and bull semen (Underwood et al., 2009). These results demonstrate that frozen-thawed ram and bull sperm can be sex sorted for either immediate or future use in an IVF system after recryopreservation. Sperm Sexing Technology (SST) will enable the producers of livestock to predetermine the sex of offspring prior to Artificial Insemination, thereby maximizing productivity, profitability and genetic potential. The technology does not involve genetic modification and is non-invasive. The ability to sex semen has a large potential for commercialization; thus, much of the research to develop and refine sperm sexing technology has been conducted in the private sector. The potential application of sexed sperm technology is (1) to obtain more female calves; (2) to obtain male calves from the very best cows in the herd to use as breeding bulls; (3) One dose of sexed sperm can be used to produce many embryos through in vitro fertilization.

The embryo transfer technology represents a powerful tool for the acceleration of various breeding programs in cattle. Known sex of embryos produced for use in ET programs can more effectively help to produce more heifer calves. This approach can improve the genetic potential of cattle herds in shorter time intervals (Lopatarova et al., 2008). Sexing of embryos before transfer and implanting has great potential for the livestock industry. Manipulating the sex of offspring has been a dream of livestock industry for decades (Chen et al., 1999). Embryo sexing has been attempted by a variety of methods, including cytogenetic analysis, assays for X-linked enzyme activity, analysis of differential development rates, detection of male-specific antigens and the use of Y-specific DNA probes. Several methods have now been reported for mammalian sex determination as following (1) Using polymerase chain reaction (PCR) to amplify a Y-chromosome; (2) PCR-based genotyping; (3) PCR-mediated approach uses three sets of primers for sex determination of pre-implantation bovine embryos; (4) rapid sexing method for preimplantation embryos of bovine using Loop-Mediated Isothermal Amplification (LAMP) reaction (Zoheir and Allam, 2010). It was the identification of bovine Y-chromosome specific DNA probes and the subsequent development of DNA amplification techniques by PCR, that made the possibility of sexing embryos into a reality. Using DNA analysis to analyse the sex of embryos has been shown to be reliable. The removal of a few cells in this procedure, caused very little trauma to the embryos. It did not alter developmental potential in vitro. Several commercial Polymerase Chain Reaction (PCR) kits are available which uses the primers specific to the Y-Chromosome Determinant (YCD) to determine sex of the embryos. The PCR product was detected by UV light in agarose gel with ethidium bromide and the embryos were scored as Y chromosome determinant positive (male) or Y-chromosome determinant negative (Lopatarova et al., 2008).

7. Stem cell Technology

A. **Embryonic stem cells** (ESCs) have capacity to differentiate into any type of cell lineage. So, they offer great potential for regenerative medicine. Genetic manipulation prior to transplantation may not only reduce the risk of rejection but transplanted embryonic stem cells may also provide a novel vehicle for gene therapy to treat genetic or non genetic diseases. Furthermore, ESC types may prove to be a valuable source of cells on which new candidate drugs can be tested, that has previously relied on animal models, limited human cell lines. In context of stem cell research, four differentiated buffalo embryonic stem cell lines have been established at NDRI, Karnal that are showing all properties of stemness, pluripotency and differentiated state. Embryonic stem cells have been used in production of 'Garima II' as the donor cell and that is as normal as the natural animal (George *et al.,* 2011) at NDRI, Karnal. The ability of buffalo ES cells to form embryoid bodies (EBs) and to spontaneously differentiate to neuron cells, germ cells, muscular cells and epithelial cells was demonstrated by our group (Verma *et al*., 2007, Singh *et al.,* 2012). In the very same way, steps are progressing to direct the buffalo embryonic stem cells towards mammary lineage so that we can imagine of artificial mammary tissue resulting higher milk production and reduced chances of mastitis in farm animals.

- An ES cell approach will reduce costs considerably by using fewer genetically valuable embryos.
- ES cells enable the researcher to place new genes in advantageous places in the genome or to remove deleterious genes.
- ES cells and homologous recombination technologies can be used to insert a transgene into a specific location in the genome. Additional milk protein could be produced if the milk gene is inserted into a high expression region of the genome.
- Another possibility is to use ES cells to "knockout" a milk protein that is detrimental to cheese or yogurt production.

B. **Spermatogonial stem cells (SSCs)** are adult stem cells of testis which are responsible for the maintenance of the spermatogenesis throughout the entire life of the male. SSCs can be used to cure infertility caused by germ cell dysfunction by their testicular transplantation resulting in restoration of fertility of recipients. Besides the possibility to study male infertility, the SSC transplantation also provides another way to conserve reproductive potential of genetically valuable individuals within or between species and, finally, can be used to produce transgenic animals after generation of transgenic sperms. As such, SSC transplantation would be an alternative strategy to the currently less efficient and costly methods of producing transgenic farm animals (Keefer, 2004). The advantage of using SSCs for transgenesis is that SSCs can be harvested, in vitro cultured, cryopreserved or transfected and still preserve the potential to colonize the recipient testes. The buffalo spermatogonial stem cells have been established (Kala et al., 2012, Sharma et al., 2017) and maintained for their use in fertility restoration in cross breed bulls under guidance of scientists team at NDRI, Karnal. These encouraging results, suggest a possibility of banking and transplanting transgenic SSCs to produce sperms having desired gene which will be used to produce transgenic founder animal.

8. Cloning

Cloning is a technique used for creating genetically similar organisms from a single parent without sexual reproduction The first ever cloned animal was 'Dolly', a sheep, which was produced by the traditional complicated method of cloning that required expensive equipment and skilled manpower.

In Hand-guided cloning, a small piece of the skin tissue of the animal which is to be multiplied is collected aseptically. The animal may be a male or a female. The tissue is cultured in the laboratory for proliferation of cells. Simultaneously, oocytes are collected from slaughterhouse buffalo ovaries and are incubated in the laboratory for maturation after which these are denuded by removing the cells surrounding them. The denuded oocytes are treated with an enzyme to dissolve the outer covering called 'zona-pellucida' following which these are given a chemical treatment which leads to protrusion of their own genetic material at one end of the periphery. The protruded portion containing the genetic material is cut off using a very sharp microblade under a dissecting microscope rendering them 'enucleated'. Two such enucleated oocytes and one somatic cell that had been obtained from the animal to be cloned are fused together using electric current. The single cell formed from the fusion of these three cells is then cultured in the laboratory for further development to the 'blastocyst' stage embryo. These blastocysts are transferred to the uterus of the recipient-buffalo (surrogate mother). A major breakthrough was made by the scientists of National dairy Research Institute, Karnal, (Indian Council of Agricultural Research, New Delhi), India by producing World's first cloned buffalo *(Bubalus bubalis)* calf through the Hand-guided cloning technique in February 6, 2009 using somatic cell of a new-born calf (Shah et al., 2009). Similarly, lateron in 2012, SKUAST, Srinagar successfully cloned a Pashmina goat 'Noori'. They used the 'hand guided technique by fusing somatic cells (from goat ear) with an ova derived from the slaughter house ovaries.

9. Transgenic animal production

Genetic engineering of livestock to produce animal with altered traits such as disease resistance, wool growth, body growth, prolificacy and reproductive efficiency and milk composition is being researched in laboratories globally (Niemann and Kues 2003, Baldassare *et al.* 2004). Numerous methods have been used to produce transgenic progeny with varying success. Techniques are; DNA transfer by retroviruses; microinjection of genes into pronuclei of fertilized ova; injection of embryonic stem cells; embryonic germ cells pre exposed to foreign DNA into the cavity of blastocysts; sperm mediated gene transfer (SMGT) during IVF; liposome mediated DNA transfer into cells and embryos; electroporation of DNA into sperm, ova or embryos and nuclear transfer of somatic cells, ES or EG cells. Among them pronuclear microinjection and somatic cell nuclear transfer (SCNT) have been suggested as the methods of choice to produce transgenic goats and sheep. Microinjection of cloned DNA into pronucleus of a zygote has been most widely used and successful method for producing transgenic mice. This method encompasses microinjection of several thousand copies of DNA into pronuclei of zygote. Offspring produced on transfer of such embryos are screened for integration and expression of foreign gene. During the present decade substantial advancement in livestock transgenesis has been made through application of somatic cell nuclear transfer (SCNT).

The merger of nuclear transfer with molecular tools such as targeted genetic modification and conditioned gene expression will be boosting the technology of transgenesis (Niemann and Kues 2003). Genetically engineered animals are a better alternative for producing proteins, which forms the basis of all biological drugs. Important Applications of transgenic technology are:

a. Bio steel from goats

b. Xenogenic cells and tissues

c. Production of a new class of antibiotics and other pharmaceuticals

9. Conclusion

Reproduction in animals offer numerous advantages and scope for the use of novel biotechniques viz. *in vitro* embryo production, ovum pick up, Artificial Insemination, embryo transfer technology, stem cell, cloning and transgenic animal production. Nevertheless, these emerging techniques should be judicially supplemented with good practices in animal health, nutrition and management at the level of stake holders for manipulation and improvement of health, production and reproductive performance of any livestock species, which will facilitate the production and dissemination of superior germplasm thereby enhancing the overall productivity of livestock species. The most emerging technology among the above is the transgenic animal production and cloning to produce the desired drug at high levels without endangering its own health and rapid multiplication of elite, transgenic and endangered breed of animals.

10. References

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NES–L–02
Molecular Diagnosis of Infectious Diseases of Pigs

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The effective control and treatment of diseases of animals requires access to diagnostic tests that are rapid, reliable and highly sensitive. Establishment of a good diagnostic laboratory for rapid and reliable diagnosis to ensure proper preventive and control measures of infectious diseases in livestock is a necessary step. The most authentic diagnosis of diseases is made by isolation and identification of the pathogen. However, it is time consuming, tedious and sometimes requires living medium. The development of various molecular techniques has revolutionized diagnostic procedures by providing specific diagnosis or detailed characterization of any pathogen or host pathogen interactions. Some of the recent molecular techniques employed in the diagnosis of swine diseases are discussed in brief.

Detection of Nucleic Acids

The use of nucleic acid-based diagnostics in veterinary medicine has increased exponentially in recent years. These techniques have redefined the level of information available for animal disease control programmes. In addition, modifications of nucleic acid detection techniques based on polymerase chain reaction (PCR) have lead to the development of rapid, specific assays. The molecular technique with the widest variety and application in veterinary diagnostics is PCR. The strength of this technique is its ability to make millions of copies of a deoxyribonucleic acid (DNA) target. This amplification enables the desired target to be readily detected by other techniques such as electrophoresis and sequencing. Some of the widely used nucleic acid based detection methods for diagnosis of swine diseases are described below:

Polymerase Chain Reaction

PCR is the best-developed and most widely used method of nucleic acid amplification. The basic technique of PCR includes repeated cycles of amplifying selected nucleic acid sequences. Each cycle consists of three steps: *(a)* a DNA denaturation step, in which the double strands of the target DNA are separated; *(b)* a primer annealing step, performed at a lower temperature, in which primers anneal to their complementary target sequences; and *(c)* an extension reaction step, in which DNA polymerase extends the sequences between the primers. At the end of each cycle (each consisting of the above three steps), the quantities of PCR products are theoretically doubled.

Real-time PCR

It is the latest improvement in the standard PCR technique to be implemented in veterinary laboratories. This technique is a single-capillary, closed assay that greatly decreases the problem of cross-contamination between samples. The fluorescence readings are plotted by computer software and results can be transmitted electronically, eliminating the need for post-PCR reaction analysis by electrophoresis (Spackman *et al.*, 2003). The development of extraction methods such as the magnetic bead technique has made it possible to use real-time PCR to test large numbers of samples in a matter of hours during disease outbreaks. In addition, realtime PCR has been adapted for use in the field through the use of portable thermocyclers and lyophilised reagents. This approach may allow for more rapid decision-making during potential disease outbreaks. The PCR is also used extensively for the genotyping and phylogenetic analysis (relatedness) of veterinary pathogens.

Diagnosis by DNA probes

In DNA probe hybridization the DNA, derived from sample suspected of containing a pathogen (the 'unknown'), bind with highly characterized DNA derived in advance from a pathogen of interest (the 'known' DNA). In conventional DNA probing the unknown DNA (or RNA), the target, is immobilsed on a solid surface e.g. a filter; and the known DNA (labelled/stagged probe) which is applied to the target, is in the liquid phase. The bound probe can be detected by addition of specific molecules/substances linked to an enzyme that generate colour or light (chemiluminescence). Detection of pathogen by this method is limited by the number of probes used.

DNA micrarray technology

A microarray comprises 20,000 or more different known DNAs, each being spotted onto glass slides, to form the array. In microarray diagnosis the known DNA that is the target, immobilsed on a glass slide, and the unknown DNA, in the liquid phase, which is labelled to make a probe. In microarray analysis, the detection of pathogen is limited only by the number of target DNAs on the array. Microarray analysis has great potential when one is investigating diseases of unknown aetiology, diseases where more than one pathogen might be present and when subtyping is required. The great advantage of microarray analysis in searching of pathogens is that hundreds of pathogens can be looked simultaneously when probing a single microarray slide. To enhance sensitivity in pathogen detection, microarrays can be coupled with PCR amplifications.

Fluorescent in situ hybridization (FISH)

FISH is a technique that can localize nucleic acid sequences within cellular material. Peptide nucleic acids, molecules in which the sugar backbone has been replaced by a peptide backbone, are perfect mimics of DNA with high affinity for hybridization that can be used to improve FISH techniques (Stender, 2003).

Nucleic acid sequence-based amplification (NASBA)

NASBA is a promising gene amplification method. This isothermal technique is comprised of a two-step process whereby there is an initial enzymatic amplification of the nucleic acid targets followed by detection of the generated amplicons. The entire NASBA process is conducted at a single temperature, thereby eliminating the need for a thermocycler. The use of this technique has been shown to detect avian and human influenza viruses (Moore *et al.*, 2004).

Nanotechnology

The term nanotechnology is broadly defined as systems or devices related to the features of nanometre scale (one billionth of a metre). This scale of technology as it applies to diagnostics would include the detection of molecular interactions. The small dimensions of this technology have led to the use of nanoarrays and nanochips as test platforms (Jain, 2003). One advantage of this technology is the potential to analyze a sample for an array of infectious agents on a single chip. Applications include the identification of specific strains or serotypes of disease agents, such as the identification of specific influenza strains, or the differentiation of diseases caused by different viruses but with similar clinical signs, such as vesicular viral diseases. Many research groups are considering the use of chip assays that detect a number of agroterrorism agents in each sample. Small, portable platforms are being designed to allow pen-side testing of animals for diseases of concern. Another facet of nanotechnology is the use of nanoparticles to label antibodies. These labelled antibodies can then be used in various assays to identify specific pathogens, molecules or structures. Examples of nanoparticle technology include the use of gold nanoparticles, nanobarcodes, quantum dots (cadmium selenide) and nanoparticle probes.

DNA Based Typing of Microorganisms

Random Amplification of Polymorphic DNA (RAPD)

RAPD typing involves the use of a short (usually 10 to 15 mers), arbitrarily chosen primer to amplify nearly homologous sequences of the genomic DNA under low-stringency conditions. RAPD has been used to differentiate strains of various species, various serotypes within species, and various subtypes within a serotype. It is, therefore, useful for determining whether two isolates of same species are epidemiologically related. RAPD has been used to evaluate outbreaks of infection of drug resistant bacteria.

Restriction fragment length polymorphisms (RFLP)

This DNA- based method is used to distinguish between isolates of closely related pathogens, whether they are viruses, bacteria, fungi or parasites. The RFLP approach is based on the fact that the genomes of even closely related pathogens are defined by variation in sequence. The RFLP procedure consists of isolating the target pathogen, extracting DNA or RNA (with subsequent reverse transcription to DNA) and then digesting the nucleic acid with one of a panel of restriction enzymes. The individual fragments within the digested DNA are then separated within a gel by electrophoresis and visualized by staining with ethidium bromide. Ideally each strain will reveal a unique pattern, or fingerprint. The results can be further analysed with the help of computer softwares.

PCR-RFLP

PCR-RFLP is a modification of the basic RFLP technique whereby the polymerase chain reaction (PCR) is incorporated as a preliminary step. The PCR method is used to amplify a specific region of the genome (known variable sequence between pathogens), which then serves as the template DNA for the RFLP technique. This new combination (PCR-RFLP) offers a much greater sensitivity for the identification of pathogens and is especially useful when the pathogen occurs in small numbers or is difficult to culture.

Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) is a nucleotide sequence-based approach to the unambiguous characterization of strains of bacterial species, or other microbial species, via the Internet. MLST involves obtaining the sequences of internal fragments of seven house-keeping genes for each strain of a particular species. The sequences of each fragment are compared with all the previously identified sequences (alleles) at that locus and, thereby, are assigned allele numbers at each of the seven loci. The combination of the seven allele numbers defines the allelic profile of the strain and each different allelic profile is assigned as a sequence type (ST), which is used to describe the strain. Nucleotide sequencing is relatively cheap, and easy to perform. The data produced by MLST are ideal for the characterization of strains of bacterial or fungal species via a web server. MLST is now widely used for molecular epidemiology as it allows strains studied by different groups to be compared and MLST schemes have been developed for several bacteria (mostly pathogens), and fungi and databases that can be queried are available.

Pulsed field gel electrophoresis (PFGE)

The limitation to separate very large DNA molecules by standard gel electrophoresis techniques can be overcome with this new technique, called pulsed field gel electrophoresis (PFGE). In PFGE an alternating voltage gradient is applied which facilitates the differential migration of large DNA fragments through agarose gels by constantly changing the direction of the electrical field during electrophoresis. The development of PFGE expanded the range of resolution for DNA fragments by as much as 2 orders of magnitude. PFGE has been successfully applied in subtyping of many pathogenic bacteria among other applications such as cloning of large plant DNA, construction of physical maps, genetic finger printings, etc. This technique is time consuming and require high-level of skill.

Peptide synthesis

Synthetic peptides or recombinant antigens produced by recombinant DNA technology offer many advantages over natural antigens isolated from other biological sources. These advantages include a high purity, high specific activity and consistency. The use of synthetic peptides or recombinant antigens in detection of animals infected with diseases such as classical swine fever, foot -and -mouth disease or endemic disease (Langedijk *et al*., 2001; Soutullo *et al*., 2001), reduces the risk involved in the production of the assay and the risk of producing kits with antigen that has not been completely inactivated and, therefore, remains potentially infectious.

Biosensors

Biosensors are sensors that transduce bio-recognition processes into measurable signals via a physico-chemical transducer, with electronic and optical techniques as two major transducers (Fan *et. al*., 2005). The development of biosensors meets the rapidly increasing need for clinical diagnostics in these days. A variety of new strategies have been developed toward biosensors with clinical applications. In principle, biosensors are analytical devices composed of a biological recognition element and an optical/electronic transducer. The biological element is in charge of capturing analytes in solution and the transducer converts the binding event to a measurable signal variation. The type of biosensors can be categorized by the nature of recognition, that is, enzyme-based biosensors, immunological biosensors, and DNA biosensors. Alternatively, based upon the type of transducers, there are electronic biosensors (electrical or electrochemical), optical biosensors (fluorescent, surface plasmon resonance, or Raman), and piezoelectric biosensors (quartz crystal microbalance).

Proteomics

In addition to the use of proteomics to identify and characterize the protein produced by pathogenic agents, proteomic technologies have great potential in veterinary diagnostic applications because they target the patterns of protein expression of the target analyte whether it is viral, bacterial, parasitic, etc. The standard proteomic approach involves the separation of proteins by two-dimensional gels with the staining of the proteins and molecular weight control. This protein pattern or fingerprint is then analyzed by performing image analysis. Proteome maps can be compared in order to find proteins that may be up- or down-regulated due to disease. A protein of interest can be cut from the gel and fully characterized using peptide-mass fingerprinting and/or mass spectrometry methods. In the future, veterinary diagnostics may make use of proteomics to identify or look for known disease markers or patterns with biochip technology and instrumentation that combines mass spectrometry with other separation chromatography or molecular techniques. These instrumentations are designed to specifically select, separate by molecular mass, and identify the complex mixture of proteins in a sample, which can then be compared to known samples for diagnostic purposes. This type of technology may be useful for identifying animals infected with agents that do not induce predictable serologic reactions. Proteome maps are being derived for a range of veterinary pathogens such as *Brucella melitensis*, *Toxoplasma gondii*, *Eimeria tenella*, *Trypanasoma brucei* and nematodes such as *Haemonchus contortus*.

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NES–L–03
Improvement of Yak for Socio-Economic Upliftment of Highlanders

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Introduction

Yak (*Poephagus grunniens* L.) is one of the most important economically useful animals for highlanders. It is the only large hairy multipurpose bovid and a unique genetic resource with an amazing ability to survive, reproduce and provide marketable products to the highlanders. Traditionally, the yaks are reared under free-ranged system in the high hills, usually between 3000 to 4500 meter above mean sea level (MSL), where air, water, and pasture are free from any pollution, and their products are organic and just natural (Pal, 1991). They even thrive at 6000 meter above mean sea level (MSL) with a temperature up to -50^{0}C. Yak population in India is 76,237 in 2012 (19th Livestock Census) distributed in Ladakh region of J&K (54,398), Arunachal Pradesh (14,061), Sikkim (4,036), Himachal Pradesh (2,895) and West Bengal (1,088). It's proved that yaks contribute to 62 per cent of their livelihood requirements (Ramesha and Bhattacharya, 2008). Economically yak rearers are poor but self sustaining due to their limited needs. There are lots of difficulties associated with yak rearing due to the nomadic lifestyle away from modern amenities, therefore, younger generations are not willing to continue this age-old yak rearing profession. Scientific exploration through advanced technologies to improve yak production is necessary. ICAR-NRC on Yak, established in 1989, chalked out issue-based strategies and research programmes, and has been working on it with holistic approach for sustainable development of yak husbandry, conservation and multiplication of elite yak germ plasm through *in situ* and *ex-situ* research and management.

Improvement of yaks - pertinent issues-

1. Elite yak conservation and multiplication

Yak farmers maintaining elite herds of yak can be utilized for male calf production through planned breeding and buy-back guarantee. Bull exchange programme is necessary for reducing inbreeding. For this purpose, elite bulls produced in ICAR-NRCY farms were distributed occasionally to the farmers, and selected bulls of the farmers are purchased from the farmers for production of semen and its preservation. Farmers are also encouraged to exchange their bulls once in a year with those of their fellow farmers.

Artificial Insemination (A.I.): Application of artificial insemination technology in yak breeding is limited as compared to cattle and buffaloes. Conception rate in yak reportedly varied from 37.5 to 94.0 per cent (Badamdorj *et al*, 2000). Consistent works on A.I. in yak have long been conducted at ICAR-NRC on yak since 2004, and encouraging 62.79% conception and 53.49% calving rate on A.I. with semen sample having minimum 45 – 50% post thaw motility has been reported by Dutta Borah *et al* (2013). Yak bull semen collection and its freezing is now a routine practice in Nyukmadung farm of ICAR-NRC on Yak, and with production of yak and yak hybrid calves through A.I., the technique finds its place of acceptability for yak improvement programmes. For effective conservation, utilization and multiplication of germ plasm, it is essential to establish nucleus breeding centres at different high altitude locations like Ladakh, Kargil, H.P., Tawang and Sikkim where yak and yak hybrid calves would be produced through A.I. In accessible hilly terrains, fixed time AI using yak frozen semen from Arunachali Yak, the first Indian Breed registered, will be a boon to produce yak and hybrids and has got tremendous potential to improve socio-economic status of yak keepers.

Advanced reproductive biotechnology: Multiple ovulation and embryo transfer (MOET) and *in vitro* fertilization (IVF) techniques, which have been standardized at ICAR-NRCY with production of calves, are important ways to generate embryos *in vivo & in vitro*, their non-surgical collection, preservation following conventional and vitrification methods, and transfer to suitable recipient yaks/ cattle / hybrid for production of yak calves as and when required.

2. *Enhancement of production and productivity.*

Nutritional intervention: Nutritional deficiency specially in winter reduce growth and reproductive performances of yak. Average winter loss in body weight of the yaks in India is 15-25%. Limited study conducted at NRC on Yak revealed that yaks could gain 350-422g/d when animals were fed complete feed block with area specific mineral mixture during winter months at mid altitudes 10,000 ft above msl (Baruah*et al,*2012a, Baruah*et al,*2012b). Considerable number of yaks reared in the Nyukmadung farm (located at an altitude of 2750 meter above msl) of NRC on yak show oestrus even in late winter. The observations have suggested that semi-intensive system of rearing with required feed & fodder or Complete Feed Blocks supplementation would help reducing the infertility and delayed breeding, thereby improving reproductive efficiency in yaks. To maintain the yaks at particular grazing field and to stabilize yak production throughout the year, yak sedentarization programmes along with development of alpine and sub-alpine pastures may be undertaken. To support the nutrient requirement during the winter scarcity period complete feed blocks (CFB) with area specific mineral mixture developed by NRCY may be supplied to sedentarized yaks. ICAR-NRC on Yak recorded beneficial effects of these CFBs with or without ASMMs in preventing the loss in body weights and milk yields due to winter feed crisis in growing and lactating yaks, respectively under field conditions. The experiments were conducted at Manadala situated at a height of 10,000ft msl. Yak husbandry facing a big challenges like degradation of natural grasslands and their encroachment by the unwanted grasses. Therefore, some suitable temperate grasses and legumes viz., *Dactylis glomerata, Lolium perenne, Setaria spp* and *Trifolium repens* were identified and tried to establish in this region and regenerate the degrading natural pastures. Besides, *Dactylis glomerata* grasses are growing for seed production in some areas of West Kameng district of Arunachal Pradesh.

Ensuring health care: It is essential to take effective measures for disease surveillance, eradication of important diseases, forecasting of disease outbreaks and awareness on prevention and control of diseases with vaccination in yak. It is also equally important to ensure the sanitation and clean milk production.

Value addition of the yak products: The productivity of the yak-based livestock production system is too low in comparison to the others bovine species. However, the return from the yak products can be enhanced by value addition and exploring their unique quality. ICAR-NRC on Yak has developed numbers of such products.

i) Functional paneer : ICAR-NRC on Yak has produces paneer having higher level of dietary fibre with low fat from yak milk and the products had found beneficial effect for cardiac patients. Yak milk paneer is usually prepared with full fat milk having as high as 8.5% fat resulting in 25-30% fat in paneer. Increasing concerns among health conscious consumers demand development of low fat paneer. Interestingly, low fat paneer prepared from yak milk having 1% fat has very hard in texture. Therefore, the effect of dietary fibers in improving the product quality of low fat paneer along with enhancement of dietary fiber content of the low fat paneer was undertaken. Increased fiber content in this product may have health benefits in terms of prevention against cardiovascular diseases and colon cancer of the consumers. This technology could make a profit of Rs. 20.16 per kg of yak milk employing domestic labour from yak farmers.

ii) Yak Hair-Jute blended Fabric and Garments: Carpets/wall hangings and foot mats are prepared from coarse yak hair fibre with beautiful designs by hand knotted method. There is a great demand of this unique product as a decorative material among the urban dweller. ICAR-NRC on Yak has long been working with ICAR-NIRJAFT, Kolkata on value addition of yak fibres. Yak hair-jute blended fabric cloths are prepared from coarse yak hair fibre blended with Jute and Yak Jute blended Fabric cloths are prepared from coarse yak hair fibre blended with Jute and cotton (40:40:20) with beautiful computer designs and made in newly installed Fabric machinery. This commercially viable blended yarn of jute and yak wool can be used for different product like Man Over coat, Table liner, Man Jacket, Man half Jacket, Women half Jacket and man over coat. This technology has overwhelmed acceptance among local farmers. Cost benefit ratio of yak can be enhanced many folds by blended fabric production with jute. Income generation of yak farmers is enhanced through this technology.

iii) *Yak as a pack animal:* In addition to milk, meat and wool production yaks are also used as pack animals in difficult hilly terrain. This may be a source of additional income. To prove this hypothesis studies on assessment of work efficiency in different categories of yak was conducted at different temperature and different altitude. It was observed that the yak bull can carry a load up to 35% of their live body weight (i.e.136 kg of load) and can walk 4-6km/h at a stretch and was found comfortable at sub-zero temperature.

Conclusion

Though the yak population in India is very less in comparison to other livestock but still it is important due to the livelihood dependency of the people associated with yak rearing across the Himalayans. Strategies and technologies developed for improving yak production are found effective. However, an effective extension setup well equipped with knowhow and solid infrastructural back up is required to cater the local needs of the yak rearers of the country.

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Advance breeding intervention for improving pig genetic resources in North-Eastregion

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Amongst the various livestock species, pig is one of the most important sources of meat besides being most efficient food-converter after the broiler chicken. Pig production in India has enormous potential to upscale and contribute to higher economic gains. Pigs have high fecundity, high feed conversion efficiency, shorter generation interval and relatively smaller space requirement. They are equally adapted to intensive and diversified agriculture. Pig farming has the potential to provide employment opportunities to seasonally employed rural farmers notably in the rain-fed areas and supplementary income generation to womenfolk in their households for improving living standards. Lately, entrepreneurs have started showing interest in pig rearing, processing, value addition and marketing of pork and pork products. For realizing the potential, this sector requires a thorough understanding, appreciation of the present scenario, meticulous planning, mobilization of resources and training of manpower on a larger scale. Sectoral analysis reveals that the pig farming constitutes the livelihood of rural poor belonging to lowest socio-economic strata who have limited means and access to undertake scientific pig farming with improved foundation stock, proper housing, feeding and management. Therefore, suitable circumspective measures are the need of the hour to popularize the piggery husbandry with adequate technical interventions to modernize this sector and improve the productivity of smaller sized rural pig farms.

1. Pig population statistics

According to the 19th livestock census of India its pig population is 10.29 million in comparison to the world population of 977.02 (FAOSTAT, 2013 data (http://faostat.fao.org) which constitutes 1.05% of world pig population. Pig constitutes a mere of two percent of total livestock population (512.05 million) of the country; still needs much attention for its improvement. A gradual decrease in population may be essentially due to diminishing population size of native pigs and higher interest of farmers towards faster growing crossbred pigs like Hampshire crosses in north and north-eastern part, Large White Yorkshire crosses in southern and central part and Tamworth crosses in central and eastern part of India.

The last livestock census of 2012 demonstrated that pig population is distributed skewedly across the country. The highest pig population is formed in eastern and north eastern (NE) states (63.10%), followed by northern (15.52%), southern (9.48%), central (5.97%) and western India (5.94%). The highest population is in the state of Assam (1.63 million), succeeded by Uttar Pradesh (1.33 million), Jharkhand (0.96), Bihar (0.65) and West Bengal (0.65 million). The northeastern part of the country houses 38.42% of the pig population of the country. Indigenous pigs (76.14%) are the cornerstone for pork production in the country followed by crossbreds and exotic germplasm (23.86%). Concentration of pig per 100 human populations in India is 0.85. Highest concentration of pig per 100 capita is in Arunachal Pradesh (25.77) followed by Nagaland (25.43) Mizoram (22.48), Meghalaya (18.33) and Manipur (10.19).

2. Pig genetic resources

Pigs are widely distributed in all the eco-regions of the country and are an important occupation of the rural society especially the tribal masses. People of certain ethnic groups prefer to keep more pigs, especially black ones, for festivals and ceremonial purposes. Interestingly, these ethnic groups are mainly concentrated in the north eastern region, where almost 38% of the country's total pig population exists. Broadly Indian pig population consists of three types; indigenous, exotic and crossbred.

2.1 Indigenous pig germplasm

Out of total pig population, 76.14% are indigenous and non-descript. Most of these breeds/varieties are yet to be characterized with proper scientific intervention. These pigs are of smaller size and almost no efforts have been made for any conservation and selection experiment to improve its economic traits, such as litter size, birth weight, weaning weight, average daily gain, feed conversion efficiency and carcass traits. These animals are well adapted to hot and humid environment and supposed to have better disease tolerance. The indigenous pigs of India identified as a distinct group as a result of gradual domestication of wild pigs to their surroundings. These pigs differ in their characteristics and colour from region to region within the country depending the topography and climatic conditions. Following are the native/indigenous pig germplasm available in different northeastern part of the country:

Dome: This strain is found in north and south districts of Tripura. Colour varies from black, grey to grey white with long thick crest bristles in the back line. Adult body weight is about 40 kg and litter size varies from 8 to10 at birth and 5to7 at weaning. Animals are commonly raised in backyard system although semi-intensive system of rearing is gradually becoming popular.

Doom: These are indigenous pigs of Assam found in very less number. Animals are also called Assam Local Gahori. These are medium sized pig with an adult weight of about 60 to 80 kg. The animals are predominantly black in colour with white patch found at fore head. Animals are occasionally reared in a group. The breed is registered with nation database with Accession number INDIA_PIG_0200_ DOOM _09006.

Ghungroo: These are found in North Bengal and adjoining district of Assam. Color of this animal varies from black to tan with occasional white patches at front and hind feet. The animals have typical bull dog type head with folded skin at face and neck. Long and cylindrical barrel belly, large and drooping ears and docile nature are other prominent characters of the breed. This breed is weighing about 80 to 100 kg at maturity. The litter size at birth of Ghungroo is comparatively higher than other indigenous pigs. This is the first recognized pig breed of India (Accession No: INDIA_PIG_2100_GHOONGROO_09001).

Lepchamoun: This breed is found in Sikkim. It is a medium sized verity with black coat colour. The animals are docile in nature. Mature weight ranges between 50 to 80 kg and litter size is about 7 to10 at birth.

Mali: The animals of this breed are found in Dhalai & North District of Tripura. Animals are black in colour with star shaped white patches on forehead. The animals are commonly raised in semi-intensive system. Adult body weight is about 50 kg and litter size varies from 8 to10 at birth and 7to8 at weaning.

Manipuri Local: *This is found in Ukhrul, Imphal East, Tamenglong, Churachandpur and Senapati districts Manipur. It is small sizes breed and usually black in colour. Adult body weight ranges from 60 to 70 kg. The numbers of these animals are decreasing rapidly due to crossbreeding.*

Niang Megha: It is a medium sized animal found in hills of Meghalaya. Animals are black in colour with diamond or star shaped white patches on forehead. They are ferocious in nature with bright, active and wild look. These animals have uniformly covered glossy coat. The midline is thickly covered with bristles. Ears are small, erect and extend in vertical direction. Adult body weight ranges between 50 to 60 kg. Litter size at birth ranges from 5 to 7. Niang Megha is the second recognized pig breed of India (Accession No: INDIA_PIG_1300_NIANGMEGHA_09002).

Tanyi-Vo: The animals are found in Nagaland and adjoining state. Color mostly black with diamond or star shaped white patches on forehead. The animals have moderately dense coat and small, erect ears. Animals are alert and ferocious with active look. The adult body weight ranges between 40 to 60 kg at maturity. Litter size is very small and ranges between 4 to 6 at birth. These animals attain early sexual maturity. They are raised on scavenging and backward system by tribal people of Nagaland. The breed is registered with nation database with

Accession number INDIA_PIG_1400_ TENYIVO _09004.

Zovawk: This is a small size breed found in Mizoram. The animals are predominantly black in colour with pot bellied appearance. The mature body weight ranges between 40 to 50 kg and litter size about 5 to 8. These pigs are mainly raised in backyard and semi-intensive system by tribal community of the state. The breed is registered with nation database with Accession number INDIA_ PIG_2700_ ZOVAWK _09007.

2.2 Exotic pig germplasm

Due to poor performance of indigenous pig germplasm and for further upscaling the performance of piggery sector in India, exotic pigs have been imported by different Government and non-Government organizations during past as per recommendation of National Commission on Agriculture (NCA). These breed were extensively used for subsequent crossbreeding programme. Berkshire, Charmukha, Duroc, Hampshire, Landrace, Large Black, Large White Yorkshire, Middle White Yorkshire, Tamworth and Wessex Saddleback are the major known exotic breeds imported in India for piggery developmental programme. These breeds have well studied in different All India Coordinated Research Project on Pig (AICRP), State Government and privet sector farms.

2.3 Crossbred pig germplasm

Piggery developmental programme undertaken by central and state departments namely DADHF of Government of India, Veterinary and Animal Husbandry Department and Animal Resource Development Departments of different states and research organization viz. ICAR, CAU, SAUs has resulted in noticeable progress over the time. During the initial period of development, focus was on genetic improvement of indigenous pig through selective breeding. Subsequently exotic breeds were imported to India and efforts were made for stabilize their performance in Indian agro-climatic condition. Consequent to slow progress of indigenous pigs and based on demand, crossbreeding of native pigs with exotic boars gained momentum in different parts of the country. Several crossbred pig germplasm has been reported till today by different organizations.

The crossbred germplasm of northeastern India includes high-producing varieties viz. Rani, Asha, HD K-75 and Lumsniang suitable for different agro-climatic condition of the country.

Conclusion:

3. Domestication and Selection

Origin of domestic pig belongs to a single wild species *Sus scrofa*, formerly existed throughout Eurasia and also found in North Africa and Nile Valley. *Sus scrofa* is a member of the Suidae family, of the order Artiodactyle. About 25 subspecies of *Sus scrofa* have been described and it is assumed that these subspecies have evolved as adaptation to localized climate. Other than these, the sub species *Sus salvanius*, is a smaller size animal, popularly known as pygmy hog, once widely distributed in foothill of the Himalayas and now restricted in Manas National Park of Assam. The Indian wild pig *Sus scrofa cristatus* is found in sub-Himalayan area including Shiwalik and Tarai area of northern India.

The establishment of agricultural economies based upon domestic animals began independently in many parts of the world and led to both increase in human population size and the migration of people carrying domestic plants and animals. There are two different opinion regarding domestication of pig. One is that they were independently domesticated at different parts of the world, whereas the other is, pigs were domesticated at one center in western Asia in 7000 BC, which gradually diffused to remaining part of Asia, Europe and Africa. Virtually nothing is known about the history of pig domestication in the Indian subcontinent, and a temporal context for when local pig domestication might have occurred remains unexplored. Although *Sus* remains are a widespread component of zooarchaeological assemblages throughout India and Pakistan, they represent such a minor proportion of the domestic and wild fauna recovered (Chattopadyaya, 2002) that the possibility of an autochthonous domestication of *S. scrofa* in India has rarely been considered. Recent genetic data have, however, suggested the possibility of an independent domestication of Indian wild boar. Modern mtDNA data (Larson et al., 2005, 2010; Tanaka et al., 2008) have demonstrated that domestic pigs in India and

Bhutan possess unique and highly differentiated haplotypes identical to those found in Indian wild boar. This suggests that Indian domestic pigs were probably not derived either from migrating east Asian or south-east Asian populations or from near eastern or European sources, but were the result of a process of local domestication, though, again, the maternal inheritance of mtDNA means that a non-native paternal origin of these pigs remains a possibility.

In India, domestication was most likely to have been during the Neolithic era and linked possibly with the Harrapan culture of India. Pigs were domesticated before initiation of the civilization of Mohenjo-Daro, probably for meat. The remains of pig were found in very earlier time of evolution in the city of Mohenjo-Daro.

4. Conventional pig breeding

Genetic improvement in pigs began several centuries ago leading to a clear transformation from the wild pig to the domestic pig. The genetic improvement at that time was based on empirical methods or due to 'unconscious selection', as termed by Darwin (1859). The methods became more scientific after the discovery of Mendel's principles and the development of genetics as a scientific discipline.

In Scientific breeding programme, pig breeding operate in a three-tier pyramidal structure (Fig. 1). At the peak of the pyramid is the nucleus breeding *farms* that actually generate the genetic changes, followed by the next tier of *multiplier farms* that carry out specific mating or crossbreeding for the production of large numbers of females. These females are then sold to *commercial producers* for *piglet production* and *finishing* to produce market pigs that are sent to slaughterhouses to produce the pork.

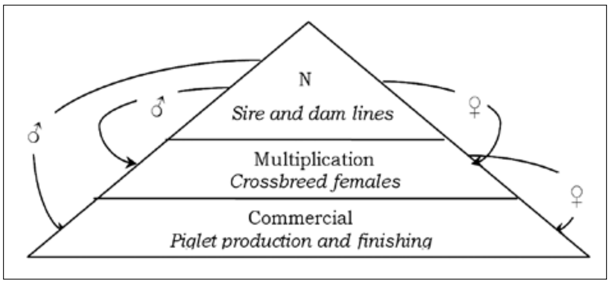


Fig. 1. Pig breeding pyramid (Source: Dekkers et al., 2011)

The nucleus farm actually conducts breeding and selection for the genetic improvement of specific breeds or lines. They target their selection programmes to the needs of their customers, the pork producers and processors. However, there is a time delay between the genetic improvement in the nucleus farms and the transfer of genetic gains to commercial producers through multipliers. This delay is typically 3–5 years and is called *genetic lag*. It is, therefore, very important for the nucleus breeders to evaluate the future needs of producers ahead of time and decide their breeding goals accordingly.

The conventional genetic improvement programmes are driven by measuring phenotypes for traits of interest on selection candidates in the nucleus or on close relatives of the selection candidates. These phenotypes are then used to estimate the breeding values of selection candidates for traits and incorporate these in a multi-trait selection index to identify individuals that best meet the breeding objective. Although this has led to impressive increases in performance for several traits, the phenotype-based approach to genetic improvement suffers from several important limitations.

- Several traits have low heritability, e.g. reproduction, disease resistance and survival traits. This limits the accuracy and efficiency of selection and, therefore, genetic improvement for these traits.
- Several traits can only be measured later in an animal's life, e.g. sow lifetime productivity. This either increases generation intervals if the choice is made to wait until the phenotype can be observed on the selection candidates, or reduces accuracy of selection if the choice is made to make selection decisions before the phenotype is observed.
- Several traits cannot be measured directly on selection candidates. For example, many meat quality traits require slaughter of the animal. Thus, for these traits, genetic evaluation is based on phenotypes of relatives that are slaughtered, limiting the accuracy of selection as well as the selection intensity, as individuals that may be potential selection candidates are slaughtered.
- Some traits are expensive to measure (e.g. disease

resistance). Phenotype-based programmes require routine collection of large numbers of phenotypes on the selection candidates themselves or their close relatives, resulting in costs that may not outweigh the benefits.

- The increased emphasis that is placed on relatives in many of the above situations also leads to greater rates of inbreeding within the selection lines. The higher rates of inbreeding result from family members having very similar estimates of breeding values (EBV) if these EBVs are heavily based on phenotypes of parents, full sibs and half sibs rather than on own performance or progeny performance. Thus, selection tends to be of families rather than individuals within a family, resulting in selected individuals being more closely related and higher rates of inbreeding.
- Whereas the main focus in pig breeding programmes has been on the genetic improvement of additive genetics through selection on EBV, most traits of interest exhibit non-additive effects, e.g. through heterosis when crossing breeds. Traditional quantitative genetics, however, offers limited opportunities to select for non-additive effects.

With the implementation of molecular genetics in the 1980s and 1990s, in particular the discovery of new classes of DNA polymorphisms, prospects to overcome these limitations of phenotype-based selection emerged with the potential to directly select on an individual's genotype for genes or genetic markers that are associated with the trait.

5. Open Nucleus Breeding Scheme (ONBS)

Open Nucleus Breeding Scheme (ONBS) is a three tier system of pig production comprising of nucleus herd, multiplication herd and commercial herd. The nucleus herd actually conducts breeding and selection for the genetic improvement of specific breeds or lines. They target their selection programmes based on the needs of customers, the pork producers and processors. However, there is a time delay between the genetic improvement in the nucleus farms and the transfer of genetic gains to commercial producers through multipliers. This delay is typically 3 to 5 years and is called genetic lag. It is, therefore, very important for the nucleus breeders to evaluate the future needs of producers ahead of time and decide their breeding goals accordingly. Open nucleus breeding schemes (ONBS) should be followed as a model for pig industry in developing countries. The reasons for following ONBS are that the breeding stock is concentrated in a few herds from where the quality animals are disseminated to other units and moreover good record keeping and data management at reasonable cost, will ease the functional operation.

In order to have a successful ONBS at a large scale, use of artificial insemination (AI) with frozen semen is essential. AI using frozen or liquid semen is a prerequisite for large scale development of any animal husbandry sector. AI in pig using frozen semen still remains as a formidable challenge in India with limited success only on experimental basis.

6. The Quantitative Trait Loci (QTL) explosion and deception

Since the late 80's and 90's, the availability of neutral molecular markers, such as the microsatellites, encouraged the development of a plethora of experimental studies to locate genomic regions and Quantitative Trait Loci (QTL) associated with traits of economic interest.

Initially, two basic designs were used. The first uses the linkage disequilibrium between markers and QTL generated by crosses. Typically, animals are generated by crossing breeds that are highly divergent for the traits of interest (for example European wild boar and domestic Large White). The second design uses mainly the within-family linkage disequilibrium. This design is especially well suited analysis of large half-sib families within a breed. This activity has been very successful. By January 2014, in the data base http://www.animalgenome.org/QTLdb/, a total of 9862 QTL for 653 different traits have been identified for pigs.

After detecting a QTL, the next task is to locate the gene responsible (causal mutation). In QTL detection studies, we can locate one QTL in a chromosome as a region of about 20-40 cM (probably harbouring 200-400 genes) which made it difficult to identify the underlying gene responsible. To refine the position several actions can be taken:

to increase the number of individuals, to carry out fine mapping or to try the 'candidate gene approach'. All these approaches are difficult, expensive in terms of time and money and success is not guaranteed, thereby making the location of the responsible gene a formidable task. The difficulties for finding the causal mutations can be exemplified by more than 9000 QTLs reported in pigs, of which less than a dozen of causative mutations have been firmly established. Interestingly, the first QTL reported in livestock was FAT1 QTL located in swine chromosome 4 (Andersson et al., 1994); however, its causal mutation is still unknown.

7. Marker-Assisted Selection (MAS)

Various candidate gene and quantitative trait loci (QTL) analyses have recognized substantial chromosomal regions and individual genes linked with traits of economic interest in pig. These comprise QTL for meat quality traits, back fat, growth and reproduction. Some commercially used traits for Marker Assisted Selection (MAS) in pig industry are given below.

Experimentations on an array of candidate genes for various economic traits including fitness and adaptability can easily be mixed in a large scale coordinated breeding programme. Combined studies and experimental work carried out at National Research Centre on Pig on a limited scale has indicated the probability of oestrogen receptor (ESR) and follicle stimulating hormone beta subunit (FSHb) genes as candidate markers in Ghungroo breed of indigenous pigs. Niang Megha and Ghungroo, the two popular local breeds have been found to be free from recessive mutation in halothane gene (candidate marker) accountable for Pale-Soft-Exudative (PSE) pork.

8. Genomic selection

Recent developments in technology have removed some of the limitations of previous applications of QTL-mapping results for MAS, which, as already mentioned, have limited the use of markers in commercial breeding. These developments include genome sequencing, the identification of large numbers of genetic markers across the genome in the form of SNPs, and the cost-effective high-throughput genotyping of tens of thousands of such SNPs on individual animals. Combined with the further development of statistical methods for analysis of molecular data, this has led to a paradigm shift in the strategy of using genetic markers for the prediction of breeding values in the form of what has been termed 'genomic selection'

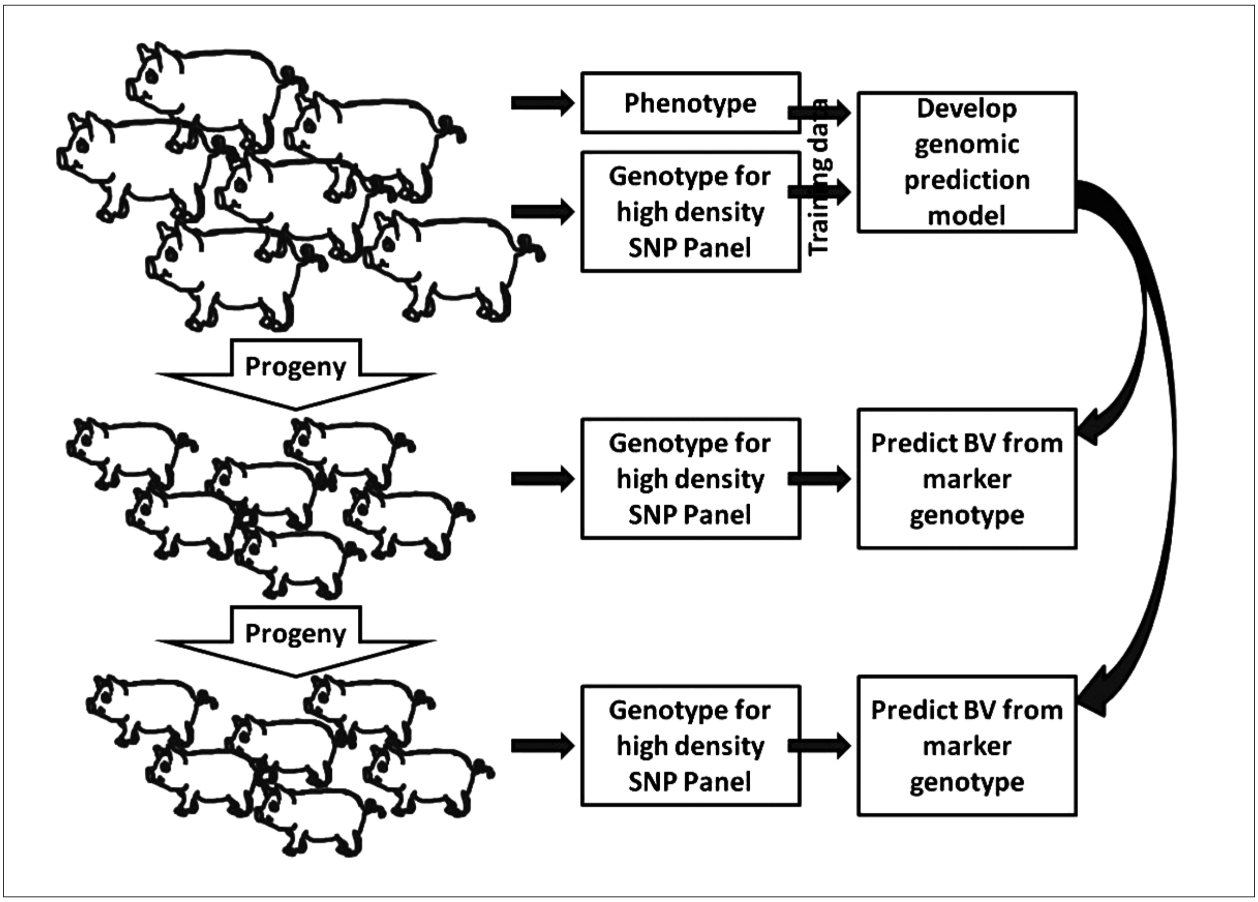
Table 1: Commercially used traits in Marker Assisted Selection (MAS) in swine industry:

Trait	Marker	Name	Chromosome	Patent
Litter size	Oestrogen receptor	ESR	1	yes
	Osteopontin	OPN	8	yes
	Prolactin receptor	PRLR	16	yes
Lean growth	Marker for fat	SO112	1	--
	Insulin like growth factor	IGF2	2	--
	Myogenic factor 3	MYF3	2	--
	Marker for fat	SO107	4	--
	Leptin receptor	LEPR	6	--
	Marker for fat	SO102	7	--
	Myostatin	GDF8	15	--
Meat quality	Skeletal muscle calpain	CAPN	1	--
	Calpastatin	CAST	2	--
	Halothane gene	RYR1	6	yes
	Rendement Napole	RN	15	--
Intramuscular fat	Heart fatty acid binding protein	H-FABP	6	yes
Immunity	Tumour necrosis factor	TNFB	7	--
	Histocompatibility	SLA-1	7	--
Coat color	Dominant white	KIT	8	yes

(Meuwissen et al., 2001). Genomic selection (GS) is an enhanced version of MAS that involves the selection of animals for breeding on the basis of their genotype for tens of thousands of 'random' SNPs that cover the genome. In GS, the association of each SNP with phenotype is estimated using sophisticated statistical and quantitative genetics models without pre-screening markers based on significance.

The first step in GS is to collect phenotypes and DNA from a large group of individuals that have been phenotyped for the trait, or that have progeny with those phenotypes, and to genotype each animal using the SNPChip. The resulting 'training data' are used to 'train' a statistical model that estimates the effect of each of the SNPs on the SNPChip with the trait phenotype. In principle, the estimate for a given SNP is based on the comparison of the average phenotypes of individuals that have alternative genotypes at that SNP, as described above, but with GS this is done simultaneously for all markers on the SNPChip. The resulting estimates can then be used to predict the 'Genomic' EBV (G-EBV) of new individuals based on their genotypes for the SNPChip.

In the pig breeding industry GS had raised great interest. The accuracy of the breeding values for the target traits, generally low compared to dairy cattle, can be significantly increased, preserving the generation interval and control of inbreeding. The effectiveness of GS depends largely on the level of linkage-disequilibrium (LD) that can be captured by markers within the target population. The higher the level of LD, the fewer markers are needed to capture the genomic regions contributing to the phenotype. In a survey of several swine lines, Deeb et al. (2010) found that a few thousand equally-spaced SNP achieved average LD of 0.2 or higher in most pure lines and crosses evaluated. Moreover, of the 62k markers on the commercially available PorcineSNP60 BeadChip, over 50k SNP had allele frequencies of 5% or higher in most pure-lines and crosses, indicating that the commercial chip provided enough markers to be effectively used for genomic



(Reproduce from: Dekkers et al., 2011)

Fig. 2. Steps in genomic selection. (SNP: single nuclear polymorphism; BV: breeding variation).

selection of all line groups. The improvement of genetic gain on the nucleus has an important impact on the large commercial populations and can make GS economically feasible, given the large influence of elite individuals. Pig breeding schemes currently have developed a very efficient data recording scheme which easily could include genomic information. However, in spite of these advantages, GS in pig has only recently be implemented and it is still not so common as in dairy cattle. First, the rather recent availability of the SNP chip technology for pigs has delayed its introduction. Second, the peculiarities of pig breeding schemes (e.g. small nucleus size, diversity of breeding goals, pyramid system) made genomic evaluation strategies not straight forward to implement. In the last years, many efforts have been done to evaluate new methods and strategies to allow efficient implementation of GS on pigs.

9. Assisted Reproductive Technologies (ART) in pig breeding

Global needs for foods and animals require the development of strategies beyond traditional breeding ensuring offspring of value characteristics, of high productivity but maintaining genetic diversity. In any livestock venture, reproductive performance is often the number one contributor to profitability. Improved reproductive performance requires an understanding and utilization of innovative approaches to the synchronization of breeding and farrowing, while maintaining adequate litter size and maximizing the contribution of elite genetics to future progeny. Below are some of the reproductive technologies that are currently being applied in swine industry.

9.1 Oestrus synchronization

Synchronization of oestrus in swine herds is essential to maintaining large farrowing groups and has permitted efficiencies in production, management and marketing. The most common method of oestrus synchronization in sows is weaning litters at the same time. Generally, most of the sows will begin oestrus within 4-7 days following weaning. This method is widely used throughout the swine industry and is consistently reliable in sows. Gilts, in contrast, represent a significant proportion of the breeding inventories for most swine herds, and synchronizing gilt oestrus can be more challenging.

In pigs, PMSG is exclusively used to induce superovulation (SO). Superovulation is stimulated in prepuberal gilts by application of 1000-1500 IU PMSG followed by an injection of 500 IU hCG 72 h after PMSG. In cycling gilts SO is induced after estrus synchronisation (feeding of Regumate® for 15 days) by administration of 1500 IU PMSG and of 500 IU hCG 78 h later. Multiparous sows are injected with 1000-1250 IU PMSG 24 h after weaning and 500 IU hCG 58 h after PMSG. The use of GnRH is an alternative to hCG in triggering ovulation. Gilts and sows are fixed-time inseminated 24 h and 38 h after hCG injection.

9.2 Artificial Insemination (AI)

AI has dramatically advanced the genetic improvement of pigs and is the standard operating procedure on major swine farms. The downside of AI in pigs is that the long uterine horns of females require a volume and sperm count that limits the boar to producing an average of 20-30 AI doses per ejaculate. To overcome this, investigators have been developing approaches to insemination that can reduce both the volume and sperm numbers required per dose. These approaches require the delivery of the semen past the cervix (i.e. post-cervical or intrauterine insemination) or further into the uterine horns (deep intrauterine insemination, also referred to as low-dose insemination). Strategies capable of maintaining average litter size while significantly reducing sperm numbers are required to advance the utilization of sex-sorted sperm and frozen-thawed semen to widespread commercial application.

Currently, swine producers typically utilize two doses of semen, each about 24 h apart, and beginning at the onset of oestrus. The reason that two doses are used is because the length of behavioural oestrus is highly variable between females (24-72 h), and, given that the timing of ovulation relative to the duration of oestrus is quite variable, it is difficult to establish appropriate insemination timing for a single dose.

9.3 Semen and embryo cryopreservation

Cryopreservation of semen and embryos from pigs has presented more of a challenge than in many other species. Although boar semen has been frozen and used for AI for 35 years, the success rates are still low. Boar semen is sensitive to changes in osmotic balance, oxidative stress, low temperatures and the toxic effects of exposure to cryoprotectants. As sperm survivability after cryopreservation can be low, deep uterine insemination is the preferred method of AI.

Similar to sperm, pig embryos are especially sensitive to a decrease in temperature. Interestingly, removal of the numerous lipid droplets appears to alleviate this sensitivity. However, the lipid removal techniques generally compromise the zona pellucida, thus creating the possibility of pathogen entry. Other strategies, such as destabilizing the cytoskeleton, altering the vitrification conditions or using a solid surface for cryopreservation, have been successfully employed. Additional research is needed to develop these technologies for widespread commercial application.

9.4 Embryo transfer (ET)

Utilization of ET in the swine industry is much less advanced than the significant progress in the beef industry. The greatest advantage of any ET programme is that it permits an increase of the genetic contributions of select females to the herd. Perhaps one of the most limiting aspects of ET in pigs is that non-surgical collection and transfer of embryos is not nearly as successful as surgically removing and transferring embryos. While investigators have been capable of producing piglets following non-surgical ET, the efficiency still lags significantly behind surgical methodologies.

More recently, pig ET has become somewhat commercialized in an effort to flush embryos encapsulated in their zona pellucida from diseased herds, appropriately wash/sanitize them and transfer them to recipient pigs that have a specific pathogen-free status. While this strategy is not economically viable for many swine herds, it is being utilized as a method for salvaging elite and/or valuable genetic stock from herds before depopulation.

10. Transgenic pigs and their development:

A transgenic pig is one whose genetic material has been altered to influence the expression and abundance of specific genes, with the ultimate objective of producing a specific, desired phenotype. This is most commonly done by introducing exogenous DNA, produced through recombinant DNA technology, into the genome of the pig. The appropriate production method and expected results can vary significantly, depending on whether the desired modification is random, targeted and/or tissue specific.

Table 2. A timeline of significant achievements leading to production of transgenic pigs

Milestone	References
Transgenic pig via pronuclear injection	Hammer et al., 1985
Cloned pig from embryonic cells	Prather et al., 1989
Sperm mediated gene transfer in pigs	Lavitrano et al., 1997
Cloned pig from fetal and adult cells	Polejaeva et al., 2000
Transgenic pig via oocyte transduction	Cabot et al., 2001
Transgenic pig via somatic cell nuclear transfer (SCNT)	Park et al., 2001
Gene 'knockout' pig using SCNT	Lai et al., 2002
Intracytoplasmic sperm injection (ICSI)- mediated transgenic pig	Kurome et al., 2007
Gene 'knock-in' pig via SCNT	Rogers et al., 2008

(Source: Ross & Prather, 2011)

Numerous milestones have been achieved in the development of methods capable of producing cloned and transgenic pigs. The development of efficient methods for producing transgenic large animals has been and still is essential to understanding the biology and increasing the utility of pigs.

Pigs have long been considered a source of organs for xenotransplantation to humans. Their anatomy, physiology and genetics are similar to those of humans (Prather et al., 2008). An initial problem with xenotransplantation is the hyper acute rejection (HAR) that occurs following transplantation from pigs to primates, as a result of natural antibodies that are capable of initiating complement activation and eliciting a cascade of events that end in immediate rejection of the organ. A large number of genetic modifications such as adding complement modifiers, remodelling the cell surface carbohydrates, preventing antithrombosis and knocking out alpha- 1,3-galactosyltransferase have been made to eliminate HAR to make the pig organs less immunogenic to the primate.

As progress continues in the production of a transgenic pig for which the organs are capable of being utilized for transplantation into humans, other issues will also need to be addressed. One of these is the matter of porcine endogenous retroviruses (PERVs). While their impact on xenotransplantation is unclear, certain types of PERVs have been documented to be transmissible across species *in vitro*. Because of their multiplicity throughout the pig genome, their entire removal by 'knocking out' each retrovirus through transgenesis or by developing a breeding strategy that is capable of producing pigs lacking all known retroviruses both represent daunting tasks. Instead, some efforts have focused on the development of short interfering RNA (siRNA) constructs whose expression can effectively knock down PERV expression in pigs.

Conclusion

The pig breeding programmes have been very successful in effecting genetic improvement of economically important traits, especially daily gain (+100%), back fat thickness (-75%), and feed efficiency during the last decade. However, this is not enough for the future. In commercial pig breeding programmes selection limits have not been reached yet. Nevertheless, there are experimental results that indicate unintended side effects of the present breeding goals. Some of the genetic alterations realised in pursuit of breeding goals may have unintended negative side effects on animal welfare and integrity. As basic scientific discovery reveals specific molecular mechanisms that can be controlled or influenced by the modification of specific genetic sequence(s), the opportunities to improve animal production agriculture and biomedicine using the pig become unlimited. While there is no doubt that scientists and producers will continue to improve pig production efficiency through better management practices that utilize novel tools in genomics and reproduction, the ability to make modifications to the pig has given us tremendous opportunities to better understand physiology and improve the health.

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Molecular typing of bacterial pathogens with special reference to *Salmonella*

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Differentiation and identification of variants among microbial isolates within a species is called typing. Traditional typing systems based on phenotypic characteristics, such as serotype, biotype, phage-type or antibiogram have been used for many years. However, these methods are generally too variable, labour-intensive and time-consuming to be of practical value in epidemiological investigations (Maccannell, 2013). They are able to differentiate between organisms in which there is marked variation in phenotypic expression, but most organisms causing infections are a small subpopulation of the numerous strains within a species that may show little diversity.

There are a number of reasons why it may be necessary to characterize a microbial isolate beyond species level and determine its sub-species, strains, or even sub-strains. For example:

- To relate individual cases to an outbreak of infectious disease
- To establish an association between an outbreak of food poisoning and a specific food vehicle
- To study variations in pathogenicity, virulence and antibiotic resistance of individual strains within a species
- To trace the source of contaminants within a manufacturing process
- To study the microbial ecology of complex communities, such as biofilms
- To characterize microorganisms with important industrial applications

In the recent time, the methods that can be used to study relatedness of isolates at the molecular level have revolutionized our ability to differentiate among bacterial types (or subtypes). The choice of an appropriate molecular typing method (or methods) depends significantly on the purpose and the epidemiological context in which the method is going to be used, as well as the time and geographical scale of its use. Importantly, pathogens of one species can comprise of a diverse types of organisms. Therefore, typing techniques should have excellent differentiating capacity to be able to type all the isolates studied (Struelens, 1996). In outbreak investigations, a typing method must have the discriminatory power needed to distinguish all epidemiologically unrelated isolates. Ideally, such a method can discriminate very closely related isolates to reveal individual-to-individual strain transmission, which is important to develop strategies to prevent further spread. At the same time, it must be rapid, inexpensive, highly reproducible, and easy to perform and interpret (van Belkum *et al*., 2007).

During the last two decades, the development and extensive use of high resolution molecular typing systems based on direct analysis of genomic polymorphism have greatly improved the understanding of the epidemiology of infectious diseases (Maslow and Mulligan, 1996). To date, many different molecular methods for epidemiological characterization of bacterial isolates have been developed. However, none of them is optimal for all forms of investigation. Thus, a thorough understanding of the advantages and limitations of the available typing methods is of crucial importance for selecting the appropriate approaches to define outbreak strains unambiguously (Sabat *et al*., 2013).

The efficiency or suitability of a typing method may be assessed based on a number of criteria. These include: *typeability, reproducibility, stability, discriminatory power* and *epidemiologic concordance* (Struelens, 1998). *Typeability* refers to the proportion of isolates that can be scored in the typing system and assigned a type, ideally for all isolates. *Reproducibility* refers to the ability of the typing system to assign the same type on repeated testing of the same strain. *Stability* is the biological feature of clonally derived isolates to express constant markers over time and generations. *Discriminatory power* is a key characteristic of typing systems, because it conditions the probability that isolates sharing identical or closely-related types are truly clonal and part of the same chain of transmission. *Epidemiologic concordance* is the capacity of a typing system to correctly classify all epidemiologically related isolates from a well-described outbreak into the same clone. Other practical aspects of typing systems include ease of performance and ease of result interpretation, as well as cost and availability of reagents and equipment. Additionally, a typing method used for surveillance should rely on an internationally standardized nomenclature, and it should be applicable for a broad range of bacterial species.

This overview aims at briefly describing the molecular methods most commonly used for subtyping of bacterial pathogens and discussing their main advantages and limitations.

Plasmid profiling

Plasmid DNA analysis has been applied for typing of diverse species of Gram-negative and Gram-positive bacteria (Wachsmuth *et al.*, 1991; Liu *et al.,* 1996). Plasmid profiling was one of the earliest genotyping methods used for epidemiological studies. However, it has a number of disadvantages, mainly intrinsically related to the properties of the plasmids. These mobile elements are transferable by conjugation, can be gained or lost spontaneously and under selective pressure they may spread rapidly from one strain to the next (Tenover *et al.*, 1997). The gain or loss of plasmids can cause confusions while determining genetic relatedness among the isolates. Moreover, plasmids may show discrepancy as time passes and either carry or lose genetic sequences such as transposons (Bopp *et al.*, 1999). However, small plasmids which appear on agarose gels as bright bands, mostly below chromosomal DNA, are suitable to be used for typing purposes because of their stability unlike larger plasmids which tend to be lost during cell storage and subculturing or extraction procedures (Farshad *et al.*, 2006).

Genotyping

The development of techniques for direct study of the microbial genome, notably amplification by PCR, has led to the introduction of a number of novel methods for genotyping microorganisms over the last 25 years (Anonymous, 2015). A multiplicity of techniques for analyzing the DNA extracted from microbial cells, often termed as DNA fingerprinting, have been developed for typing. Some of the widely used methods among them are outlined below:

1. **Pulsed-field gel electrophoresis (PFGE)** – This technique allows the electrophoretic separation of low numbers of large DNA fragments produced by digestion with restriction enzymes to generate a highly discriminatory genetic fingerprint. It is based on the study of total bacterial DNA restriction patterns, which was first developed by Schwartz and Cantor at Columbia University in 1984 (Schwartz and Cantor, 1984). This method is widely used and is still considered as the method of choice for typing of a number of bacterial pathogens and for investigation of disease outbreaks. PFGE is relatively a costly method and requires at least 3-4 days for obtaining a result. The degree of discrimination also depends on choice of restriction enzymes. PFGE is a valuable tool for assessing interrelatedness among pathogens. It has made a tremendous impact in the field of molecular biology by making possible the separation of large DNA fragments.

   In conventional agarose gel electrophoresis, DNA molecules bigger than 40 - 50 kb in size fail to migrate efficiently and appear in the gel as a single large diffuse band due to their size-independent co-migration, known as *reptation* (Slater, 2009). By periodically applying changes in the direction of the electrical field in which large DNA molecules are suspended, PFGE allows the separation of DNA molecules over 1000 kb. PFGE used with various restriction enzymes is considered the gold standard for subtyping of bacteria and is the currently used method in the PulseNet USA network (http://www.cdc.gov/pulsenet), the molecular surveillance network for foodborne infections in the United States (Swaminathan *et al.*, 2001; Gerner-Smidt and Scheutz, 2006).

2. **Multilocus Sequence Typing (MLST)** – This method involves sequencing of 400-500 base pair fragments of DNA at seven different conserved genes that allows detection of small variations within a species. This method is quite time-consuming and costly, but can be highly discriminatory if the genes are correctly chosen. The method has the advantage of being easily standardized and automated. MLST involves PCR amplification and sequencing of 5–10 loci of ~500 bp in length, with each sequence variant assigned a unique locus variant or allele number. Each unique combination of locus variants is assigned a sequence type (ST), which is then used to denote a precise set of sequences. Public MLST databases are used to store and share information linking DNA sequences to locus variant numbers and sequence types and are available for > 85 bacterial species including important human pathogens such as *Staphylococcus aureus, Haemophilus influenzae* and *Neisseria* species (http://pubmlst.org). This format allows quick, simple and direct comparison of bacterial populations analysed in different laboratories and over time (**Inouye** ***et al.*****, 2012).** MLST has now become the gold standard for the analysis of bacterial populations (Maiden, 2006).

3. **Multilocus variable number of tandem repeats analysis (MLVA)** – This is a technique related to MLST which is based on PCR amplification and sequencing of rapidly mutating repetitive DNA sequences called tandem repeats. MLVA is faster and easier to perform than MLST, but there are issues with reproducibility and validation. The method involves determination of the number of repeats at multiple loci, thereby providing a powerful tool for assessing the genetic relationships between bacterial strains of the same species. In a study of the highly clonal organism *Bacillus anthracis*, 426 isolates that were previously homogeneous by other molecular subtyping methods including PFGE could be separated into 89 distinct genotypes by MLVA (Keim *et al.*, 2000). MLVA has several advantages over PFGE because the output is highly objective, making the data amenable to automated computer analysis for the rapid detection of outbreaks and easy to compare across laboratories.

4. **Random Amplified Polymorphic DNA (RAPD)** - Random amplification of polymorphic DNA (RAPD) is based on the parallel amplification of a set of fragments by using short arbitrary sequences as primers (usually 10 bases) that target several unspecified genomic sequences. Amplification is conducted at a low, non-stringent annealing temperature, which allows the hybridisation of multiple mismatched sequences. When the distance between two primer binding sites on both DNA strands is within the range of 0.1–3 kb, an amplicon can be generated that covers the sequence between these two binding sites. The number and the positions of primer binding sites are usually unique to a particular bacterial strain. RAPD amplicons can be analysed by agarose gel electrophoresis or DNA sequencing depending on the labeling of primers with appropriate fluorescent dyes. The genetic variation analysis based on RAPD allows proper genetic diversity due to its capacity to generate random markers from the entire genome (Lin *et al.*, 2014). Although it is less discriminatory than PFGE, RAPD has been widely used for the typing of bacterial isolates in cases of outbreaks (Chang *et al.*, 2009; Lanini *et al.*, 2011) as it is simple, inexpensive, rapid and easy to use. The main drawback of the method is its low intra-laboratory reproducibility since very low annealing temperatures are used. RAPD also lacks inter-laboratory reproducibility since it is sensitive to slight differences in reagents, protocols, and machines.

5. **Ribotyping** – Ribotyping is a method that can identify and classify bacteria based upon differences in rRNA (16S and 23S rRNA). Ribotyping is one of the typing techniques based on RFLP analysis of ribosomal DNA. It simplifies the restriction fragments pattern generated by enzymatic digestion of the chromosomal DNA selecting only those containing the gene sequences of the ribosomal

RNA (rRNA). DNA is extracted from a colony of bacteria and then restricted into discrete-sized fragments that are then separated by electrophoresis. The resulting fingerprint is visualised using fluorescent probes. The variations that exist among bacteria in both the position and intensity of rRNA bands can be used for their classification and identification. Ribotyping has been developed as an automated system that is commercially available with dedicated databases [RiboPrinter Microbial Characterization System (Qualicon Inc., Wilmington, DE, USA)]. It is comparatively more rapid (takes < 24 hours to obtain result), reproducible and works for a wide range of bacterial species, but relatively costly in terms of equipment, if automated system is to be used. Ribotyping has higher discriminatory power at the species and subspecies levels compared to the strain level (Shi *et al.*, 2010).

Although ribotyping is a powerful method for determining the molecular epidemiology of bacterial pathogens, technical difficulties limit its application. As an alternative, polymorphisms in the 16S-23S spacer regions of bacterial rRNA genes amplified by PCR can be used. Chromosomal DNA from isolates of bacterial pathogens are used as a template in the PCR with oligonucleotide primers complementary to highly conserved sequences flanking the spacer regions of the rRNA genes. Length polymorphisms in the amplified DNA can distinguish unrelated isolates of the organism. This method is called PCR-ribotyping. This has been described as a reliable technique for typing different pathogens such as *Clostridium difficile*, *Pseudomonas cepacia*, *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli* and *Enterobacter* sp.

6. **Repetitive sequence-based PCR (rep-PCR)** – Bacterial and fungal genomes contain numerous non-coding, repetitive DNA sequences separating longer, single copy sequences and their arrangement varies between strains. The rep-PCR technique relies on amplifying these repetitive sequences to produce amplicons of varying length that can be separated by electrophoresis giving a fingerprint comprising of bands that fluoresce at different intensities after binding with an intercalating dye. Multiple families of repeat sequences have been used successfully for rep-PCR typing, such as the 'enterobacterial repetitive intergenic consensus' (ERIC), 'the repetitive extragenic palindromic' (REP), and the 'BOX' sequences (Versalovic *et al.*, 1994). As this typing approach is based on PCR amplification and subsequent DNA electrophoresis, the results of rep-PCR can be obtained in a relatively short period of time. This is also the reason why this approach is very cheap. The system is widely used for molecular typing of a number of human pathogens. Discriminatory power of rep-PCR depends on the method used and the number of repetitive sequences present in a strain. Reproducibility of Rep-PCR can be affected by variability in PCR reagents, thermal cycling and gel electrophoresis conditions. Rep-PCR has also been developed into a commercial automated typing system (*e.g.* DiversiLab System) giving rapid results using dedicated software for analysis of the banding patterns.

Molecular typing of *Salmonella*

Salmonella infections are one of the major global public health problems. During the last decade, antibiotic resistance and multi-resistance of *Salmonella* spp. have increased to a great extent, especially in the developing countries with an increased and indiscriminate use of antibiotics in the treatment of humans and animals. Identification of an epidemic strain of *Salmonella* is often critical to the success of epidemiological investigations aimed at preventing the spread of infection and eradicating its source.

Different molecular typing methods have been applied by various workers for epidemiological investigation on *Salmonella.* Plasmid profiling and plasmid restriction analyses have been extensively used for characterization of bacterial foodborne pathogens, such as *Salmonella* spp. (Threlfall *et al.*, 1982; Nayak *et al.*, 2004). Many investigators have found RFLP suitable for typing of foodborne pathogens. RFLP analysis was first used to differentiate *Salmonella* serotypes, such as Typhimurium, Dublin and Enteritidis (Tompkins *et al.*, 1986). The IS-RFLP method has also been used for typing *S. enterica* serovars Typhimurium and Enteritidis by targeting the IS200 sequences in their bacterial genomes (Olsen *et al*., 1997; Millemann *et al*., 2000). In India, molecular methods like REP-PCR and ERIC-PCR have been tried by a few workers to discriminate *Salmonella* Bareilly and other poultry isolates (Saxena *et al*., 2003; Arora *et al*., 2008).

In our study, the efficacy of three molecular typing methods, *viz*. rep-PCR, PFGE and plasmid profiling was compared for discriminating *Salmonella* isolates (n = 88) from faecal samples of human, animals and birds collected from different parts of Assam and Kolkata city.

Phylogenetic analysis based on plasmid profiling showed that the *Salmonella* isolates from various sources could be differentiated into two distinct clusters. However, the method couldn't clearly discriminate between serotypes or among the strains within a serotype. In the PFGE analysis, the most common serotypes of *Salmonella* strains (n = 28) isolated from Kolkata and Assam were considered. *Salmonella* Newport strains isolated from human, poultry and cattle and *S.* Weltevreden isolated from poultry, cattle and wild birds of Assam were identical in their respective clusters, *i.e.* A and B. However, S. Weltevreden strains isolated from diarrhoeal patients of Kolkata were identical and placed in cluster C, which were different from Assam strains.

PFGE is considered as the 'gold standard' of molecular typing methods, which is accurate, reliable, reproducible, highly discriminatory and easy to perform (Olive and Bean, 1999; Olsen, 2000). However, the method is limited with regard to being less effective in discriminating closely related strains (Lindstedt *et al*., 2004; Wattiau *et al*., 2011). It is observed that no apparent correlation exists between the results of one molecular typing method and those of the others, suggesting that a combination of multiple methods is needed to differentiate *S. enterica* serovars (Foley *et al*., 2006).

In our study, rep-PCR was able to clearly discriminate between strains within a particular serovar. Hence, it could be suggested that for molecular typing of *Salmonella* isolates during epidemiological investigations, a combination of PFGE and rep-PCR would prove to be more useful and appropriate. Rep-PCR fingerprinting is stated to offer an attractive choice as a primary method for the discrimination of various *Salmonella* serotypes as well as isolates within a serotype (Beyer *et al*., 1998). Based on the present study, it may be concluded that PFGE is a better method for molecular typing of *Salmonella* compared to rep-PCR and plasmid profiling. PFGE could clearly discriminate between strains belonging to different serovars, *i.e.* PFGE could discriminate the isolates in a serotype-specific manner. However, in the present study it was observed that this method could not efficiently discriminate among the strains within a serovar. The PFGE is an approach to measure the genetic diversity of the entire genome, which may have arisen as a result of mutation. Such mutations may remove or create recognition sites for restriction enzymes through insertion, deletion or by mobile genetic elements, and as time passes divergence may occur in the population (Bakeri *et al.,* 2003). However, if the genetic variation does not significantly impact the size or electrophoretic mobility of a restriction fragment, then the change may not be identified as a separate pulsotype (Foley *et al.,* 2006).

Johnson *et al.* (2001) reported that minimal subserotype discrimination was obtained with modified rep-PCR typing and the method was highly reproducible and simple to perform. It may allow serotype-level discrimination of *Salmonella* spp. in any laboratory equipped for diagnostic PCR. Contrary to this, Oliveira *et al.* (2007) found that rep-PCR performed as described in the present study was not useful for *S.* Enteritidis typing, because it was not able to discriminate potentially different isolates. However, the combined use of genotypic and phenotypic methods allowed a more accurate discrimination between isolates. Albufera *et al.* (2009) reported that REP-PCR had greater discriminatory power and was better able to differentiate between closely related *Salmonella* isolates as compared to RAPD method.

Our study also showed that the semi-automated DiversiLab rep-PCR system using micro-fluidic chips provides a standardized, semi-automated fingerprinting for pathogens including *S. enterica*. It is a rapid (requiring less than 4 hr) and standardized method for sub-typing isolates of *S. enterica* (Ben-Dariff *et al.,* 2010).

Conclusions

Several detection and typing methods have been developed during the last two decades, and are widely used to detect, differentiate, type and/or to classify pathogens for efficient identification, outbreak investigations, clinical treatments and/or epidemiological studies. In recent years, substantial technical improvements have been made in the existing methods for typing of bacterial isolates. A few completely new technologies have also emerged that may make identification and differentiation of pathogenic microorganisms much easier and rapid. This has also involved the efforts towards automation of some of the typing methods, the improvement of their resolution and throughput, and the design of adequate bioinformatics tools for analysis and interpretation. Although many techniques are available, each of them has its advantages and limitations that make it useful in some studies and restrictive in others. Although a particular typing method may have high discriminatory power and good reproducibility, the complexity of the method and interpretation of results as well as the costs involved in setting up and using the method may be beyond the capabilities of the laboratory.

The choice of a molecular typing method, therefore, will depend upon the skill level, and resources of the laboratory and the aim and scope of the study. Depending on the setting (local, national or international), one or more different typing methods need to be applied (Sabat *et al.*, 2013). If rapidity of typing a pathogen is important for controlling a local disease outbreak, a PCR-based method with high discriminatory power, such as MLVF and/or DiversiLab, may work well for characterization of the isolates. However, if an outbreak of bacterial disease is disseminated among various geographical locations, a more robust typing approach, such as PFGE, will be needed to allow reliable comparison of the results obtained in different laboratories. As different typing methods are based on detection of different genomic target sequences, strain variations detected with one method may remain undetected when applying another approach. Therefore, in certain situations, the combined use of several different typing methods may lead to a more precise discrimination of bacterial isolates than the use of a single method. Molecular typing systems are undergoing rapid technical improvements.

Recent advances in **whole genome sequencing (WGS)** are rapidly resulting in a reduction of costs and time of sequencing. The cost and time required to sequence a typical bacterial genome is now less than $100 and one single day. As a result, WGS is increasingly becoming competitive with any diagnostic technology, including traditional methods of culturing bacteria. According to some researchers, in a few years, WGS could become the sole diagnostic and molecular epidemiological tool for identification, genetic characterization and drug susceptibility testing of pathogens (Schurch *et al.*, 2018).

Advances in the understanding of biological basis of microbial biodiversity at subspecies levels will certainly lead to proper epidemiologic interpretation of typing results. Wider application of these systems is expected to improve tremendously our understanding of the epidemiology of hospital and community-acquired infections and, therefore, allow for more effective control and prevention strategies.

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ORAL PRESENTATION

NES–O–01

Prevalence and Pathological detection of Bluetongue virus in Mithun in North East India

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Bluetongue (BT) is an arthropod born, non-contagious viral diseases affecting many species of domestic as well as wild ruminants. In India, BT is endemic all over the country. however, the clinical outbreak of the diseases occurs only in small ruminants mainly sheep more frequently in the southern states of the country. BT often occurs as asymptomatic form of the diseases in other ruminants. In the present study, prevalence and molecular detection of BT was carried out in Mithun (Bos frontalis) in the north eastern region of the country. A total of 297 apparently healthy mithuns from all the Mithun rearing state of the country i.e. Arunachal Pradesh, Nagaland, Nagaland, Manipur and Mizoram representing different agroclimatic conditions were screened for the presence of BTV specific antibodies using competitive enzymes-linked immunosorbent assay (c-ELISA). Very high prevalence of 93.27 % (277/297) was absorbed in free ranging mithuns. Animals showing high titre of BT antibodies were also screened for the presence of BT genome in RBCs using published primers for VP7 genes for RT-PCR AND 26.4% (33/125) of seropositive and.1% (33/297) of total mithuns were observed positive for BT specific products of 222bp were amplified and sequenced. Real time PCR analysis revealed moderate to high Ct values ranging from 22 to 30 using specific primers for NS3 gene. Further, tissues samples (lings, spleen, and lymph nodes) of the necropsied mithuns were also screened for the detection of BT genome by RT-PCR and specific products of 222bp were amplified in 20% (11/55) necropsied mithuns. Histopathological examination of the tissues revealed edema, congestion and haemorrhages and immunohistochemical examination using anti rabbit anti BTV polyclonal antibody revealed positive signals for BTV antigen in the lungs, spleen and lymph nodes. From the study, it can be concluded that BTV is highly prevalent in the north eastern region of the country. Therefore, it is required to carry out systematic investigation of the diseases in different species of domestic and free ranging animals in the north eastern region of the country to establish the prevalence and dynamics of the spread of the disease. It will great help to understand the epidemiology of BTV in North East region and to formulate effective control and preventive strategies.

NES–O–02

Seroprevalence of selected viral pathogens of pigs in Mizoram, India

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Virus co-infection has become a common phenomenon that involves viruses of same or different type or closely related species. Porcine circovirus 2 (PCV2) being immunosuppressive in nature was found persistently co-infected with other viruses such as Porcine Parvovirus (PPV), classical swine fever virus (CSFV), and Porcine respiratory and reproductive syndrome (PRRS) resulting in the outbreak and pig mortality. Thus, coinfection can be considered as a serious threat to animal husbandry as well as public health. A pilot study was undertaken to investigate the seroprevalence of important viral diseases of pigs in four districts (Aizawl, Champhai, Kolasib, Serchhip) of Mizoram, India. Serum samples (n=169) and clinical samples from 4 animals displaying symptoms suggestive of PRRS (n=16) were collected randomly from pigs irrespective of their age, sex and breed from organized and unorganized farm from four districts of Mizoram during February, 2018 to June, 2018. The serum samples were analyzed by commercially available enzyme-linked immunosorbent assays (ELISA) (OIE approved) according to the manufacturer's instructions; Out of 169 samples, 67 samples were found positive for PRRSV, 44 samples for CSFV, 94 samples for PCV2 and 29 samples for PPV. Indicating a mean positivity of 39.64% (67/169), 26.04% (44/169), 55.62% (94/169) and 17.17% (29/169) respectively. Clinical tissues samples were analyzed by conventional PCR method using previously published primer set based on ORF6 gene segment of PRRSV. Presence of mixed infection was observed in many cases, a total of 11.24% (19/169) showed the presence of PRRS, CSFV and PCV2 whereas 10.39% (18/169) showed the presence of PPV and PCV2; 2.37% (4/169) showed the presence of PCV2 and CSFV. The hidden threats of PCV2 can be recognized from co-infection point of view. Thus the study prioritize on the proper control measure such as maintenance of farm hygiene, seromonitoring, and restricted animal movement from affected farms and culling of infected animals but regular seromonitoring to check this dreaded diseases in correct time otherwise it has huge potential to increase the disease burden to the pig industry of the state. The present study had able to resent the endemicity of CSFV and PRRSV in organized and unorganized farms of Mizoram, which is retarding the growth of swine industry in the state; simultaneously establishing the presence of hidden threat of PCV-2 which is silently affecting the pig population of the region.

NES–O–03

Prevalence of ESBLs and Class-I integrons producing *Escherichia coli* isolated from faeces of pigs in NER states of India

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Integrons are short sequences of deoxyribonucleic acid (DNA) like transposes with the capability of transferring genes of antibiotic resistance from one to other bacteria. Studies revealed that these elements could bear the extended spectrum beta lactamase (ESBL) genes that are transmitted to many strains of bacteria especially among the members of *Enterobacteriaceae*. The aim of this study was to determine the prevalence of ESBLs and integron elements in *Escherichia coli* from faeces of pigs in NER states of India. A total of 790 faecal samples were collected from pigs maintained under organized as well as individual house hold irrespective of age, sex and with or without history of diarrhea from all the eight states of NER India. A total of *E. coli* 2291 were isolated and identified. All the isolates were subjected for antibiotic susceptibility test against cefotaxime, ceftriaxone, cefpodoxime, aztreonam, ceftazidime, cefpodoxime/clavulanate by disk diffusion method. The selected ESBLs (CTXM, TEM, SHV) genes and integron (*IntI1* and *IntI2*) genes were detected by specific PCR assay. A total of 366 (15.98%) *E. coli* isolates were found to be positive for Class 1 integrons (*IntI1*). However, Class 2 integrons (*IntI2*) were not detected in any of the isolates. Altogether, 3.49% isolates were positive for both ESBLs genes and Class 1 integrons. A total of 24 (1.05%) isolates positive for Class 1 integrons were carrying multiple ESBLs genes. Otherwise, independently, 0.17%, 1.92%, 0.17% and 0.17% *E. coli* isolates, positive for Class 1 integrons, were also positive for bla_{SHV}, bla_{TEM}, bla_{CMY} and $bla_{CTX\text{-}M}$ genes, respectively. The results indicated that Class 1 integron were more commonly associated with the bla_{TEM} gene compared to other three genes, which might be due to close genetic linkage between them. The present finding also suggested that *E. coli* isolates with multiple ESBL genotypes have a greater opportunity to carry Class 1 integron. Therefore, bacteria carrying both integrons and ESBL genes have stronger multi-resistance activity.

NES–O–04

Prevalence and Pathology of Mycoplasma bovis in Mithun in North East India

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Mycoplasma bovis (*M.bovis*) is an important and emerging pathogen of bovines and is associated with variety of clinical manifestations including pneumonia, mastitis, arthritis and reproductive problems etc. In India, *M.bovis* is endemic and Prevalence of *M.bovis* have been reported based on serological studies in cattle and buffaloes, however, there are no reports of prevalence of *M.bovis* in Mithun. In the present study, Prevalence and molecular detection of *M.bovis* was carried out in Mithun (*Bos frontalis*) in the north eastern region of the country. A total of 265 apparently healthy and 55 necropsied mithuns from different mithun rearing states of North East India were screened for the presence of *M.bovis specific* antibodies using commercial enzyme-linked immunosorbent assay and a prevalence of 44.9% (119/265) was observed in Mithun population. Tissues samples (lungs and lymph nodes) from 55 dead mithuns and milk samples (37) from seropositive mithuns were screened for the detection of *M.bo*vis genome using PCR using specific primers of *uvr*C gene and amplicon of 690bp amplified in tissues of 29.1% (16/55) dead mithuns and from milk of 24.3% (9/37) seropositive mithuns. Real time PCR analysis of tissues and milk samples using specific primers for 16S *RNA* revealed moderate to high value of Ct ranging from 25-30. Histopathological examination of the tissues revealed chronic lymphocytic bronchointerstitial pneumonia with varying degree of hyperplasia of the bronchiolar epithelium, moderate to extensive proliferation of peribronchiolar lymphocytes and type II pneumocytes and infiltration of mononuclear cells, macrophages and lymphocytes in the interstitium. Immunohistochemical examination of the tissues sections revealed positive signals for the presence of *M.bovis* specific antigen using rabbit anti *M.bovis* polyclonal antibody. From the study, it can be concluded that *M.bovis* highly prevalent in the Mithun population of the north eastern region of the country. Therefore, it needs systematic investigation for the detection of *M.bovis* in the northern region of the country to establish its prevalence and dynamics of spread in different species of animals in the region, which will help to formulate the effective therapeutic and prophylactic strategies for the diseases.

SESSION – IX

Presentation of Society Awards (Young Scientist, Mid-career Scientist and other Awards)

AS-01

Evaluation of surface glycoproteins of classical swine fever virus as an immunogen and an effective diagnostic for pig: A recombinant Newcastle disease virus approach

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Classical swine fever (CSF) is an important viral disease of domestic pigs and wild boar. The structural proteins E2 and Erns of classical swine fever virus (CSFV) are immunogenic surface glycoproteins, which participate in the attachment of the virion to the host cell surface and its subsequent entry. The E2 and Erns proteins are used in the diagnosis and development of the vaccine against CSFV infection in swine. Newcastle disease virus (NDV) has been successfully used as a viral vector to express heterologous proteins. In the present study, E2 and Erns glycoproteins of CSFV are expressed using recombinant NDV (rNDV) platform. The rescued rNDV expressing E2 and Erns proteins showed effective CSFV neutralization antibody titer upon intranasal vaccination in pigs. In addition, the vaccinated serum samples showed neutralization of both homologous and heterologous CSFV strains. Furthermore, the rNDV comprising E2 and Erns proteins of CSFV were used to develop an indirect ELISA. This proposed methodology showed an insight that the E2 protein-based ELISA is better as compared to Erns. Furthermore, the addition of Erns along with E2 protein could reduce the efficacy of E2 based ELISA. The result suggests a possible vaccine strategy and an effective diagnostics for CSFV infection. This could be an economical and better alternative for existing vaccine and diagnostic for CSFV detection and control, respectively, in pigs.

AS–02

Experimental Immune Sensitisation Potentiates Innate Immune Response to Secondary Challenge by Pathogen and Pathogen Product

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Vertebrate immune system has multipronged effectors to enable survival against the myriad pathogenic challenges it encounter through different forms and routes, is primarily classified into two arms i.e. innate immune response and adoptive immune response. Of the two arms, adoptive immune response is bestowed with properties of specificity and memory whereas the innate immune response, the first line of defence against unwarranted attack by pathogenic crucial in encountering the intruder is considered to be amnestic in its response. However increase body of evidences has pointing towards existence of anamnestic response in innate immune response. Here we used experimental animal model of malaria infection using nonlethal murine malaria parasite *Plasmodium chabaudi* in Balb/C mice, to pre-sensitise the immune system and subsequently challenged with secondary bacterial assault (live bacteria). The mortality, estimation of bacterial load in different vital organs i.e spleen and liver and in circulation, moreover the host cytokine response were scored. The data suggest that existence of memory in innate immune system to subsequent pathogen or pathogen product like Lipopolysachharide (LPS) challenge which is now coined as innate immune memory or trained immunity which provides survival advantages to host to an immune assault once the immune system is primed by earlier stimulus.

AS–03

Molecular insights into the ImmunoDefence during novel Japanese encephalitis virus infection

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Japanese encephalitis (JE) is an arboviral disease which accounts for significant mortality annually in Asia and Australia, including India. Our laboratory has discovered a novel strain of JEV (JEV-GP05, accession no: FJ979830) which has significant variation as compared to JEV-GP78 (endemic strain of India). Infected individuals represented with range of symptoms such as fever, headache, reduced level of consciousness, vomiting, altered sensorium, tremors and convulsions. Encephalitis is merely the result of viral invasion in CNS and pathological consequences. Neuronal loss is the outcome of both JEV infection of neurons and bystander damage caused by activated microglia. Recent studies have shown that microRNAs (miRNAs) holds a major platform during JEV infection and modulate cellular pathways to determine the pathological condition. Therefore, we have characterized the GP-05 and investigated the role of miRNA in controlling JEV infection in macrophages, which, if unregulated, might be the probable reason for the adverse inflammatory condition present in the disease pathology.

AS–04

Development Of Probiotic Applications For Endometritis Of Cows

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In the present study twenty four, Lactic Acid Bacteria (LAB) were isolated from bovine vagina and their probiotic properties evaluated to develop a consortium of probiotic organisms for use as a complementary therapy for endometritis in cows. All 24 LAB were assessed for their antibacterial activity, hydrogen peroxide production levels, stability at alkaline pH, adhesion capacity to bovine endometrial epithelial cells and surface hydrophobicity. A probiotic scoring system was developed for relative comparison of their probiotic potential. The LAB was ranked based on their scores and the first four bacterial strains were selected for the formulation of the probiotic product. These probiotic microorganisms were subjected to batch fermentation followed by spray drying. Application of probiotics as intra-uterine delivery for 3-5 consecutive days in cases of laboratory-confirmed bovine clinical endometritis showed improvement in mild and moderate cases but not in severe cases. The parameters analyzed included the nature of cervico vaginal mucus (CVM), White side (WS) test scores, uterine cytology (CY) and leucocytic infiltration of endometrial biopsy (HP score). The overall mean scores before and after treatment with probiotics was 1.76 ±0.6 and 0.29 ± 0.7 for CVM, 1.76 ± 0.6 and 0.29 ± 0.7 for WS test, 1.76 ± 0.6 and 0.82 ± 0.8 for CY, 1.76 ± 0.6 and 0.82 ± 0.8 for HP scores, respectively. Statistical analysis revealed highly significant reduction ($P \leq 0.01$) in the clinical scores before and after probiotic treatment. Percent reduction in the pathogenic bacteria counts after probiotic therapy ranged between 11 and 100. Lowest reduction of bacterial counts was seen in cases infected with *T.pyogenes*.

AS-05

Application of lytic bacteriophages in the treatment of mastitis induced by *Proteus vulgaris* in mice

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Proteus vulgaris accounts for 4.5% of environmental mastitis in bovines. The organism exhibits intrinsic high antibiotic resistance and becomes refractory to treatment. In the present study 185 lytic bacteriophages were screened and based on plaque morphology five were selected as therapeutic agents. The five lytic bacteriophages named ϕPV1, ϕPV2, ϕPV3, ϕPV4 and ϕPV5. These were characterized for host range on the reference strain of *Proteus vulgaris* and also on strains isolated from the cases of mastitis and observed 80 to 92.5%. Biophysical characterization of phages was done to ascertain their stability in hostile environment and observed stability at pH 4, 7, 9 and temperature 16°C, 37°C and 42°C. On morphological characterization the phage mixture belonged to order *Caudovirales* and one phage PV3 to family *Siphoviridae*. Molecular characterization showed DNA as nucleic acid and phage PV3 measured about 28.9Kbp and showed digestion with restriction endonuclease. Therapeutic activity of phage mixture was evaluated in lactating Swiss albino mice that were divided into control, infected and treatment groups. At multiplicity of infection 10 lytic bacteriophage mixture eliminated infection (milk bacterial load) and observed reduction in swelling of mammary gland. This experiment may form a base line for further evaluation of therapeutic efficacy of lytic bacteriophages.

AS-06

Evolutionary Epidemiology of Emerging Enteric Viruses in Wildlife-animals and their Relationship to Human Strains

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Acute viral gastroenteritis remains a major health problem worldwide, especially among young animals and children. Wild animals are always considered as an important source of zoonotic infectious diseases. Most of the emerging infectious diseases (70%) reported so far have originated in wildlife. In present situation zoonoses with wild life reservoir constitutes the most of the public health problems reported world over. Recent advances in molecular diagnostic techniques have allowed recognizing viruses as major cause of diarrheal illnesses globally and identification of several new virus families or novel virus members. We worked on a virus "Picobirnaviruses" (PBVs) which belongs to a recently identified family "*Picobirnaviridae*" and is a dsRNA virus. To date, it is being considered to be an opportunistic pathogen commonly found in the cases of diarrhoea. PBVs have been reported from different animal species worldwide including wild animals. In India, PBVs have been reported from human, cattle and buffalo, horse and pig. There are no reports of PBV from wildlife species. Non-human primates (NHP) play one of the most important roles as reservoir and transmission of several zoonotic viral diseases is due to their similar physiology with humans. Rhesus macaques are unthreatened, though their original habitat is increasingly losing due to the development. We have screened a dozen of wild Rhesus macaques (*Macaca mulatta*) samples for the detection of enteric viruses, and detected PBV in through RT-PCR. To our knowledge, this is the first ever report of PBV strains in free range monkey species in India. Genotyping of detected PBV reveals presence of both genogroup I and II. Furthermore, in phylogenetic analysis the monkey PBV sequence was showing similarity with human strains, predicting the interspecies transmission between wild monkeys and humans. Whereas, GG-I monkey PBV shows higher similarity with gorilla PBVs. Furthermore, histopathological investigations of their small intestines show lesions indicative of enteritis.

Further, we extended our investigation to screen wildlife in captivity inhabiting a high altitude area in temperate Himalayan ecosystem. From August 2014 to May 2015, 53 faecal samples were collected from healthy ruminant (4 species) and non-ruminant (8 species) wild captive animal host species and subjected for molecular screening of enteric viral infections (calicivirus, astrovirus, PBV, rotavirus A and coronavirus), where only PBV infection was seen in captive ruminant species while none of the non-ruminant species was found positive. PBVs detected in captive ruminant host species namely Himalayan goral (*Naemorhedus goral*) and sambar deer (*Rusa unicolor*). Notably, non-ruminant species in same ecosystem were found to be negative of PBV infection. Himalayan goral and sambar deer showed high positivity for PBV infection. Sequencing of the genome segment 2 encoding RdRp gene from Himalayan goral and sambar deer's in the ecosystem confirmed predominance of genogroup- 1 (GG) and circulation of GG-II virus. Phylogenetic analysis shows the high genetic divergence among the PBV strains circulating in wild captive species. Notably, GG-II PBVs clustered with human PBV isolate from India and also exhibited high sequence similarity at nt (77.2-78.2) and aa (83.9-84.7) levels. This finding shows chances of zoonotic transmission of PBVs between wild captive animals and humans. The conserved motif, DFSKFD, was seen in GG-I and GG-II isolates. This appears as the first detection of PBV in these species, which increases the host range of PBV and further supporting the high diversities among PBVs.

Another important family where a number of emerging viruses have been identified is *Picornaviridae*. The genus under this family named Kobuvirus infects humans and animals. It has been reported in several animal species including cattle, sheep, pig, black goat, dog, cat, wild boars and bats. The host range of kobuvirus is expanding with new reports of detection in different wild mammals. Pygmy hog (*Porcula salvania*) is the smallest and rarest wild suid in the world, categorized as 'Critically Endangered' as per the Red list of the International Union for Conservation of Nature (IUCN). To date, the population of pygmy hogs is only in hundreds and is continually waning. Even if, certain pathogens might be posing a major threat to the survival of these pygmy hogs, only a few potential pathogens have been reported in pygmy hogs so far. We took over the work to screen the Pygmy hogs from NE region for enteric viruses. We were successful in detection of kobuvirus (genus *Aichivirus* C) in the critically endangered pygmy hogs. Sequencing of its partial 3D gene revealed close identity with porcine kobuviruses from Czech Republic (JX232619, 90.6-91.6%) and Hungary (NC_011829, 89.8-91.6%) and clustering with Czech Republic (JX232619) strain. The findings support that this report could be a start point to further investigate the role of kobuvirus in health and disease of pygmy hogs. We couldn't detect any other enteric virus in the stool samples including rotavirus, picobirnavirus, astrovirus, coronaviruses etc.

Overall, the molecular investigation clearly points towards circulation of novel enteric viruses belonging to picorna and picobirna virus group's Molecular characterization reported a great diversity of strains. Furthermore, due to the closeness of several wild animal viruses with human virus isolates in this study, the importance of zoonoses is increasingly being recognized and addresses a need for more attention in this area. Therefore, virological surveillance of wild animals is of great interest to establish the repertory of viruses associated with healthy as well as clinically diseased wild hosts. The findings will be important to guide for designing prevention and treatment strategies and vaccine development.

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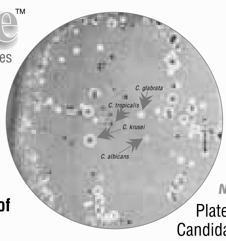

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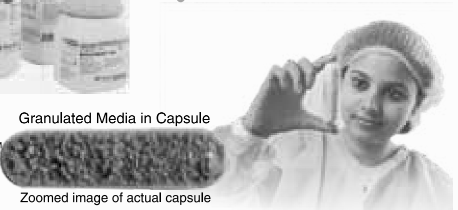

Zoomed image of actual capsule

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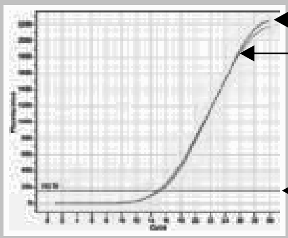
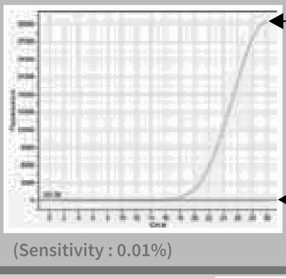
Sample	Ct value
Positive control	14.89
Salmonella spiked in milk	15.32
Negative control	N/A



Detection limit in Milk : upto 1 cell (pre-enrichment)

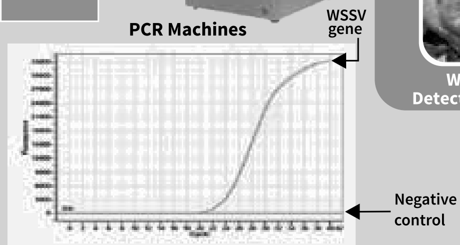
Sample	Ct value
Pork	16.56
Negative control	N/A



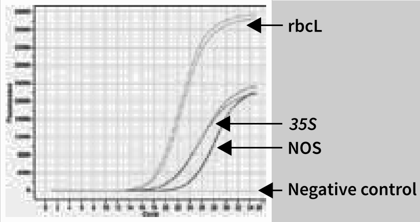
(Sensitivity : 0.01%)

Sample	Ct value
WSSV gene	19.49
Negative control	N/A

PCR Machines



Gene	Ct Value
35S	15.43
NOS	20.19
rbcL	13.9
Negative Control	N/A



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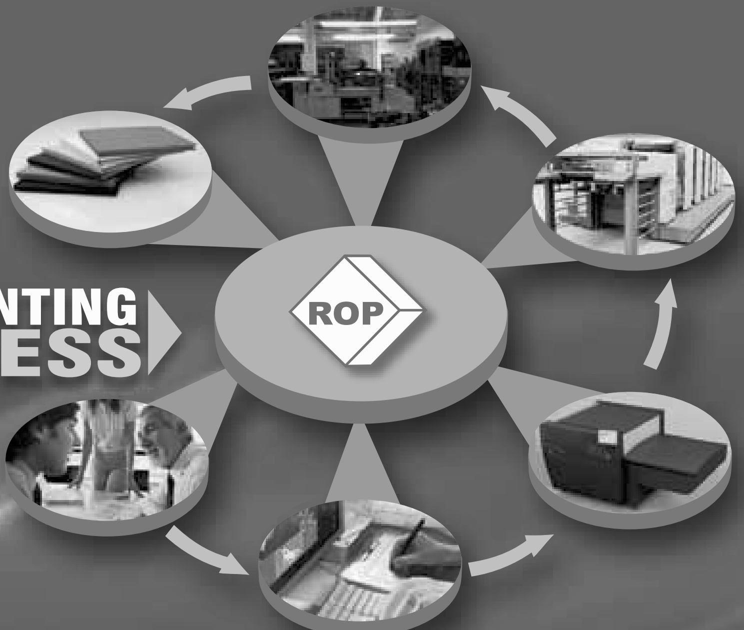
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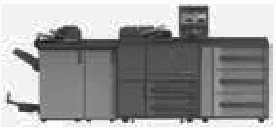

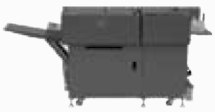


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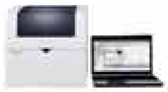

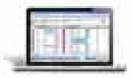

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